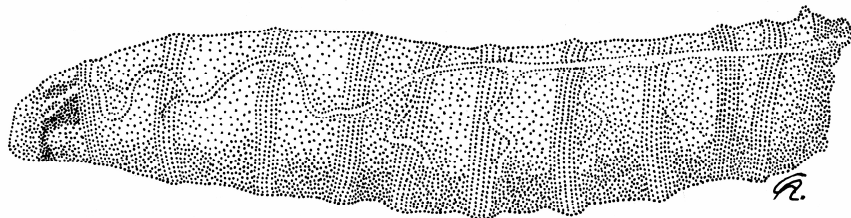


Influence of the microbial environment  
on the interaction  
of a saprophagous insect  
and its parasitoids



A thesis submitted to the  
Christian-Albrechts-University of Kiel  
for a doctoral degree  
in the faculty of science

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*"As for me I am proud of my close kinship with other animals.*

*I take a jealous pride in my Simian ancestry.*

*I like to think that I was once a magnificent hairy fellow living in the trees, and that my  
frame has come down through geological times via sea jelly and worms and*

*Amphioxus, Fish, Dinosaurs and Apes.*

*Who would exchange these for the pallid couple in the Garden of Eden?"*

*[WNP Barbellion, pseudonym of Bruce Frederick Cummings]*

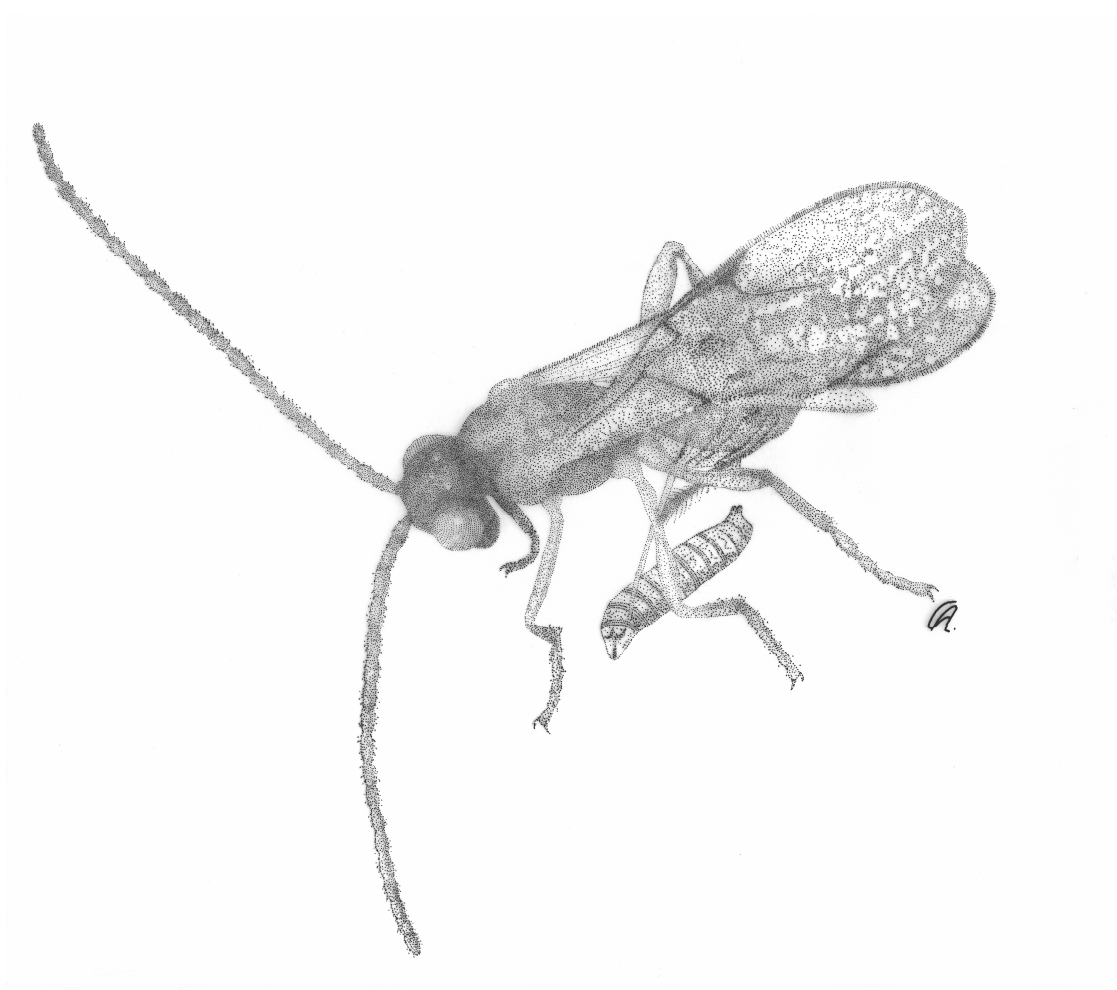






## List of abbreviations used in the text

*	statistical interaction between factors
∅	diameter
%	per cent
ANCOVA	Analysis of covariance
°C	degrees Centigrade
cm	Centimetre ( $10^{-2}$ m)
d	day(s)
df	degrees of freedom
DMSO	dimethyl sulphoxide
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German collection of microorganisms and cell cultures)
F	F-value
Fig.	Figure
g	gram
GLM	Generalised linear model
hrs	hours
l	litre
L/D	light/dark
ME	Malt extract
mg	milligram
ml	millilitre
μl	microlitre
μm	micrometre
mm	millimetre
N	nitrogen
n	number of replicates
n.a.	not analysed
NaOCl	sodium hypochlorite
n.s.	not significant
p	p-value
R <sup>2</sup>	Coefficient of determination
SE	standard error



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## 1 Abstract

*The more you say, the less people remember [François Fénelon].*

*It is better to understand a little than to misunderstand a lot [Anatole France].*

*Why is this thus? What is the reason of this thusness? [Artemus Ward].*

The interactions between hosts and parasites have been observed to be characterised by a genetic variation (genotype-by-genotype interaction) in resistance, tolerance and susceptibility on the host's side and virulence on the side of the parasite, as an outcome of coevolutionary processes. Besides these genetic aspects, various ecological forces may drive evolutionary host-parasite interactions (genotype-by-genotype-by-environment interactions) affecting the strength of and response to selection. These forces may comprise abiotic factors such as environmental temperature or humidity, as well as biotic factors such as predators, superinfection with additional parasites or pathogens and the presence of alternative hosts. Nutrition, and here especially the host's nutritional status, may play a pivotal role in host-parasite relationships, since a parasite usually utilises the host as food source. Additionally, nutrition may have a drastic impact on the host's defence mechanisms and the host's resource allocation to immune defences. Thus, nutrition may modulate both the parasite's virulence and the host's resistance. Saprophagous insects like *Drosophila melanogaster* spend most of their life cycle in, on and in close association to decaying organic material (preferably fruits), which always harbours a plethora of different microorganisms like bacteria and fungi. Especially various yeast species play an important role in both the larval and adult diet of *Drosophila*. Are these different yeast species, which often differ in their metabolic features, all one and the same to a *Drosophila*? The present study revealed that the dietary yeast species composition had a substantial impact on various fitness traits of *Drosophila melanogaster*, such as survival, development time, size parameters and also immune characteristics measured as encapsulation ability. Surprisingly, it also turned out that larvae of *Drosophila melanogaster* chose to devour those yeast species which supported their own development and immunocompetence. Hereby, inter-individual differences occurred regarding the preferred yeast species and their influence on fitness traits of *Drosophila melanogaster*. The microbial influence even carried forward to a third trophic level, parasitoids of *Drosophila*. Since parasitoids (especially endoparasitoids) derive the resources for their development directly from host tissues, the species composition of the host's diet may directly influence parasitoid fitness. Via the impact of nutrition on the host's immune defence, the parasitoid may also be indirectly affected. This study revealed that the dietary microbial species composition also had a substantial impact on various life history traits of the parasitoids *Asobara tabida* and *Leptopilina heterotoma*. Hence, a different microbial species composition in the nutritional environment may modulate the outcome of host-parasitoid interactions and play a role in host-parasitoid dynamics and coevolution between *Drosophila melanogaster* and its parasitoids *Asobara tabida* and *Leptopilina heterotoma*.

### Zusammenfassung

Als Folge koevolutionärer Prozesse sind Interaktionen zwischen Wirt und Parasit durch eine genetische Variation (Gen-zu-Gen Interaktion) bezüglich Resistenz, Toleranz und Anfälligkeit von Seiten des Wirtes und der Virulenz von Seiten des Parasiten gekennzeichnet. Neben diesen genetischen Aspekten, vermögen verschiedenste ökologische Faktoren die evolutionären Vorgänge in Wirt-Parasit Interaktionen anzutreiben (Gen-zu-Gen-zu-Umwelt Interaktionen), und die Stärke der Selektion wie auch selektionäre Antworten zu beeinflussen. Diese ökologischen Faktoren können sowohl abiotische Faktoren wie z.B. Temperatur und Feuchtigkeit, als auch biotische Faktoren wie z.B. Prädatoren, Superinfektionen mit anderen Parasiten oder Pathogenen, und die Anwesenheit alternativer Wirte umfassen. Die Nahrung, und insbesondere der Ernährungszustand des Wirtsorganismus, können eine entscheidende Rolle in Wirt-Parasit Beziehungen spielen, da ein Parasit den Wirt in der Regel als Nahrungsressource nutzt. Außerdem kann die Ernährung einen drastischen Einfluss auf Abwehrmechanismen des Wirtes nehmen und darauf, wie viel seiner Ressourcen er in eine Immunabwehr investiert. Saprophage Insekten wie beispielsweise *Drosophila melanogaster*, verbringen einen großen Teil ihres Lebens in, auf oder in enger Verbindung zu verfaulem organischen Material (vorzugsweise Früchten), das stets eine Fülle unterschiedlichster Mikroorganismen wie z.B. Bakterien und Pilze beherbergt. Insbesondere verschiedenste Hefearten spielen hierbei eine wichtige Rolle als Nahrungsressource, sowohl für Larven als auch Adulttiere von *Drosophila melanogaster*. Haben diese verschiedenen, sich häufig in ihrem Stoffwechsel voneinander unterscheidenden Hefearten, eine unterschiedliche Bedeutung für *Drosophila*? In der vorliegenden Arbeit zeigte sich, dass die mikrobielle Zusammensetzung der Nahrung einen erheblichen Einfluss auf verschiedenste Fitness-Parameter von *Drosophila melanogaster* hatte, wie beispielsweise auf Überleben, Entwicklungszeit, Körpergröße- und gewicht, und ebenfalls auf als Einkapselungsfähigkeit erfasste Immunparameter. Überraschenderweise zeigte sich auch, dass Larven von *Drosophila melanogaster* bevorzugt die Hefearten verspeisten, die für ihre Entwicklung und Immunabwehr förderlich waren. Hinsichtlich dessen, welche Hefearten sie bevorzugten und welche für ihre Fitness förderlich waren, traten jedoch Unterschiede zwischen einzelnen Individuen auf. Der Einfluss der Mikroorganismen setzte sich sogar in die nächste trophische Ebene fort, Parasitoide von *Drosophila*. Da Parasitoide (insbesondere Endoparasitoide) die Ressourcen für ihre Entwicklung direkt vom Gewebe ihres Wirtes beziehen, vermag die mikrobielle Zusammensetzung der Nahrung des Wirtes die Fitness des Parasitoiden direkt zu beeinflussen. Über den Einfluss der Ernährung des Wirtes auf dessen Immunabwehr kann die Fitness des Parasitoiden auch indirekt beeinflusst werden. In der vorliegenden Arbeit zeigte sich, dass die mikrobielle Zusammensetzung der Nahrung auch erhebliche Auswirkungen auf Fitness-Parameter der beiden Parasitoide *Asobara tabida* und *Leptopilina heterotoma* hatte. Folglich vermag eine unterschiedliche mikrobielle Umwelt den Ausgang einer Wirt-Parasit Interaktion zu modulieren und kann bezüglich der Dynamik und Koevolution zwischen dem Wirtsorganismus *Drosophila melanogaster* und dessen Parasitoiden *Asobara tabida* und *Leptopilina heterotoma* von Bedeutung sein.



*„Health food makes me sick“  
Calvin Trillin, American Journalist,  
humorist and novelist.*





## 2 General Introduction

*"Don't forget that the flavours of wine and cheese  
depend upon the types of infecting microorganisms"  
[Martin Henry Fischer]*

It is probably rather hard to imagine a saprophagous insect as the dew fly *Drosophila melanogaster* as gourmet with a refined smell and taste, enabling it to discriminate between specific compounds of its diet. The majority of saprophagous (Greek: σαπρός meaning “rotten” and φαγεῖν meaning “devour”) animals, or “detritivores”, as they are mostly called in English, consume both the detritus itself (dead organic matter of different kinds) and the associated microbial flora (bacteria and fungi), and usually a great diversity of microbial species with different metabolic features is involved in the decomposition of dead material (DREW *et al.* 1983; BEGON, TOWNSEND & HARPER 2006). Many of these microorganisms serve as nutritional environment for *Drosophila melanogaster*, besides having the potential of being pathogenic. If we were at least as small as Tom Thumb it would probably be easier for us to put ourselves into the position of a *Drosophila* larva facing this microbial diversity. Many species of *Drosophila* are reported to exhibit specific preferences for particular categories of rotting fruit and vegetables (OAKESHOTT *et al.* 1982), which in themselves usually carry a specific associated microflora, mainly dominated by yeasts. Are these different microorganisms all one and the same to a *Drosophila*?

Phenotypic traits of organisms are usually determined by an interplay of genetic and environmental influences. Even traits with a strong genetic determination may experience a severe influence by environmental conditions, so that the same genotype may exhibit different phenotypes under different environmental conditions (LAZZARO *et al.* 2008). An organism's nutritional environment undoubtedly plays a pivotal role for all physiological and ecological processes, determining growth, reproduction and maintenance (FROST *et al.* 2005). *Drosophila melanogaster* is faced with being dependent on ephemeral and patchily distributed resources and additionally on the presence of microorganisms that process the plant raw material into important dietary factors (STARMER 1981).

In this study, attention was turned to specific microbial species in the potential microbial environment of *Drosophila melanogaster*, and their impact on certain parameters in the life-history of this saprophagous insect. Of particular interest hereby was the impact on the immune system of *Drosophila melanogaster* as it is challenged by a third trophic level – parasites. One crucial question absorbing evolutionary ecology with regard to immune defence in hosts is to explain, why the observed immune responses vary widely across species and situations (SCHMID-HEMPEL 2003). Besides various other factors (e.g. costs of

## 2 *General Introduction*

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immune defence, sexual selection), the availability and quality of nutritional resources are assumed to act as potential modulators inducing the observed variation in immune defence. Additionally, since parasites live on and inside their hosts, from which they derive resources for their own development and reproduction, the food of their host may have a crucial effect on the parasite's own life history, both in a direct way (food is assimilated by the parasite via host tissues) and an indirect way (food affects the host's immune system). Thus, the specific microbial environment of a saprophagous insect may be a key factor and modulator in host-parasite interaction.

The aspects I intended to tackle in my study were (i) whether different microbial species in the larval diet of *Drosophila melanogaster* have a different impact on life-history traits (such as survival, development time, body weight, immune defence) both of unparasitised and parasitised larvae, (ii) if so, what possible mechanisms might account for the observed differences, (iii) whether larvae of *Drosophila melanogaster* are able to distinguish between the different yeast species, detect potential differences in their quality and choose to devour those which best meet their nutritional demands, and (iv) whether different microbial species in the larval diet of *Drosophila melanogaster* also have a variable impact on life-history traits of a third trophic level, parasitoids of *Drosophila melanogaster*. The two most common parasitoid species of *Drosophila melanogaster* on fermenting substrates in Europe are the braconid wasp *Asobara tabida* and the chalcidoid wasp *Leptopilina heterotoma*. These two parasitoids were chosen for the present study. Interestingly, larvae of *Drosophila melanogaster* are mainly able to exhibit a genetically determined successful immune defence (as encapsulation ability) against *Asobara tabida*, but rarely against *Leptopilina heterotoma*. For this reason, most of the experiments presented in my study were carried out with *Asobara tabida*, in order to observe possible impacts on the immunocompetence of *Drosophila melanogaster*.

## 2.1 Parasites and pathogens – a success story?

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### 2.1 Parasites and pathogens – a success story?

*He [Einstein] is supposed to have imagined that he was travelling through space riding on a beam of light. This fantasy adventure led to the discovery of the theory of relativity. My version of an Einsteinian trip was to imagine that I was travelling through our biosphere riding on parasites. What I saw was astounding! I saw very clearly that parasites were running the world, holding it together and managing it [Donald A. Windsor].*

*Although the natural history and identity of parasitoids are little known among nonbiologists, they are of immense importance in natural and agricultural ecosystems where they influence or regulate the population density of many of their hosts [H.C.J. Godfray].*

#### 2.1.1 *Creaturus omnipraesens*

From the point of view of most human ethics parasitism may be a rather condemnable way to lead ones life. Yet, parasitism is probably the most common lifestyle on our planet and one of the most successful modes of life, at least when considering how often it evolved and how many parasitic species presently exist (POULIN & MORAND 2000).

But what actually is a *parasite*? To define the term parasitism or parasite is not as easy as it might seem at first glance, when trying to separate parasitism from other inter-organismal interactions (such as commensalism or mutualism). PRICE (1980) even stated that “There are probably as many definitions of a parasite as there are books on parasitism”. To make things easier and shorter, I will merely give the following most widely accepted definition according to (and slightly modified after) POULIN (2006): “A parasite (Greek παρά = beside; σίτος = food) is defined as an organism that lives in or on another living organism (the host) deriving its nutrients from it (usually one or a very few host individuals), showing some degree of structural adaptation to it and normally causing it some harm”. It is sometimes helpful to think of parasitism not as a distinct category, but rather envisage it as part of a continuum connecting different kinds of species interactions.

Often a parasite is referred to as *parasitoid* (see 2.1.2) when the harm inflicted on the host regularly leads to its death. Once a parasite colonises a host, i.e. enters the body (and multiplies), that host is said to harbour an infection (BEGON, TOWNSEND & HARPER 2006). Sometimes parasites are also referred to as pathogens, since they may elicit a disease and thus cause a state of distress (Greek: παθογένεια = that which creates suffering; πάθος = suffering, disease; γεννώ = give birth to). Pathogens, however, may comprise inanimate infectious substances as well as organisms (viruses, prions, bacteria, protozoa, fungi etc.) and the term pathogen is preferably used for microbial agents (agents too small to be seen without a microscope). The term parasite, in turn, is often used for both microbial

### 2.1.1 *Creaturus omnipraesens*

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pathogens (*microparasites*) and multicellular parasites (*macroparasites*). Parasites are often classified due to the mode of interaction with their host and their life cycle. Often, parasites are divided into those, developing and living within internal organs or tissues of their host (*endoparasites*), and those, developing and living on their host's exterior (*ectoparasites*).

Parasites and pathogens are stated to pose a ubiquitous threat to all organisms and it is assumed that probably no organism exists that does not harbour any parasites (WINDSOR 1998; WILSON 2005). In view of the fact that most parasites are host specific, probably more than 50% of all species and individuals on earth are parasites (BEGON, TOWNSEND & HARPER 2006). WINDSOR (1998) concludes the number of parasite species being much greater than that of free-living species, due to the fact that “some parasite species may have several species of hosts, some host species several species of parasites and that parasites themselves can have parasites”.

Also in human history, infectious diseases have played an undeniably important role by influencing the biology and culture of human beings – “from the plagues of biblical times to the HIV pandemic of today” (KLINKOWSKI 1970; ANDERSON & MAY 1991; DOBSON & CARPER 1996). To humans, parasites also are of considerable agricultural concern, since they may challenge the lives of domestic and wild animals, fish stocks and food crops.

Besides mutualistic associations, parasitic ones are addressed to be of major importance as drivers of ecological function and evolutionary processes (WINDSOR 1998; THRALL *et al.* 2007). Being such an important feature in the host's selective environment, parasites are even supposed to have driven the evolution of sex and host's sexual selection behaviour, and the coevolution (reciprocal evolutionary change in interacting species driven by natural selection) of hosts with their parasites seems to be one reason for the predominance of sexual reproduction throughout the animal kingdom (HURST & PECK 1996; LYTHGOE 2000; MARTINS 2000; THOMAS *et al.* 2002; HOWARD & LIVELY 2003; KURTZ 2003; COOPER *et al.* 2005; FISCHER & SCHMID-HEMPEL 2005; AGRAWAL 2006; MILINSKI 2006). Parasites are hypothesised to play a vital role in sexual selection and the maintenance of secondary sexual traits (hypothesis of parasite-mediated sexual selection or *Hamilton-Zuk hypothesis*) (HAMILTON & ZUK 1982). This hypothesis predicts that females of a given species should favour to mate with ‘bright’ males in order to gain a selective advantage for their offspring, whereby ‘brightness’ is correlated with parasite resistance. The females are hereby supposed to detect the male's ‘brightness’ via male signals that are energetically costly such as bright plumage, elaborate song, athletic behaviour etc. (HAMILTON & ZUK 1982; MILINSKI 2001; ROLFF & SIVA-JOTHY 2003) Parasite-host interactions are supposed to play an important role for the maintenance of genetic diversity in natural populations in general (not merely in reference to the evolution of sex and sexual selection) (see *Red Queen Hypothesis*) (LIVELY 1996, 2001). Although parasites are usually documented to

### 2.1.1 *Creaturus omnipraesens*

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reduce the fitness of their host there is some evidence that other ecological factors may determine whether a parasite is detrimental or beneficial to host net fitness. In sexual selection processes, for example, males having genes to survive parasitism and having experienced parasitic infection may provide an 'honest signal' to females that they possess genes for parasite resistance (THOMAS *et al.* 2000).

Parasitism is thought to have occurred at an early stage of evolution, soon after the formation of self-reproducing systems, which were exploited by the parasitic system for its own replication (BREMERMAN 1983). This would, however, imply a broader definition of parasitism that also comprises molecules. As indicated by the available fossil evidence, the origins of parasitism go back several million years, up to 400 million years e.g. for a parasitic fungus (TAYLOR *et al.* 1991), with parasites having probably evolved from free-living ancestors. Parasitism is supposed to have evolved independently several times in many different organismal lineages and has sometimes arisen more than once within one taxon (POULIN 2007).

In adaptation to the parasitic lifestyle, organisms have undergone a variety of changes including their physiology, morphology and life history traits (POULIN 1995a). They must above all have the ability to infect and damage their host by invading its immune response (ZELMER 1997). In order to achieve this, **virulence** is supposed to be a parasite's key property. Virulence (Latin: virulentus = poisonous) is referred to as the relative degree of damage (loss of fitness) (*quantitative property*) done to a host by the pathogen, whereas pathogenicity is the qualitative capacity (*qualitative property*) of an infectious agent to inflict a disease on an organism (SACRISTÁN & GARCÍA-ARENAL 2008). The probability of infection, however, does not only depend on traits of the parasite (pathogenicity, virulence and avirulence), but also on traits of the host (susceptibility, resistance and tolerance) (ROY & KIRCHNER 2000; GANDON *et al.* 2002), whereby host resistance is considered to lead to host-mediated loss of fitness in the parasite. According to empirically supported models (gene-for-gene and matching-allele models) the outcome of an infection is hereby supposed to be determined by specific combinations of host and parasite genotypes (**genotype-by-genotype interaction**) (RESTIF & KOELLA 2003; LAMBRECHTS *et al.* 2006). Yet, these host-by-parasite interactions may be of alternate influence on variance in parasite virulence and host resistance (GRECH *et al.* 2006). Host-parasite coevolution may additionally be shaped by the spatial structure of the interaction both on a narrow and broad geographic scale, and may be dependent on metapopulation dynamics manifested in gene flow, extinction and colonisation events (BOOTS & SASAKI 1999; LIVELY 1999; THOMPSON 1999, 2001; GANDON & MICHALAKIS 2002; THOMPSON & CUNNINGHAM 2002; THRALL & BURDON 2002). Several studies have also revealed that a variation in environmental conditions can affect host-parasite coevolution. Thus, variation in factors such as environmental temperature and nutrient availability, may affect the outcome of infection, overriding the genetic basis, not

### 2.1.1 *Creaturus omnipraesens*

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necessarily in a linear way, but as a complex interaction of the genotypes of both host and parasite and the environment (*genotype-by-genotype-by-environment interaction*) (THOMAS & BLUNFORD 2003; LAINE 2007; LAZZARO *et al.* 2008; MCKEAN *et al.* 2008).

### 2.1.2 Parasitoids – aliens from inner space

GODFRAY (1994) introduces his milestone book on parasitoids by referring to the motion picture “*Alien*” produced in 1979 where occupants of a spaceship get parasitised by an alien life form which develops and grows in human bodies. Fortunately, no such creature has been detected on our planet so far. Although human beings have been spared the fate of being attacked by parasitoids, probably almost every species within the huge taxon of insects suffers from parasitoid attacks. Now, what actually is a *parasitoid*? Parasitoids are usually insects whose larvae develop by feeding on the bodies of other arthropods - usually also insects - and the larval feeding results in the death of the parasitoid’s host (GODFRAY 1994; WHITFIELD 2003). Most parasitoids are found among the order Hymenoptera (within the monophyletic Apocrita) and are mostly referred to as *parasitoid wasps*. Female wasps have specialised ovipositors, allowing them to lay their eggs on or inside their host or their host’s food plant. Parasitoids may also be found among other insect orders such as Diptera, Coleoptera, Lepidoptera, Trichoptera, Neuroptera and Strepsiptera (GODFRAY 1994; PENNACCHIO & STRAND 2006).

The number of parasitoid species can yet only be guessed. Since the total number of insect species on earth is yet not known (and will probably never be), the number of parasitoid species (themselves being mainly insects) is an enigma. Nevertheless, parasitoids are assumed to constitute 20%-25% of all insect species (GODFRAY 1994) and estimates suggest that 10%-20% of all insects are parasitoid wasps, i.e. belonging to the order Hymenoptera (WHITFIELD 2003), although the proportion may be much higher considering that nearly every insect species may harbour one parasitoid species.

Most parasitoids attack a specific life stage of their host, i.e. either eggs, larvae, pupae or adults. Adult parasitoids are free living organisms that may feed - among other things - on pollen and nectar. Similar to predators, parasitoids always kill their host. In contrast to predators, however, that usually attack several prey individuals during their lifetime, parasitoids require only one host to fulfil their development (BEGON, TOWNSEND & HARPER 2006). Parasitoids may either live and feed inside their host (*endoparasitoids*) or live externally and feed from the outside (*ectoparasitoids*). One host may harbour merely one parasitoid (*solitary parasitoids*) feeding on it, or up to several thousand (*gregarious parasitoids*) sharing one host. Parasitoids may prevent their host’s further development after parasitisation (by *permanent paralysis*) feeding merely on the resources available at ovi-

### 2.1.2 Parasitoids – aliens from inner space

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position (*idiobiont parasitoid*). Others, however, allow further development of their host after parasitisation and only start to feed invasively and destructively until the host either pupates or becomes an adult (*koinobiont parasitoid*). Most koinobionts are endoparasitoids, attacking hosts during their larval stage. Idiobionts can be either ectoparasitoids that paralyse their hosts, or endoparasitoids, mostly attacking sessile developmental stages of their hosts, such as eggs or pupae (GODFRAY 1994; WHITFIELD 1998; MAYHEW & BLACKBURN 1999; RONQUIST 1999; PENACCHIO & STRAND 2006; JERVIS *et al.* 2008). WHITFIELD (1998) suggests that permanent paralysis of the host or even death by venoms is the ancestral state in parasitic Hymenoptera. The benefit of temporal paralysis in hosts of koinobiont parasitoids is yet not exactly known.

Female parasitoids may be characterised by the timing of egg maturation (*ovigeny*). Parasitoid species may be *proovigenic*, meaning that all eggs are mature at the time of eclosion of the adult female. This also includes that these females have already reached their potential lifetime fecundity at the time of eclosion and that it is limited. The other extreme may be that parasitoid species are *synovigenic*, meaning that egg maturation occurs after eclosion, often after the female has had access to high energy food (e.g. host feeding). Nevertheless, ovigeny should be seen as a continuum, with proovigeny and synovigeny at the outer ends. It may also be expressed in terms of ‘ovigeny-index’, defined as the proportion of the potential lifetime complement of eggs that is mature upon female emergence, whereby in proovigenic species ovigeny index = 1, in synovigenic species ovigeny index = 0 (JERVIS *et al.* 2001).

In order to be able to develop inside their host, parasitoids are forced to face, evade or suppress the host’s immune defence (see chapter 2.2), i.e. exhibiting some level of *virulence* (see chapter 2.1.1). Hosts not able to exhibit an immune defence against parasitoids are only very seldom found. Such a rare exception is the dew fly *Drosophila subobscura* that is a natural host for several species of hymenopteran parasitoid wasps. It is not able to encapsulate parasitoid eggs or larvae, probably due to a deficiency in a category of haemocytes that are supposed to be the main cell type of *Drosophila* involved in encapsulation (lamellocytes; see chapter 2.2.2), thus possessing an innate immunodeficiency against a wide range of parasites (ESLIN & DOURY 2006). To overcome immune responses of immunocompetent hosts, especially parasitoid wasps have evolved numerous methods, including (virulence) strategies for *passive evasion* and *active suppression* (STRAND & PECH 1995; SCHLENKE *et al.* 2007). Passive mechanisms comprise developing in locations that protect the parasitoid from encapsulation, or possessing features on the surface of the wasp’s egg as a type of ‘molecular mimicry’, preventing haemocytes from recognising the parasitoid as non-self (VASS & NAPPI 2000; SCHMIDT *et al.* 2001; PREVOST *et al.* 2005). Active mechanisms refer to parasitoids paralyzing their host, disrupting its development or interfering with one or more distinct elements of its immune system (often called immune-

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suppressive factors, ISFs) (MOREAU *et al.* 2002; MOREAU & GUILLOT 2005; MOREAU *et al.* 2005). As active protection some female parasitoid wasp species rely on maternal protein secretions, including symbiotic viruses (Polydnviruses, PDVs) or virus-like particles (VLPs) that resemble PDVs, produced in the wasp's ovaries and transmitted into the host during oviposition (LAVINE & BECKAGE 1995; SHELBY & WEBB 1999; SCHMIDT *et al.* 2001, 2005; FREDERICI & BIGOT 2003; LABROSSE *et al.* 2003; MORALES *et al.* 2005; ASGARI 2006; BECK & STRAND 2007; BECKAGE 2008). Further virulence factors used by hymenopteran parasitoids are ovarian fluids, teratocytes (cells originating from serosal cells of the parasitoid embryo, being released into the host haemolymph) and other venoms (DAHLMAN 1990; ASGARI *et al.* 1998; VINSON 1990; VASS & NAPPI 2000; BECKAGE & GELMAN 2004; NAPPI *et al.* 2004; LABROSSE *et al.* 2005; MOREAU & GUILLOT 2005).

Female parasitoids face the problem of having to locate suitable hosts for oviposition, since they often emerge distant to host populations. The location process leading to successful parasitisation has been characterised by (a) host habitat location, (b) host location, (c) host acceptance and (d) host suitability, with chemical and physical factors being involved in this process. Many parasitoids have been reported of being able to distinguish between various types of hosts and preferring those most suitable to the development of their offspring (VINSON 1976).

## 2.2 Ways to defend oneself

### 2.2.1 The art of self-defence – Notes on innate immunity (with a focus on insects and cellular immunity)

Facing the immeasurable variety and number of organisms exhibiting a parasitic lifestyle, the availability of an efficient defence mechanism permitting the recognition and elimination of such invaders must have been of a highly adaptive value and in the course of evolution led to the defence mechanism called “immune system” (BAYNE 2003; SCHULENBURG *et al.* 2004).

Prior to the immune system taking action against invading parasites and pathogens, organisms possess a variety of defence mechanisms to impede penetration and invasion. These comprise e.g. *physical barriers* provided by an organism's tegument such as a cuticle and cuticular layers, chitinous membranes, epidermis etc. (HOFFMANN & REICHHART 2002; TZOU *et al.* 2002; VODOVAR *et al.* 2004; ELIAS 2007). The usually low acidic pH in an organism's gastrointestinal tract and the secretion of antimicrobial factors, such as lysozymes, creates and maintains a hostile environment and can thus act as *chemical barriers* against invaders (HARRISON 2001; HOFFMANN & REICHHART 2002; TZOU *et al.* 2002; VODOVAR *et al.* 2004; SAURABH & SAHOO 2008). When parasites and pathogens neverthe-



### 2.2.1 *The art of self defence*

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less succeed in breaching these barriers, an organism's often multifaceted immune reactions are induced. Immune systems are supposedly amongst the most diverse biological systems (KURTZ 2003). SOMPAYRAC (2003) compares the immune system to a football team comprising a network of players who cooperate to get things done, i.e. in a direct sense, to recognise and fend off pathogens and tumor cells. This network of different physical, cellular and molecular mechanisms has traditionally been divided into the two components *innate immune system* and *adaptive immune system*, mainly stated to differ in the mechanisms and receptors that are used to recognise antigens (MEDZHITOV & JANEWAY 2000; GOVIND & NEHM 2004; SCHMIDT *et al.* 2008). Probably all metazoans (plants, fungi and animals) possess an innate immune system which is the evolutionary older system of the two (HOFFMANN *et al.* 1999; JANEWAY & MEDZHITOV 2002; HOFFMANN 2003; BEUTLER 2004). Even though it is still not known, when and where the so called *adaptive immune system* emerged, it is thought to be unique to jawed vertebrates (KASAHARA *et al.* 2004). The key components of the adaptive immune system comprise the Major Histocompatibility complex (MHC) and a mechanism of genetic recombination (V(D)J recombination) and somatic mutation (see also *somatic hypermutation*) that allows the generation and somatic diversification of a great repertoire of antigen receptors of lymphocytes and immunoglobulins (KASAHARA *et al.* 2004). Additionally, immunological memory (which cannot be seen in disassociation to specificity) is mostly supposed to be a key property of adaptive immunity. The term "adaptive" refers to this high adaptability of the adaptive immune system to a plethora of specific antigens. Often, the term 'specific immune system' is (incorrectly) used as a synonym for the adaptive immune system. Specificity, however, needn't be bound to the mechanisms evolved within the adaptive immune system, since specificity is also known from invertebrates (SCHMID-HEMPEL & EBERT 2003; KURTZ & ARMITAGE 2006). There are also hints of immunological memory to exist in innate immunity (SCHMID-HEMPEL 2003; SCHMID-HEMPEL 2005; KURTZ 2005). How the specificity within the innate immune system of invertebrates is generated, is still not known. A very promising mechanism is, however, seen in the *Dscam* (Down Syndrome Cell Adhesion Molecule) gene, being a *Drosophila* homologue of the human DSCAM, and encoding for surface receptor molecules belonging to the immunoglobulin superfamily. By alternative splicing (modification of pre-mRNA into different mRNAs), more than 38.000 different splice variants and thus different isoforms of the receptor molecule can be generated out of a single gene. The *Dscam* isoforms have been shown to play a role in neuronal circuit guidance and assembly (SCHMUCKER *et al.* 2000; HATTORI *et al.* 2007), but are also expressed by immune-competent cells of *Drosophila* (WATSON *et al.* 2005) and other insects (DONG *et al.* 2006) and arthropods (BRITES *et al.* 2008) and are excreted into the haemolymph.

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As suggested by SCHMID-HEMPEL (2005), when viewing immune systems from an evolutionary ecology perspective, it would be reasonable to complement the division of immune systems into innate and adaptive immunity by a classification that charts two major functional determinants of defence, namely speed and specificity. Components of fast versus slow as well as specific versus non-specific can be found both among the constitutive (represented by innate immune responses) and the induced arm of the immune defence (SCHMID-HEMPEL 2005).

The major problems for an immune system are to recognise and destroy all potential pathogens and to be able to discriminate between alien structures invading an organism and those of its own, i.e. 'non-self' and 'self' (KIMBRELL & BEUTLER 2001; HOFFMANN & REICHHART 2002; BEUTLER 2004).

The *innate immune system* is capable of recognising a wide spectrum of conserved microbial structures or products of microbial metabolism that are usually termed as *pathogen associated molecular patterns (PAMPs)*. These are unique to microorganisms (also non-pathogenic ones) and are not present in the host (BEUTLER 2004; SCHMIDT *et al.* 2008). However, the term PAMPs is criticised by Beutler, since it is not only pathogens but all microbes that are sensed and it is individual molecules and not molecular patterns that are recognised (BEUTLER 2004). Additionally, innate immune responses are obviously more differentiated towards microorganisms than PAMPs predict, since commensal microorganisms do not elicit an immune response. How the host distinguishes between pathogenic microorganisms which should elicit an immune response and commensal microorganisms is yet not known (MEDZHITOV 2001). The specific microbe-associated molecules, however, ensure a distinction of *self* from *non-self*. Recognition is accomplished via a set of receptors encoded in the germ line, often termed *pattern recognition receptors (PRRs)* or peptidoglycan recognition proteins (*PGRPs*) (also criticised by Beutler, reason see above) that bind to molecules (peptidoglycans and glucans) associated with microbes (GILLESPIE *et al.* 1997; MEDZHITOV 2000; MEDZHITOV & JANEWAY 2002; HULTMARK 2003; KURATA 2004, 2006; KURATA *et al.* 2006; BANGHAM *et al.* 2006; IRITI & FAORO 2007; SCHMIDT *et al.* 2008).

The principle components of both the innate and the adaptive immune system include *cellular elements* (blood cells, haemocytes) and *humoral elements* (soluble non-cellular components in the blood/haemolymph or other body fluids) (BEUTLER 2004; LAVINE & STRAND 2002; SCHMIDT *et al.* 2008; STRAND 2008a & b). Among these two elements, sensing (recognition) mechanisms as well as effector (destroying) mechanisms are found. In the cellular immunity, however, these two mechanisms are often blurred in contrast to the humoral immunity, where molecules sensing microbes are not necessarily the same that kill them (BEUTLER 2004).

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As effectors within the cellular component of the immune system myeloid cells play a major role. Humoral components include antimicrobial peptides, reactive intermediates of oxygen and nitrogen (ROI & RNI), complement-like proteins and products generated by complex enzymatic cascades regulating coagulation and melanisation (e.g. the phenoloxidase pathway) (LAVINE & STRAND 2002; BLANDIN & LEVASHINA 2004; IMLER & BULET 2005; STRAND 2008b). Even though experimental evidence is comparably scant, the results achieved so far suggest, that the humoral and cellular components are well coordinated with one another, act in concert and are both essential for survival (ELROD-ERICKSON 2000; LAVINE & STRAND 2002).

In different insect species a number of different haemocytes can be identified and classified, by morphological, histochemical and functional characteristics. The bulk of haemocyte types found in most insect species are *plasmatocytes*, *granular haemocytes*, *oenocytoids* and *spherule cells*. Additionally, *lamellocytes* and *crystal cells* (see chapter 2.2.2) can be found as a peculiarity in *Drosophila* (RIBEIRO & BREHÉLIN 2006; STRAND 2008a & b). Haemocyte mediated defence responses comprise phagocytosis, encapsulation, nodulation, melanisation and coagulation (LAVINE & STRAND 2002; THEOPOLD *et al.* 2002, 2004; STUART & EZEKOWITZ 2005; JIRAVANICHPAISAL 2006; STUART *et al.* 2007; STRAND 2008a & b). Hereby, *encapsulation* (see chapter 2.2.2) is the most important defence mechanism of insects against parasitoids, nematodes and other foreign bodies that are too large to be phagocytosed (KRAAIJEVELD & GODFRAY 1999; CARTON *et al.* 2008). The particular haemocytes involved in this encapsulation process vary among different insect species (CARTON *et al.* 2008). The formation of a multicellular capsule is very often accompanied by *melanisation*, the production of melanin (more correct *eumelanin*) and deposition on the encapsulated target. Melanin is a phenolic biopolymer widely distributed throughout the animal kingdom. It can be found in skin, hair and eyes of animals providing pigmentation, but it is also involved in immune defence reactions, wound healing and cuticular hardening (sclerotisation) (SUGUMARAN 1998; THEOPOLD *et al.* 2002, 2004; FRANSSSENS *et al.* 2008). Together with asphyxiation (lack of oxygen; Greek  $\alpha$  = without and σφῆγγμός = pulse), antibacterial peptides, ROI and RNI, melanin is supposed to function as a killing agent in the encapsulation process which usually leads to the death of the encapsulated organism (LAVINE & STRAND 2002). Melanin synthesis involves a series of enzymatic and non-enzymatic reactions (CHRISTENSEN *et al.* 2005). The initial substrate of melanogenesis is the amino acid tyrosine (itself formed by the hydroxylation of phenylalanine) whose hydroxylation is catalysed by the enzyme phenoloxidase (PO), being the invertebrate equivalent (insects and other arthropods) of the mammalian tyrosinase. PO itself is the active form of the zymogen (inactive precursor) prophenoloxidase (PPO) (SUGUMARAN 1998). Several enzymatic reactions follow, generating dopa, dopaquinone, dopachrome

### 2.2.1 *The art of self defence*

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and finally various indolquinones which undergo a non-enzymatic polymerisation to eumelanin (CHRISTENSEN 2005; NAPPI & CHRISTENSEN 2005).

**Recognition** in adaptive immunity relies on a wide spectrum of randomly generated antigen receptors (T-cell and B-cell receptors) (MEDZHITOV 2001; ROYET 2004). As already mentioned before, innate immune recognition is supposed to be based on the detection of indispensable molecular components of microbial metabolism (PAMPs) - conserved across a great variety of taxa - by a limited number of receptors (PRRs), expressed on cell surfaces, in intracellular compartments or secreted into the blood or tissue fluids (MEDZHITOV 2001; BEUTLER 2004). One family of such receptors found among very diverse species is the family of **Toll-like receptors** (TLRs), appearing e.g. in mammals, plants and insects. Each TLR is thought capable of recognising specific ligands (PAMPs), such as peptidoglycan, lipoteichoic acids, flagellin, nucleic acids, etc., not only of bacteria, but also protozoa, fungi and viruses (MEDZHITOV 2001; BEUTLER 2004). Genes (Toll genes) encoding members of the class of TLRs were originally identified in *Drosophila melanogaster*, and their name derives from the exclamation of Christiane Nüsslein-Volhard of the Max Planck institute in Tübingen “Das war ja toll!” in reference to a mutant fly larva (mutation of the Toll gene) in which the ventral part of the body was underdeveloped (HANSSON & EDFELDT 2005). Additionally to the importance for dorsoventral polarity in *Drosophila melanogaster*, the role of Toll in *Drosophila* immune defence was discovered several years later. TLRs are transmembrane receptors with an extracellular domain with many leucine-rich repeats (LRR) and an intracellular cytoplasmic receptor domain (TIR, derived from Toll/IL-1R resistance), which they share with receptors of the Interleukin-1 receptor (IL-1R) family (specific to vertebrates) and proteins involved in plant disease resistance (R) (IMLER & ZHENG 2003; DOYLE & O’NEILL 2006). After the stimulation of TLRs by PAMPs, transcription factors such as NFκB are activated leading to the production of effector molecules. Toll receptors in insects are hypothesised to mainly carry developmental functions, whereas in mammals the main function of Tolls seems to be connected with immune response (IMLER & ZHENG 2003; DOYLE & O’NEILL 2006).

There is still far less known about the molecular basis for how invertebrates recognise foreign objects in their haemocoel (ranging from parasitoid eggs to artificial structures which have both been shown to be encapsulated), and how the signalling mechanism takes place (CARTON *et al.* 2005; SCHMIDT 2008). One mechanism might involve the recognition of structures that are usually only found outside the haemocoel. Egg chorion and larval cuticle have been shown to contain such structures (e.g. galactosamine-specific glycomodifications) (THEOPOLD *et al.* 2002; SCHMIDT 2008). Obviously, however, innate recognition capacities independent of recognition proteins and cell-bound receptors must be present, since also synthetic objects can be recognised and encapsulated. Here, sensor particles (e.g. lipid or lipid-containing particles) responding to a wide range of environmental changes

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have been shown to be involved (SCHMIDT 2008). These are part of coagulation and melanisation reactions, which in invertebrates are not only used for wound-healing but obviously also in immune defence (THEOPOLD *et al.* 2004; SCHMIDT 2008).

Maintaining such a complex mechanism as the immune system is supposed to demand a lot of energy and resources. Thus, optimal immune defence may not necessarily mean maximum immune defence (ZUK & STOEHR 2002). Having in mind that an organism has to spend energy on several other life history aspects affecting its overall fitness, an optimal immune response is most likely context specific and depends on the specific circumstances of an organism, his life history characteristics and *trade offs* between immune function and other life history traits (VAN BOVEN & WEISSING 2004; VINEY *et al.* 2005). Trade offs are a term used for the conflict that arises due to the cost in *fitness* that an organism has to pay when a beneficial change in one fitness trait is linked to a detrimental change in another fitness trait (STEARNS 1989). Some organisms for example have to pay for increased reproduction with decreased chances of survival and a shorter lifespan (STEARNS 1999). There are two main issues assessing the costs and benefits of investing in immune defence: (i) **Operational** or *use* or **deployment costs/benefits**: Costs/benefits of mounting an immune response in the presence of an elicitor. These costs may be due to the organism actually responding immunologically and using up resources, or as a consequence of immunopathology associated with the activation of an immune defence. (ii) **Maintenance costs/benefits**: Costs/benefits arising as a consequence of investing energy and resources in the defence machinery and keeping it ready for use both in presence *and* absence of an infection. Besides (i) and (ii), a distinction between **physiological costs/benefits** and **evolutionary costs/benefits** also has to be made. Linking (i) and (ii) with physiological costs is, however, easier and more plausible than linking (i) and (ii) with evolutionary costs, since use and maintenance can be seen as a physiological task tackled by an organism. Evolutionary costs imply an underlying *genetic basis* to the observed cost. Over evolutionary time (not necessarily a long time!), negative genetic correlations between immunocompetence and other fitness traits (such as longevity, reproduction, etc.) may arise in response to selection (STEARNS 1992; FELLOWES & GODFRAY 2000; ROLFF & SIVA-JOTHY 2003; SCHMID-HEMPEL 2003, 2005; WILSON 2005; MCKEAN *et al.* 2008). The costs, which are often easiest to detect in organisms, are the operational-physiological costs. Nevertheless, potential operational costs may be invisible if an organism has the opportunity to compensate for the extra demand posed by using its immune defence mechanisms with an extra intake of resources (SCHMID-HEMPEL 2003), or if the fitness trait revealing the costs has not been found or looked at yet. Thus, costs may be context-dependent (SANDLAND & MINCHELLA 2003). As could be shown in *Escherichia coli*, costs may not necessarily arise, when genes that mediate compensation for the costs are simultaneously favoured together with the genes which are selected for and which render increased resistance (SCHRAG *et al.*

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1997). Operational costs may be partly compensated for by the evolutionary costs, and immune systems may have evolved thus, that operational costs are minimised.

In a number of studies in both vertebrates and invertebrates, a *sexual dimorphism in immune response* was found, with males appearing to exhibit lower immune response and resistance to infection than females (ZUK & MCKEAN 1996; SHERIDAN *et al.* 2000; ADAMO *et al.* 2001; ROLFF 2002). In vertebrates, that in contrast to insects and other vertebrates possess the hormone testosterone, this phenomenon is often hypothesised to be an outcome of an increased testosterone level in males which is thought to increase mating success but suppress immune functions (*immunohandicap hypothesis*) (FOLSTAD & CARTER 1992). In invertebrates, this phenomenon has been tried to explain in reference to *Bateman's principle* (ROLFF 2002). BATEMAN himself could show that sexual selection favours promiscuity in males rather than females as a result of differences in parental investment in gametes (anisogamy). In males, mating rate seems to be a major determinant for fitness, whereas in females longevity is a major determinant, since they have to make greater physiological sacrifices for the production of each surviving offspring (BATEMAN 1948; TRIVERS 1972; ROLFF 2002; WADE & SHUSTER 2005). Thus, it was hypothesised, that females should invest relatively more in costly immune defences than males in order to increase their survival probability and their longevity. VAN BOVEN & WEISSING (2003), however, argued, based on the outcome of their model scenarios, that long-lived organisms do not necessarily have to invest more in immune function than short-lived organisms, and that rather the opposite might be the case.

## 2.2.2 Immune defence mechanisms in *Drosophila*

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### 2.2.2 Immune defence mechanisms in *Drosophila*

As invertebrates, who are supposed to solely rely on innate immunity as defence mechanism against parasites and pathogens, also insects merely exhibit innate immune responses, with both cellular and humoral elements, which are supposed to play discrete roles in the defence against invaders. There is, however, a strong indication that there is a cross-talk between haemocytes and other components of the immune system and that cellular and humoral immune responses in *Drosophila* act in concert (ELROD-ERICKSON *et al.* 2000; LEMAITRE & HOFFMANN 2007).

Since there is an ever growing pile of literature on *Drosophila* immune defence, I'd merely like to give a brief overview with focus on cellular immune defences, and otherwise refer to the reviews of HOFFMANN & REICHHART (2002), TZOU *et al.* (2000, 2002), HOFFMANN (2003), HULTMARK (2003), BRENNAN & ANDERSON (2004), MEISTER (2003, 2004), THEOPOLD *et al.* (2004), KIM & KIM 2005, CHERRY & SILVERMAN 2006, FERRANDON *et al.* (2007), LEMAITRE AND HOFFMANN (2007), WOOD & JACINTO (2007) for detailed information on this topic.

Immune responses of *Drosophila* comprise a number of mechanisms: **Integumental defences** and **epithelial barriers** constitute the first line of defence against organisms attempting to enter the insect's haemocoel (CARTON & NAPPI 1997). **Clot formation** occurs during wound healing (see e.g. THEOPOLD *et al.* 2004). Once a foreign organism has succeeded in entering the haemocoel, **phagocytosis**, **encapsulation**, **melanisation** and the production of **antimicrobial peptides** in the fat body are further immune responses exhibited by *Drosophila* (GOVIND & NEHM 2004). **Fig. 1** gives a schematic overview of immune defence mechanisms in *Drosophila*.

Hitherto, three signalling cascades regulating humoral and cellular immune responses in *Drosophila* are known: The **Toll-pathway**, the **Imd-pathway**, and the **JAK/STAT pathway**. The receptors (**pattern recognition receptors, PRRs**) that trigger signalling in response to sensing microbial ligands belong to the two structural families: **peptidoglycan recognition proteins (PGRPs)** and the **gram-negative binding proteins (GNBPs)**. They may be transmembrane receptors (like PGRP-LC), they can be associated to the plasma membrane, or be secreted and function as soluble receptors. Microbial detection usually requires direct contact between the host pattern recognition receptors (PRRs) and a microbial molecule. Recognition is achieved through sensing of specific forms of peptidoglycan (PGN) by PGRPs. The forms of peptidoglycan sensed in the Toll-pathway (Lys-type PGN; Lys for lysine) and the Imd pathway (DAP-type PGN; DAP from meso-diaminopimelic acid) differ hereby (FERRANDON *et al.* 2007; LEMAITRE & HOFFMANN 2007).

The **Toll pathway** with its transmembrane Receptor Toll is the eponym for the family of Toll-like receptors found among organisms as diverse as plants, insects and mammals (see

### 2.2.2 Immune defence mechanisms in *Drosophila*

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chapter 2.2.1). Besides its role in immune function, the Toll-pathway plays a key role in the establishment of the dorso-ventral axis in *Drosophila* and in other developmental processes. In contrast to Toll-like receptors in vertebrates, the Toll-receptor in *Drosophila* is not activated by directly recognising pathogens, but by its ligand the cytokine Spätzle, processed by proteolytic cascades activated by secreted recognition molecules such as PGRPs. The Toll-pathway is supposed to be largely activated by fungi and gram-positive bacteria (MEDZHITOV 2001; CHERRY & SILVERMAN 2006; FERRANDON *et al.* 2007; LEMAITRE & HOFFMANN 2007).

The *Imd-pathway* (defined by the identification of a mutation named *immune deficiency*), however, is supposed to be mainly activated by gram-negative bacteria. The PGRP-LC receptor of this pathway directly binds to bacterial elicitors (CHERRY & SILVERMAN 2006; FERRANDON *et al.* 2007; LEMAITRE & HOFFMANN 2007).

The precise role of the *JAK/STAT pathway* (Janus Kinase/Signal transducer and activators of transcription) with its receptor domeless is yet not known. It is thought to be activated by viruses and tissue damage (via a cytokine secreted by plasmatocytes) (LEMAITRE & HOFFMANN 2007).

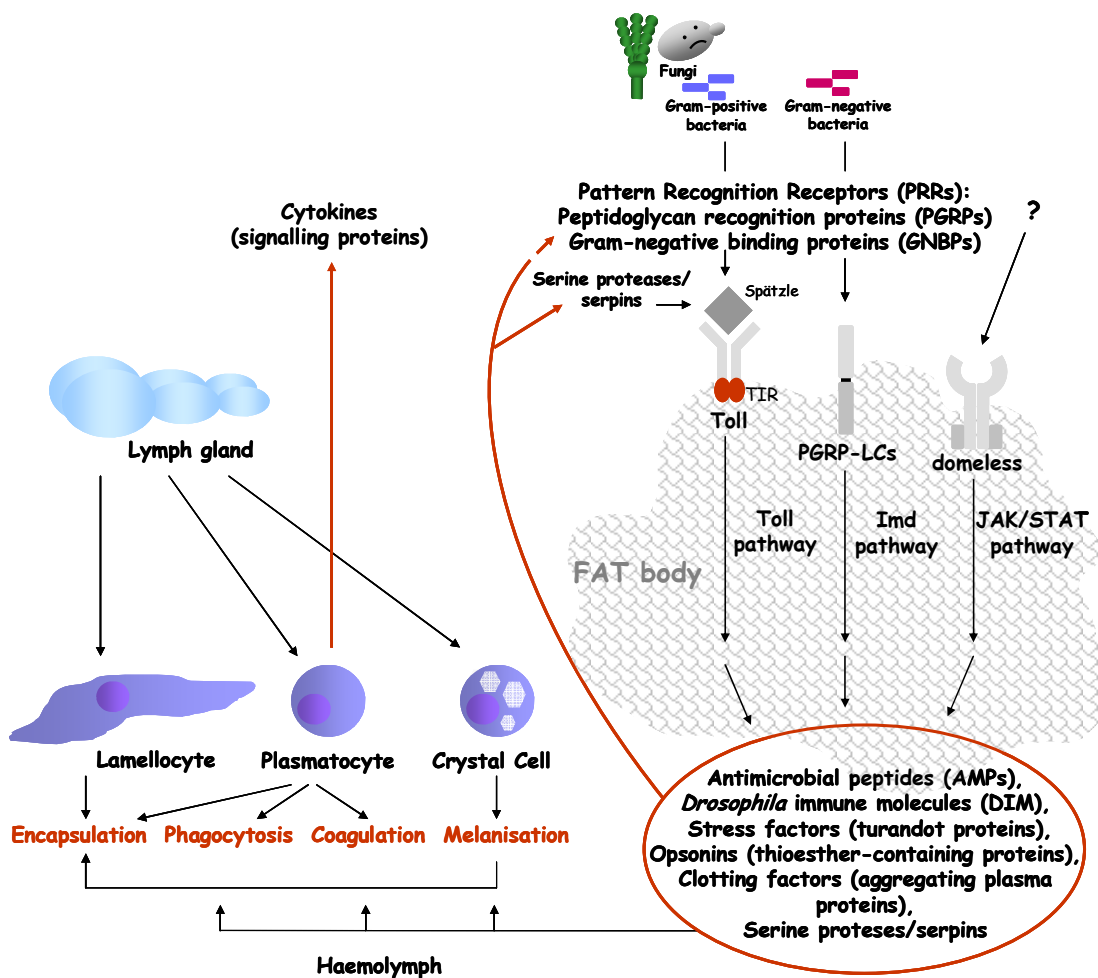
The activation of these pathways culminates in the production of various effectors, which are partly specific to each pathway. These humoral immune effectors and effector mechanisms of *Drosophila* are: *Antimicrobial peptides* (usually small, cationic peptides such as *Diptericin*, *Attacin*, *Drosocin* and *Cecropin* which are mainly active against gram-negative bacteria, *Defensin*, mainly active against gram-positive bacteria, and *Drosomycin* and *Metchnikowin*, mainly active against fungi), which allow the permeation and disruption of target membranes, *Drosophila immune molecules*, *Turandot proteins* (small peptides of unknown function), *Thioester containing proteins* (opsonisation: they promote phagocytosis and/or protease inhibitors), *Aggregating plasma proteins* (clotting factors in coagulation), *Reactive oxygen species*, *Iron sequestration factors*, *Serine proteases/serpins* (MEDZHITOV 2001; CHERRY & SILVERMAN 2006; FERRANDON *et al.* 2007; LEMAITRE & HOFFMANN 2007).

The haemolymph of *Drosophila* contains several thousand blood cells (*haemocytes*), both free floating and sessile. These are generated through haematopoiesis. *Drosophila* haematopoiesis consists of *embryonic haematopoiesis* taking place in the head mesoderm of the early embryo and of *larval haematopoiesis* taking place in the larval lymph gland. Haematopoiesis generates three types of haemocytes: *plasmatocytes*, *crystal cells* and *lamellocytes*. Plasmatocytes constitute approx. 90% - 95% of all mature haemocytes, their major function being *phagocytosis*, e.g. of bacteria, fungi, dead cells and other entities, but they also take part in *encapsulation* and the production of antimicrobial peptides. Crystal cells make up about 5% of the haemocyte population and are named after the crystalline structures they contain, which are mainly composed of prophenoloxidase (proPO). This is a



## 2.2.2 Immune defence mechanisms in *Drosophila*

precursor of the active enzyme phenoloxidase which catalyses the oxidation of mono- and diphenols to orthoquinones that polymerise to melanin. Crystal cells are supposed to be involved in the *melanisation* process during encapsulation and in wound repair (LANOT *et al.* 2001; EVANS *et al.* 2003; MEISTER 2003, 2004; LEMAITRE & HOFFMANN 2007, WILLIAMS 2007; WOOD & JACINTO 2007; STRAND 2008b). Lamellocytes occur merely in larvae and have mainly been observed in immune-challenged ones where they rapidly differentiate from prohaemocytes and form a multilayered capsule around e.g. the parasitoid eggs or larvae and other large foreign targets (STRAND 2008b). Lamellocytes have also been found to express proPO genes, thus, permitting melanisation in the encapsulation process (IRVING *et al.* 2005). So far, a haematopoietic organ in adult *Drosophila* has not been detected (CROZATIER & MEISTER 2007, LEMAITRE & HOFFMANN 2007, WILLIAMS 2007). Circulating haemocytes in the larval phase stem from embryonic haematopoiesis, whereas haemocytes generated by the larval haematopoiesis do not enter circulation until metamorphosis (LEMAITRE & HOFFMANN 2007).



**Fig. 1:** Schematic overview of immune defence mechanisms in *Drosophila*; slightly modified after LEMAITRE & HOFFMANN (2007). For a more detailed description see text.

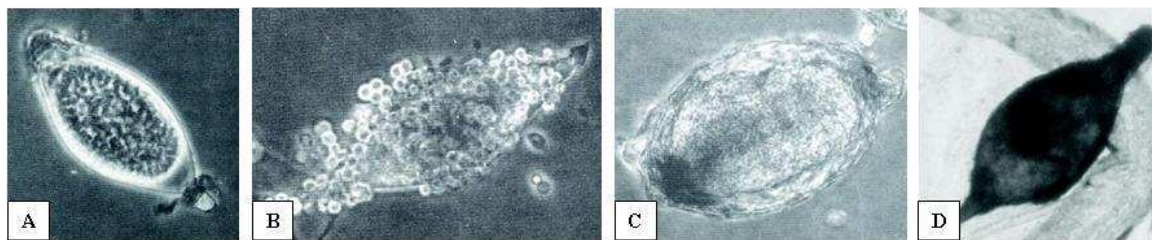
### 2.2.2 Immune defence mechanisms in *Drosophila*

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**Coagulation** in *Drosophila* is important in wound healing and also as an immune mechanism, forming a barrier to infection by immobilising bacteria and other agents. Upon injury, plasmatocytes release proteins that form clot fibers. These trap haemocytes and are cross-linked with the help of enzymes and other proteins, also including proPO released by crystal cells (THEOPOLD *et al.* 2002, 2004; LEMAITRE & HOFFMANN 2007).

The defence mechanism of *Drosophila* against parasitoids and other foreign objects too large for being phagocytosed is **encapsulation** (**Fig. 2**). The mechanism by which the foreign objects are recognised, and the signalling and molecular mechanisms lying behind the process of encapsulation, are still unclear (for just a few more details see chapter 2.2.1). In *Drosophila* specifically, the destruction of the host's tissue basement membrane is suggested to be sufficient for inducing an encapsulation reaction. Upon sensing the foreign object, plasmatocytes are then supposed to send a cytokine to the signalling center in the lymph gland and thus induce lamellocyte proliferation and differentiation (LEMAITRE & HOFFMANN 2007).

In a genome-wide microarray analysis, WERTHEIM *et al.* (2005) could identify genes specifically expressed by *Drosophila melanogaster* following parasitoid attack by *Asobara tabida*; the genes also differed from those induced by microbial infections.



**Fig. 2:** **A)** Parasitoid egg of *Asobara tabida* shortly after deposition in the haemocoel of a *Drosophila melanogaster* larva. **B)** Haemocytes have aggregated and adhered to the parasitoid egg. **C)** Haemocytes have formed a multilayered cellular capsule (20-70 or more layers of lamellocytes) around the parasitoid egg. **D)** Melanised cellular capsule. The encapsulation process may start approx. 4 hours after parasitisation and last until approx. 72 hours after parasitisation. Picture source: WERTHEIM *et al.* 2005, *Genome Biology*

The encapsulation response is supposed to begin approx. four to six hours after parasitisation. Plasmatocytes recognise the foreign object and induce proliferation and differentiation of lamellocytes in the lymph gland. The haemocytes adhere to the foreign object (**Fig. 2A**) and one another, forming a cellular capsule comprised of 20 to seventy or more layers of haemocytes (**Fig. 2B & C**). These cellular capsules primarily consist of lamellocytes, though the other two cell types may also be involved. After approx. 24 hours after parasitisation, the cellular capsule starts to melanise (**Fig. 2D**). Here, crystal cells are involved, releasing phenoloxidase (see chapter 2.2.1 and this chapter) and probably other factors. Melanisation may also be induced by humoral components present in the haemolymph. The melanisation process is supposed to last until approx. 72 hours after parasitisation. In

### 2.2.2 *Immune defence mechanisms in Drosophila*

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case a live organism has been encapsulated (parasitoid egg or larva, other large parasites like nematodes), it is finally killed either by asphyxiation, intermediates of the melanisation cascade, necrotising compounds or reactive oxygen species (WERTHEIM *et al.* 2005; LEMAITRE & HOFFMANN 2007; STRAND 2008b).

Populations of *Drosophila melanogaster* in the wild vary in their level of resistance against parasitoids (KRAAIJEVELD *et al.* 1998). It has been found that there is a high correlation between the number of circulating haemocytes in the host's haemolymph and its ability to encapsulate parasitoid offspring (ESLIN & PREVOST 1998). Lines selected for higher parasitoid resistance had approximately twice the density of circulating haemocytes compared with control lines (KRAAIJEVELD *et al.* 2001). Increased defensive ability against parasitoids, however, is associated with the cost that larvae are poorer competitors under situations of reduced food supply (KRAAIJEVELD *et al.* 2001). The following costs and benefits of immune defence in *Drosophila* have been detected so far:

**Operational costs:** A sexual dimorphism in *Drosophila melanogaster* referring to the costs of parasitism has been found: female flies having survived a parasitoid attack as larvae are reported to have a shorter thorax and wing length and to be less fecund; male flies mainly suffer from shorter thorax lengths (CARTON & DAVID 1983; FELLOWES *et al.* 1999b). It could be shown that in larvae exposed to environmental stress, such as high larval densities, insecticides and nutritional deficiencies, resistance ability was reduced (WAINBERG *et al.* 1985; DELPUECH *et al.* 1996; VASS & NAPPI 1998b).

**Maintenance costs:** *Drosophila melanogaster* lines selected for increased resistance to *Asobara tabida* or *Leptopilina boulardi* showed lower competitive ability under resource stress, due to a reduction in the feeding rate of more resistant larvae (KRAAIJEVELD & GODFRAY 1997; FELLOWES *et al.* 1998a; FELLOWES *et al.* 1999c). FELLOWES & GODFRAY (2000) argue that an explanation for this might be that a high risk of parasitism favours the selection of lower metabolic rates. It has indeed been observed that lower metabolic rates evolve in insects selected for higher tolerance to various kinds of stresses, and a combined data analysis suggested, that encapsulation actually seems to be negatively associated with adult metabolic rate (FELLOWES & GODFRAY 2000). KRAAIJEVELD *et al.* (2001) could show that *Drosophila melanogaster* flies resistant to the parasitoid *Asobara tabida* have more circulating haemocytes than their more susceptible conspecifics. Thus, an explanation, why resistant larvae are poorer competitors than their more susceptible conspecifics, could be that they invest fewer resources in feeding efficiency and more in the production of haemocytes. The trade-off may also occur during morphogenesis, since both haemocytes and the musculature involved in feeding originate from the embryonal head mesoderm, and resources allocated to producing haemocytes would be made unavailable for the feeding muscles (TEPASS *et al.* 1994, KRAAIJEVELD *et al.* 2001).

### 2.3 Food for fitness

*The whole of nature ... is a conjunction of the verb to eat, in the active and passive [William Ralph Inge].*

*Food is our common ground, a universal experience [James Beard].*

*Food is an important part of a balanced diet [Fran Lebowitz].*

*I have been a success: for sixty years I have eaten, and have avoided being eaten [Logan Pearsall Smith].*

*Part of the secret of success in life is to eat what you like and let the food fight it out inside [Mark Twain].*

*We are indeed much more than what we eat, but what we eat can nevertheless help us to be much more than what we are [Adelle Davis].*

*Let thy food be thy medicine and thy medicine be thy food [Hippocrates].*

*One cannot think well, love well, sleep well, if one has not dined well [Virginia Woolf].*

As people who live in an affluent environment where there usually is a grocery nearby to acquire nearly any food one may wish for, we probably very easily forget how essential an ample and regular food intake is to keep up vitally important bodily functions and also those going beyond the basic needs, such as reproduction. Looking at organisms among us and even at ourselves and the history of mankind just a little more attentively, it immediately catches the eye that everything in nature centres on food and how to get it (HERNES 2000).

It has long been recognised that there is a close relationship between the host's nutritious state and its ability to overcome infection, though nutrition does not influence all infections equally (CHANDRA 1996, 1997, 1999, 2002). Already in ancient writings, admonitions about food and health are found, long before there was first scientific evidence for a link between nutrition and the immune system. It is reported, for example, that Hippocrates instructed physicians to evaluate the diet in order to understand disease (BEISEL 1992). Nutrition itself may be a source of antigens to which the immune system must become tolerant, but it may also provide immune modulating nutrients and factors that influence the intestinal flora (CUNNINGHAM-RUNDLES *et al.* 2005; CALDER *et al.* 2006). Undernutrition or malnutrition may impair the immune system and suppress immune functions either due to insufficient intake of energy and macronutrients (chemical compounds needed in large quantities such as proteins, carbohydrates, fats and some minerals) and/or due to deficiencies in specific micronutrients (vitamins and minerals) (KELLEY & BENDICH 1996; CALDER & JACKSON 2000; CALDER & KEW 2002). However, excess amounts of some nutrients may also impair immune function (CHANDRA 1993; CHANDRA & KUMARI 1994; CALDER & KEW 2002). Probably all aspects of the immune system are in some way or an-

## 2.3 Food for fitness

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other influenced by nutrients, comprising macro-and micronutrients and deficiencies therein, whereby there is more good evidence of specific micronutrients influencing immunity than of macronutrients (HUGHES & KELLY 2006). Humoral-mediated immunity is supposed to be less sensitive to deficiencies in macronutrition than cell-mediated immunity (MCDADE 2005). Deficiencies in micronutrients in general, are supposed to have a wide-spread effect on nearly all components of the innate immune system (RIVERA *et al.* 2003). For a lot of amino acids their role in the metabolic pathways of immune response could be unfolded in recent years, thus explaining the deleterious effect protein deficiency may have on immune function (LI *et al.* 2007). Besides the direct metabolic nutritional requirements of a functioning immune system, pathogenic and parasitic infections may also disrupt normal processes of nutrient digestion and absorption (MCDADE 2003). Host nutrition may also influence the genetic make-up of viral genomes and increase viral virulence due to increased oxidative stress in host animals caused by host nutritional deficiencies (BECK *et al.* 2004).

Most studies on the link between nutritional status and immune status have been carried out in vertebrates, especially humans, farm animals and birds. Still, there are also a few studies on the relationship between diet and immune functions in invertebrates. In insects, food deprivation may also lead to impairment of immune functions, such as lower production of antimicrobial peptides (FEDER *et al.* 1997; LEE *et al.* 2006), reduced melanisation response (SUWANCHAICHINDA & PASKEWITZ 1998; KOELLA & SØRENSEN 2002) reduced lysozyme – and PO activity and encapsulation response (LEE *et al.* 2006). In *Drosophila melanogaster*, the presence of yeast itself, irregardless of a particular species, turned out to enhance the encapsulation ability of larvae parasitised by *Leptopilina boulardi* (VASS & NAPPI 1998b). Supplementation of larval diet with tryptophane was found to increase the encapsulation ability of *Drosophila melanogaster* against the parasitoid *Leptopilina heterotoma* (WALKER 1959).

Nutrition may also affect trade-offs due to having an impact on the costs of fitness traits, such as immunity. Food deprivation, for example, may lead to a downregulation of immune functions to minimise costs (SIVA-JOTHY & THOMPSON 2002). In general, costs may only become visible with poor nutrition, when the organism does not have the opportunity to compensate for the extra demand posed by the trait (MORET & SCHMID-HEMPEL 2000). Access of hosts to *ad libitum* food may even imply that selection for increased resistance against parasites may proceed independently of effects on other fitness traits such as fecundity (MCKEAN *et al.* 2008).

There is increasing evidence that probiotic bacteria improve host immune function. Indigenous bacteria in the intestinal tract are believed to contribute to the immunological protection of the host by effecting the immunophysiological reaction in the intestine's mucosal barrier against colonisation by pathogenic bacteria. This barrier can be disrupted by disease

### 2.3 *Food for fitness*

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and by use of antibiotics. It is now believed, and several studies confirm this, that this barrier can be maintained by providing supplements containing 'live' desirable bacteria or other microorganisms, so called 'probiotics' (CALDER & KEW 2002; DUGGAN *et al.* 2002; SAAVEDRA & TSCHERNIA 2002; RAUTAVA *et al.* 2005). The mechanisms behind the beneficial effects of probiotics are largely unknown, but they may comprise modifying pH in the gut, suppressing pathogens by antimicrobial compounds, stimulating cells involved in immune defence, affecting cells in the intestinal lumen, modulating the endogenous microflora, competing for pathogen binding and receptor sites as well as for nutrients (MARTEAU *et al.* 2004; PARVEZ *et al.* 2005). Most of the studies on probiotics have been carried out in humans, domestic animals and in aquaculture. Next to nothing is known about whether, which and how specific microorganisms in the diet of other animals such as saprophagous insects may affect immune defence mechanisms in particular. In *Drosophila* it could merely be shown that the presence of bacteria during the first week of adult life can enhance another fitness trait, namely lifespan, despite unchanged food intake. Later in life, the presence of bacteria can reduce lifespan (BRUMMEL *et al.* 2004).

In a host-parasite interaction, food for fitness may not only refer to the host's fitness and the impact of nutrition on host immune defence mechanisms. The parasite's fitness may also experience a modulation by the host's nutritional status. Host nutritive resources and immunity are among other host qualities of prime importance to parasite fitness (BIZE *et al.* 2008). A parasite probably faces the dilemma that it has to balance these two host components, since the host's body condition and thus parasite fitness increases with quantity and quality of host nutritive resources. The host's defence mechanisms (behavioural, physiological and immunological), however, usually also increase with good nutritional condition, which may in turn reduce the parasite's reproductive success (TSCHIRREN *et al.* 2007; BIZE *et al.* 2008). It is predicted that parasites may choose to parasitise energy deprived hosts if the fitness gain due to reduced host immune defences is higher than the fitness loss due to lower quality of the resources extracted from the host (KRASNOV *et al.* 2005). Low food availability may not only have a negative effect on the ability of the host to fend off parasites, but may also reduce fitness of the surviving parasite. As could be shown in *Drosophila melanogaster* and its parasitoid *Leptopilina boulardi*, availability of nutritional resources may result in a poor weight gain of the host, but may also reduce the size and the time of development of the parasitoid, (SALT 1941; BOULÉTREAU 1986; WAJNBERG *et al.* 1990; VASS & NAPPI 2000).

## 3 Organisms involved in the study

### 3.1 *Drosophila melanogaster* – the host

*Time flies like an arrow; fruit flies like a banana [Groucho Marx].*

*Busy, curious, thirsty fly, drink with me and drink as I! Freely welcome to my cup, could'st thou sip and sip it up; make the most of life you may; life is short and wears away [William Oldys].*

*Drosophila melanogaster* Meigen (Diptera: Drosophilidae), the black-bellied dew loving fly (Latin scientific adaptation for Greek δρόσος meaning “dew”, φίλος meaning “loving”, μελανός meaning “black” and γαστήρ meaning belly) is a cosmopolitan species suggested to have originated from tropical Africa (AULARD, DAVID & LEMEUNIER 2002). Together with more than 3000 other species it belongs to the acalyprate family Drosophilidae (ASHBURNER 1998). The genus *Drosophila* contains well over 1500 species (MARKOW & O'GRADY 2006). Often referred to as “fruit flies” the scientifically correct name is “dew flies” or “vinegar flies” since the term “fruit fly” is used for the family Tephritidae. *Drosophila melanogaster* was first described by the German entomologist JOHANN WILLHELM MEIGEN (1830). Larvae of *Drosophila melanogaster* develop in various kinds of injured and decaying plant material such as fruits, slime fluxes, exudates, mushrooms, etc. (GANTER 2006), in contrast to larvae of the Tephritidae, that develop in fruits still attached to the mother plant. Thus, *Drosophila melanogaster* is also often found in human homes, especially in the kitchen area, attracted by organic waste. It is generally very closely associated with humans and has been spread all over the world by human activities (KELLER 2007).



**Fig. 3:** Adult *Drosophila* flies: Left side: male fly with black sex combs (arrows), right side: female fly.



### 3.1 *Drosophila melanogaster* – the host

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The adult flies are of a yellowish brown colour and have blackish colouration on their tergites. They exhibit sexual dimorphism. The female fly is approx. 2.5mm long, the male is slightly smaller. The (dorsal) tip of the male's abdomen is more distinctly and extensively black in colour than the female's abdomen. Male flies possess a row of blackish bristles on the base of the protarsus, so called sex combs (**Fig. 3**). Only 12-14 hours (at 25°C) after eclosion, female flies become receptive and may mate. The males usually perform an interesting courtship including a song played by wing vibration. The females lay the fertilised eggs in the adequate substrates, usually preferring to insert them slightly into the medium. The eggs are about half a mm long and possess two appendages, serving as breathing tubes for the embryo (**Fig. 4**).



**Fig. 4:** Eggs of *Drosophila melanogaster* with two breathing tubes each.

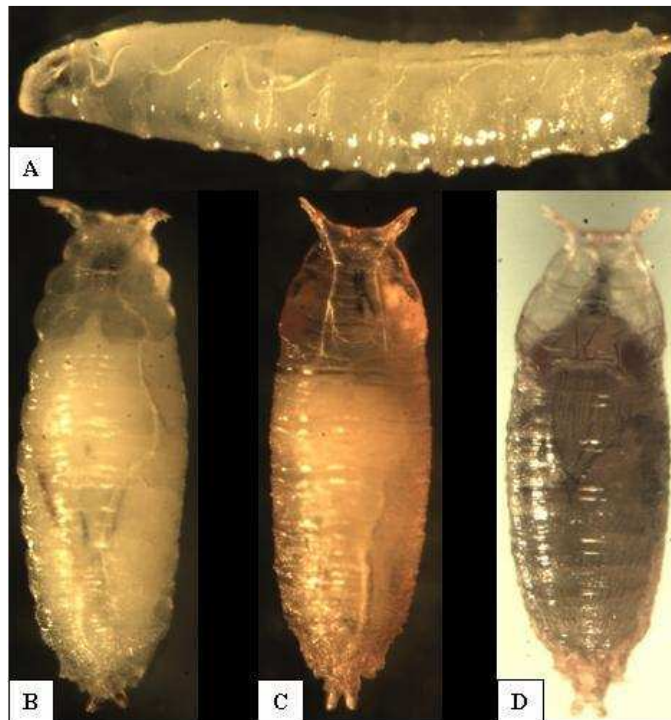
The time of development from egg to adult varies with prevailing ambient temperature, with food availability and food quality. At 25 °C and *ad libitum* food conditions, larvae usually hatch after approx. 18-24 hours and immediately start to feed. In case oviposition substrates are not available, female flies can retain their eggs, which are then laid at a more advanced developmental stage and hatch in a shorter time after laying (PRASAD & JOSHI 2003). The larval phase lasts about four days during which larvae moult twice. They have three larval stages, L1, L2 and L3 (1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> instar) that last for 24hrs (L1 & L2) and 48hrs (L3). The 1<sup>st</sup> larval instar merely feeds on the surface of the medium. After moulting to L2, the larvae begin to burrow into the medium and continue to do so during most of the remaining larval phase. Early in the 3<sup>rd</sup> instar, larvae reach a critical developmental stage, appearing to be correlated with a certain critical weight/size, after which larvae pupariate after a fixed time, even if all food is withdrawn. The time taken from hatching to the attainment of critical weight/size, however, is highly sensitive to nutritional levels and can be lengthened greatly at suboptimal diets (PRASAD & JOSHI 2003). Female larvae have a greater growth rate than male larvae, are heavier at the time of pupariation, and eclose as



### 3.1 *Drosophila melanogaster* – the host

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heavier and larger flies than males (PRASAD & JOSHI 2003 and references therein). The larva, being a typical fly larva (also called maggot), does neither possess a chitinous head capsule nor any legs. As mouthparts which to feed with, it merely has two chitinous hooks that can be thrust out and retracted. Their body is nerved with two main tracheal stems, opening out into two anterior and two posterior spiracles. The latter ones are positioned on small humps and are the only larval structure exposed while the larva feeds borrowed into the medium. In the mid of the 3<sup>rd</sup> instar, larvae leave the food medium and begin to wander about until they finally find a suitable place to pupate. Hereby, it is reported that there is a genetically manifested behavioural polymorphism (foraging locus), with larvae carrying the rover allele exhibiting significantly longer foraging path lengths than those carrying the so called sitter allele (SOKOLOWSKI 1980; PEREIRA & SOKOLOWSKI 1993; SOKOLOWSKI *et al.* 1997). First, the puparium is formed by hardening of the larval skin. After retraction of the epidermis from the cuticle of the 3<sup>rd</sup> instar, the actual pupa forms and undergoes metamorphosis within four days. The adult fly emerges by simply walking out of the puparium which opens up at the front (operculum) (**Fig. 5** shows a few developmental *stages of Drosophila melanogaster*).



**Fig. 5:** Developmental stages of *Drosophila melanogaster*. **A)** 2<sup>nd</sup> instar larva. **B)** Freshly pupated 3<sup>rd</sup> instar larva with still unhardened and light-coloured puparium. **C)** Fresh puparium with hardened and brownish cuticle. **D)** Adult fly approx. one day before eclosion.

### 3.1 *Drosophila melanogaster* - the host

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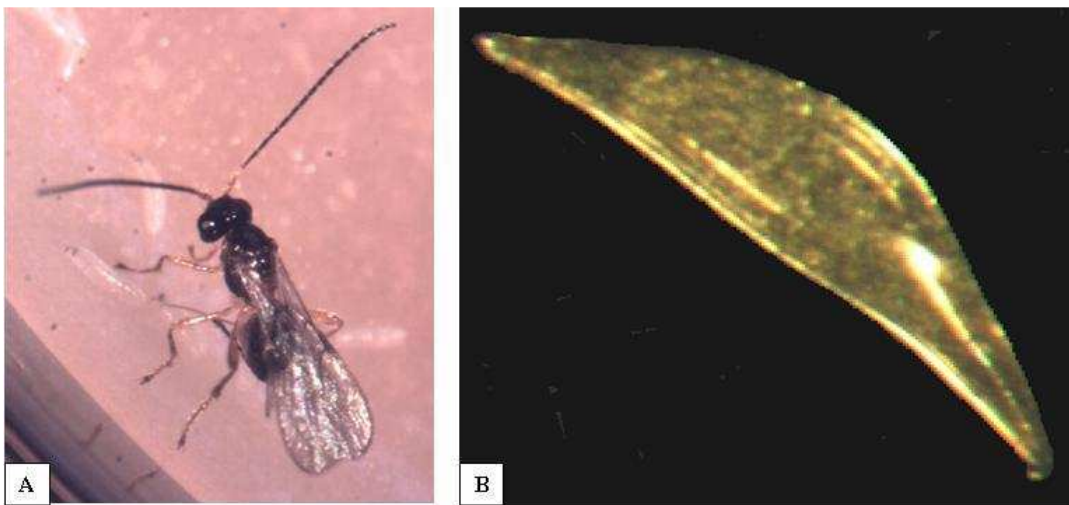
Usually the organic substrates the larvae develop in are rich in microorganisms such as bacteria and fungi, especially yeasts, and it is mainly those microorganisms which act as actual nutrient resource, processing the plant raw material into important dietary factors (STARMER 1981) and providing additional nitrogen for the developing larvae, since often the plant material itself is poor in nitrogen compounds. Especially yeasts are considered as a major food source for the majority of species of *Drosophila* in both adult and larval stages (BEGON 1982). The yeast species may hereby differ in composition and quality, depending on both the yeast itself and the environment in which the yeast grows (GANTER 2006). Various species of *Drosophila* exhibit a specific relation to particular yeast species or groups of yeast species, depending on substrate composition or the biogeographic distribution of the *Drosophila* species (STARMER *et al.* 1990). It could be shown, that different species of *Drosophila* exhibit preferences for different yeast species, whereby larvae tend to prefer those yeast species that best support their own development. Adults, however, often show a preference that is not identical to that of the larvae. The observed preferences might have evolved, since they supported larval development and/or reduced intra - (and also inter -) specific competition (LINDSAY 1958; COOPER 1960; DORSCH 2007). *Drosophila* is reported not to feed on *Cryptococcus* species, but readily feed on *Saccharomyces cerevisiae* as adults and larvae (SPENCER & SPENCER 1997).

Parasitoids are known to be a major cause of larval mortality of *Drosophila melanogaster* (and some other species) in the field (CARTON *et al.* 1986). Compatibility in associations between *Drosophila* and parasitoid wasps has been shown to have a genetic basis, being both species - and strain-specific (CARTON & NAPPI 2001). There is evidence for both genetic variation in resistance in *Drosophila melanogaster* and in virulence amongst *Drosophila* parasitoids (DUPAS *et al.* 1998; FELLOWES & GODFRAY 2000; GREEN *et al.* 2000; DUPAS *et al.* 2003), additionally showing a distinct geographic pattern (KRAAIJEVELD & GODFRAY 1999). The success of the host cellular immune response of *Drosophila melanogaster* against *Leptopilina boulardi* e.g. has been demonstrated to depend on the genetic status of host and parasitoid (CARTON & NAPPI 2001). In *Drosophila melanogaster* the gene *Rat* on chromosome 2R has been described, which confers resistance to *Asobara tabida*, whereby the resistant allele shows complete dominance over the susceptible allele (BENASSI *et al.* 1998; CARTON *et al.* 2005).

### 3.2 The parasitoids

#### 3.2.1 *Asobara tabida*

*Asobara tabida* Nees von Esenbeck, 1834 (Hymenoptera: Braconidae, Alysiinae) has a holarctic distribution. Within Europe it occurs from Scandinavia to the Mediterranean (though it appears to be lacking from most of the Iberian Peninsula) (KRAAIJEVELD 1994). Alysiinae in general are solitary parasitoids of several cyclorrhaphous Diptera (GAULD & BOLTON 1996). As described by CARTON *et al.* (1986) *Asobara tabida* is a solitary koinobiont endoparasitoid, parasitising different species of *Drosophila*, such as *Drosophila subobscura*, *Drosophila melanogaster* and *Drosophila immigrans*. In Europe, *Asobara tabida* is (besides the cynipid wasp *Leptopilina heterotoma*, see chapter 3.2.2) one of the most important parasitoids on fermenting substrates. *Asobara tabida* mainly attacks hosts developing in such substrates. First and early second instar larvae of *Drosophila* are easiest to attack and handle for oviposition by *Asobara tabida* females (Fig. 6). This is mainly due to the fact that older larvae possess a thicker cuticle (hampering penetration by the ovipositor), are all in all larger and exhibit higher activity and strength, enabling them to carry away the parasitoid with them while being punctured.

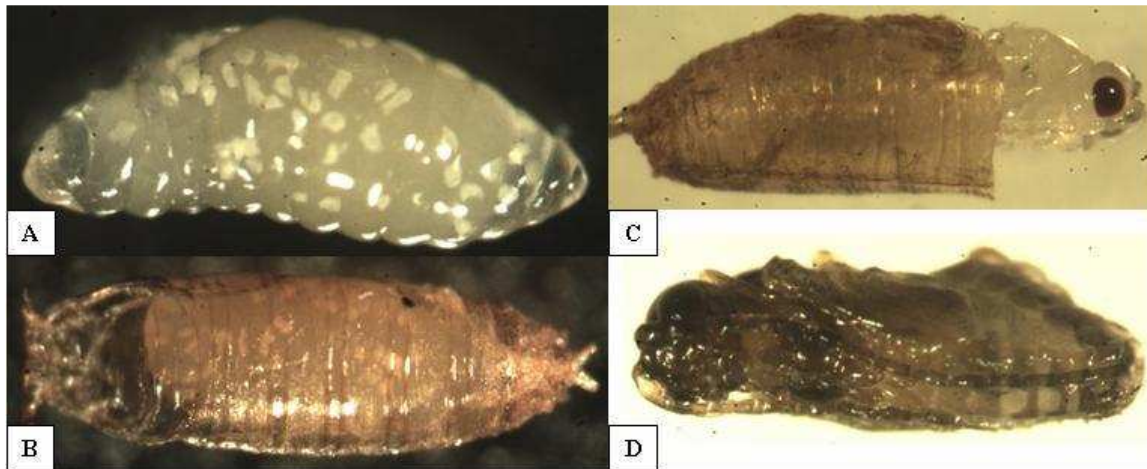


**Fig. 6:** A) Adult *Asobara tabida* female attacking a 1<sup>st</sup> instar *Drosophila melanogaster* larva for oviposition. B) Egg of *Asobara tabida* shortly after deposition into the haemocoel of a 1<sup>st</sup> instar larva of *Drosophila melanogaster*.

As koinobiont endoparasitoids, *Asobara tabida* larvae feed internally and remain quiescent while their host larva grows to full size. During this quiescent stage, the parasitoid is exposed to the host's immune defences (KRAAIJEVELD & GODFRAY 1999). After pupation of the host (often coinciding with the parasitoid larva's first moult), second and third instar larvae feed on the host's tissues until they themselves pupate inside their host's puparium.

### 3.2.2 *Leptopilina heterotoma*

The parasitoid's entire development takes approx. 17 to 24 days at 25 °C, with *Drosophila melanogaster* as host (see **Fig. 7** for developmental stages of *Asobara tabida*).



**Fig. 7** : Developmental stages of *Asobara tabida*. **A**) 3<sup>rd</sup> instar larva. **B**) 3<sup>rd</sup> instar larva shortly before pupation as it is oriented inside the puparium of *Drosophila melanogaster*. **C**) Adult parasitoid, yet without a hardened and melanised cuticle. **D**) Adult parasitoid shortly before emergence from the host puparium.

Male parasitoids usually hatch one or two days earlier than the female parasitoids (*protandry*), and often there is a female bias in sex ratio, probably due to *local mate competition* (LMC) between males (HAMILTON 1967; CHARNOV 1982; NADEL & LUCK 1992). Adult parasitoids leave the host's puparium by gnawing a hole through the operculum, to which the parasitoid's head is usually oriented (CARTON *et al.* 1986). Although female *Asobara tabida* wasps are able to distinguish unparasitised host larvae from those previously parasitised by themselves or conspecifics, it may occur under specific circumstances (e.g. when the wasp to host ratio is high), that more than one parasitoid egg is laid into a single host by the individual of one parasitoid species (*superparasitism*) (VAN ALPHEN & NELL 1982; CARTON *et al.* 1986). Nonetheless, merely one wasp may develop in a single host, thus, super numerous parasitoid larvae are killed by their conspecific sooner or later (CARTON *et al.* 1986). Adult females of *Asobara tabida* are reported to be ready to lay eggs at the time of emergence (*proovigenic*) (CARTON *et al.* 1986; ELLERS & VAN ALPHEN 1997). In other references, however, *Asobara tabida* is referred to as *synovigenic*, though not strictly, meaning that there are mature eggs at the time of eclosion, but more eggs may mature during imaginal life. Additionally, differences in ovigeny in different *Asobara tabida* lines have been found, with an ovigeny index ranging from 0.24 – 0.36 (ELLERS & VAN ALPHEN 1997; JERVIS *et al.* 2001). Mating may directly occur after female emergence with male adults that have already emerged one or two days earlier. Similar to most *Drosophila* hymenopteran parasitoids, *Asobara tabida* is an *arrhenotokous* species: Fertilised eggs develop into diploid females, unfertilised ones into haploid males (*Arrhenotoky*).

### 3.2.2 *Leptopilina heterotoma*

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For oviposition, adult female parasitoids usually have to search for suitable host larvae. Locating the host's habitat is usually the first step. Female *Asobara tabida* have been shown to be attracted by odours emitted by the food of their host, mostly volatiles produced by microorganisms that take part in the plant material's fermentation. These odours seem to be the major cue for parasitoids to locate the microhabitat of their host (VET *et al.* 1984). Besides volatiles produced by microorganisms, odours emitted by fermenting substrates may include volatiles produced during fermentation (e.g. ethanol), but also odorous substances emanating from the host itself or produced as a result of its feeding and roving activity (*kairomones*). *Asobara tabida* is e.g. able to distinguish specific host species according to their kairomones (VAN ALPHEN & VET 1986). Once the host habitat is reached, *Asobara tabida* locates host larvae again via kairomones, and additionally via *vibrotaxis* and partly by *ovipositor searching*. When the host is punctured by the female's ovipositor, the parasitoid may still decide not to lay an egg into the host, in case it does not seem to be suitable, either since it is not the proper species or it is not in a condition allowing potential successful development of the parasitoid's offspring. It could be shown in *Asobara tabida*, that the degree of preference for a specific host is linked to the degree of survival probability of the parasitoid's offspring. Females of a less virulent strain of *Asobara tabida* e.g. exhibited a greater preference for the host species *Drosophila subobscura*, where their offspring had a high survival probability, over the sympatric species *Drosophila melanogaster*, where the survival probability of the parasitoids' offspring was low (KRAAIJEVELD *et al.* 1995). In general, parasitism by *Asobara tabida* of a specific host species was shown to increase with survival probability in this host, and that *Drosophila subobscura* is preferred over *Drosophila busckii*, *D. funebris*, *D. immigrans* and *D. melanogaster* (VAN ALPHEN & JANSSEN 1981). To assess the host's suitability, female parasitoids may make use of certain chemical or physical cues, especially via sensillae on their ovipositor (*internal examination*) (CARTON *et al.* 1986; GODFRAY 1994). These sensillae also enable them to discriminate between parasitised and unparasitised hosts due to a marking substance released from the accessory glands and injected into the host's body after depositing an egg (*internal marking*) (CARTON *et al.* 1986).

*Asobara tabida* has evolved a mechanism of *passive evasion* to avoid and hamper encapsulation of its eggs by the host. The eggs possess a sticky chorion which permits their attachment to host tissues, such as intestinal tube and fat body (ESLIN & PRÉVOST 2000; MOREAU *et al.* 2003; PRÉVOST *et al.* 2005). This attachment leads to the parasitoid egg being totally embedded in the tissues of its host, which could prevent haemocytes from agglutinating at the egg's surface. The eggs' sticky chorion is argued to be the mechanism determining *virulence* in *Asobara tabida*, and the degree of 'stickiness' is supposed to be directly correlated to the degree of virulence exhibited by this parasitoid (KRAAIJEVELD & VAN ALPHEN 1994). It has been hypothesised that the elicitation of the encapsulation reac-

### 3.2.2 *Leptopilina heterotoma*

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tion may result from a race between the haemocytic reaction of *Drosophila melanogaster* and the embedment of the egg of *Asobara tabida* within host tissues (ESLIN & PRÉVOST 2000). Thus, hosts with a higher number of circulating haemocytes may have an advantage over larvae with fewer haemocytes. *Drosophila melanogaster* lines selected for increased resistance did actually exhibit a significant increase in haemocyte number (KRAAIJEVELD *et al.* 2001).

*Asobara tabida*, however, is also reported to exhibit mechanisms of *active suppression* of its host's immune defence. MOREAU *et al.* (2000) could show that contrary to an avirulent strain of *Asobara tabida*, a virulent strain induced a deleterious effect on host Phenoloxidase activity (see chapter 2.2). Additionally, MOREAU *et al.* (2004) detected an aspartyl-glucosamidase-like protein (one of the main venom proteins of parasitic wasps) in *Asobara tabida* and hypothesised, that it might be involved in a potential active suppression of the host's immune system or in temporary paralysis of the host at the early stage of parasitisation. Thus, these two findings might suggest the presence of other virulence factors in *Asobara tabida* besides the eggs' adhesiveness. Since the nature of virulence in *Asobara tabida* has not been entirely revealed and is thus not easily measured, virulence has also been indirectly measured by the parasitoid's ability to survive encapsulation (KRAAIJEVELD & VAN ALPHEN 1994).

Both in *parasitoid virulence* as well as *host resistance*, geographic patterns have been found. Thus, there is a cline in *Asobara tabida* *virulence* from the north (low) to the south (high) of Europe. Parasitoids from northern, western and central Europe usually have a low virulence (typically about 20% survive encapsulation) whereas parasitoids from the Mediterranean have a much higher virulence (60% - 95% survive encapsulation). Virulence of *Asobara tabida* may be influenced by complex local factors superimposed on broad geographic trends (KRAAIJEVELD & GODFRAY 1999). An explanation for the cline in *Asobara tabida* virulence could be, that in the north, where virulence is lowest, the main host of *Asobara tabida* is *Drosophila subobscura*, a species much rarer in the south and one that has never been recorded to encapsulate any native parasitoids that attack it or a range of exotic parasitoids in the laboratory (KRAAIJEVELD & VAN DER WEL 1994; KRAAIJEVELD & GODFRAY 1999).

The geographic pattern of *Drosophila melanogaster* *resistance* is more complex than that for *Asobara tabida* virulence. Resistance is strongest in central southern Europe but weaker in the north, in the Iberian Peninsula, and in the southeast (KRAAIJEVELD & VAN ALPHEN 1995a). There is a trend for *Drosophila melanogaster* resistance to *Asobara tabida* to be higher where virulent *Asobara tabida* strains occur (KRAAIJEVELD & GODFRAY 1999). *Drosophila melanogaster* resistance is low in northern Europe where *Asobara tabida* preferably attacks *Drosophila subobscura* (KRAAIJEVELD & GODFRAY 1999).



### 3.2.2 *Leptopilina heterotoma*

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The most important explanation for this virulence-resistance pattern is supposed to be geographic differences in host-parasitoid community structure (KRAAIJEVELD & VAN ALPHEN 1994; KRAAIJEVELD & GODFRAY 1999). There is also some evidence for a positive geographic correlation between puparial thickness and encapsulation (KRAAIJEVELD 1994). Additionally, it has often been observed, that encapsulation is more effective at higher temperatures - a generalisation that is normally, though not always, supposed to apply to *Drosophila* (KRAAIJEVELD & GODFRAY 1999). Parasitoid virulence as well as resistance often seems to be species specific, probably due to different virulence mechanisms in the parasitoid species, so that an increase in resistance to one parasitoid may not result in a correlated change in the resistance to another parasitoid (KRAAIJEVELD & GODFRAY 1999, FELLOWES et al. 1999). *Drosophila melanogaster* lines selected for increased resistance against *Leptopilina boulandi* did show large increases in resistance against *Leptopilina boulandi*, *Asobara tabida* and *Leptopilina heterotoma*. Lines selected for increased resistance against *Asobara tabida*, however, only showed increased resistance against *Asobara tabida* and *Leptopilina heterotoma*, but only a small change in their ability to survive attack by *Leptopilina boulandi* (FELLOWES et al. 1999).

*Asobara tabida* individuals are co-infected by three strains of the bacterial genus *Wolbachia*, two of which are facultative parasites inducing cytoplasmic incompatibility (CI) (crosses between *Wolbachia*-infected males and uninfected females are sterile by postzygotic reproductive isolation), and one of which is necessary for oogenesis to occur in *Asobara tabida* females (MOUTON et al. 2004).

### 3.2.2 *Leptopilina heterotoma*

In Europe, *Leptopilina heterotoma* Thomson 1862 (Hymenoptera: Cynipoidea, Figitidae) is - besides *Asobara tabida* - the second most important parasitoid wasp on fermenting substrates (**Fig. 8**). Similar to *Asobara tabida*, *Leptopilina heterotoma* is a generalist solitary koinobiont endoparasitoid, parasitising at least nine other *Drosophila* species besides *Drosophila melanogaster*, such as *Drosophila subobscura*, *Drosophila obscura*, *Drosophila immigrans* and others (CARTON et al. 1986). *Leptopilina heterotoma* occurs mainly from Western Europe till northern Africa.

The developmental characteristics of *Leptopilina heterotoma* inside the host are similar to those of *Asobara tabida*. *Leptopilina heterotoma*, however, is reported to perform better at higher temperatures: When competing with *Asobara tabida* in multiparasitised hosts, *Leptopilina heterotoma* was more successful at 20°C and 25°C, and was outcompeted at 15°C (VAN STRIEN-VAN LIEMPT 1983).

Host habitat location in *Leptopilina heterotoma* resembles that of *Asobara tabida*: Odours emanated by fermenting substrates and microorganisms as well as kairomones attract fe-

### 3.2.2 *Leptopilina heterotoma*

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male parasitoids (VET *et al.* 1984; CARTON *et al.* 1986; NUFIO & PAPAJ 2001; WERTHEIM *et al.* 2003; DELPUECH *et al.* 2005).



**Fig. 8:** Female adult *Leptopilina heterotoma*.

WISKERKE *et al.* (1993) could show, that *Leptopilina heterotoma* can use aggregation pheromones produced by male flies in host finding. The pheromone (cis-vaccenyl acetate, cVA) is transferred to the female fly during mating and further on to the oviposition sites. Aggregation of flies may aid mate finding but may also discourage males from courting other males or recently mated females (BARTELT *et al.* 1985; WISKERKE *et al.* 1993; HEDLUND *et al.* 1996). On-patch host location, however, is mainly achieved by **ovipositor searching** and not vibrotaxis as in *Asobara tabida* (KRAAIJEVELD & VAN ALPHEN 1995b). Ovipositor searching is characterised by pricking the ovipositor rhythmically into the host substrate (VAN LENTEREN *et al.* 2007). The female's ovipositor is furnished with various numbers and types of sensilla, comprising mechano- and chemosensilla used in host finding and selection of suitable hosts, as well as a structure to grip escaping host larvae (VAN LENTEREN *et al.* 1998, 2007). In case the host is accepted by the parasitoid female, an egg is deposited into the haemolymph and additionally a marking substance is injected, informing the same female or a conspecific female about whether and even how often the larval host has been parasitised (NUFIO & PAPAJ 2001; VAN LENTEREN *et al.* 2007). *Leptopilina heterotoma* has been reported to only very rarely superparasitise at all (VARALDI *et al.* 2005). It has been shown to be able to discriminate between hosts with different numbers of eggs already present in the host and to prefer ovipositing into hosts containing fewer eggs (VAN ALPHEN & NELL 1982; BAKKER *et al.* 1990).



### 3.2.2 *Leptopilina heterotoma*

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Compared to *Asobara tabida*, *Leptopilina heterotoma* exhibits a different mechanism to evade and/or suppress immune defence of its host. In contrast to *Asobara tabida*, *Leptopilina heterotoma* eggs are found floating freely in the larval haemolymph (SCHLENKE *et al.* 2007). *Leptopilina heterotoma* females are, however, able to avoid the destructive effect of the host's cellular immune defence on their offspring, by injecting virus-like particles (VLPs) which are stored in the reservoir of an accessory gland associated with the female's reproductive system. These particles (formerly also called lamelloylysin) selectively destroy lamellocytes of *Drosophila* (see chapter 2.2.2), causing them to undergo morphological changes that make them incapable of forming cellular capsules and may even cause them to lyse (RIZKI & RIZKI 1990, 1991, 1992; RIZKI *et al.* 1990; CHIU *et al.* 2006; SCHLENKE *et al.* 2007). The VLPs also cause apoptosis of prohaemocytes in the lymph gland (SCHLENKE *et al.* 2007). These VLPs pose the virulence of *Leptopilina heterotoma*. Wild type *Drosophila* larvae are reported to only rarely encapsulate eggs of *Leptopilina heterotoma* (MORALES *et al.* 2005). Nevertheless and rather surprisingly, higher encapsulation rates may occur as in the study of FELLOWES *et al.* (1999a), where *Drosophila melanogaster* lines selected for increased larval resistance to *Asobara tabida* and *Leptopilina bouvardi* as well as control lines exhibited encapsulation abilities against *Leptopilina heterotoma* of mostly far more than 10% depending on rearing temperature. It has been observed, that the encapsulation rate of *Leptopilina heterotoma* eggs by *Drosophila melanogaster* increases with increasing age of the parasitoid female, in contrast to the related species *Leptopilina bouvardi* (STREAMS 1968; STREAMS & GREENBERG 1969; VASS & NAPPI 1998a; LABROSSE *et al.* 2003). WALKER (1959) kept parasitised larvae at temperatures ranging from 16°C to 28°C and observed that encapsulation ability of *Drosophila melanogaster* against *Leptopilina heterotoma* appeared to be optimal between temperatures of 18°C to 20°C.

Similar to *Asobara tabida*, *Leptopilina heterotoma* harbours three different strains of the bacterial genus *Wolbachia* which, when all three strains are present, induce a complete CI (FLEURY *et al.* 200; VAVRE *et al.* 2001; MOUTON *et al.* 2005).

In **Table 1** characteristics of the two parasitoids *Asobara tabida* and *Leptopilina heterotoma* are summarised.

### 3.2 *Asobara tabida* & *Leptopilina heterotoma*

**Table 1:** Summary of characteristics of the parasitoid's *Asobara tabida* and *Leptopilina heterotoma* including references

Characteristic	<i>Asobara tabida</i>	References	<i>Leptopilina heterotoma</i>	References
<b>A few basics</b>	solitary	CARTON <i>et al.</i> 1986	solitary	CARTON <i>et al.</i> 1986
	endoparasitoid	CARTON <i>et al.</i> 1986	endoparasitoid	CARTON <i>et al.</i> 1986
	koinobiont	CARTON <i>et al.</i> 1986	koinobiont	CARTON <i>et al.</i> 1986
	arrhenotokous	CARTON <i>et al.</i> 1986	arrhenotokous	CARTON <i>et al.</i> 1986
	protandry	CARTON <i>et al.</i> 1986	protandry	CARTON <i>et al.</i> 1986
<b>Ovigeny</b>	proovigenic,	CARTON <i>et al.</i> 1986; ELLERS & VAN ALPHEN 1997	proovigenic	CARTON <i>et al.</i> 1986
	moderately synovigenic Ovigeny index: 0.24 – 0.36	ELLERS & VAN ALPHEN 1997; JERVIS <i>et al.</i> 2001	<i>no information found</i>	-
<b>Adequate developmental temperature</b>	In superparasitised hosts (with <i>Leptopilina heterotoma</i> ): more successful at 15°C	VAN STRIEN-VAN LIEMPT 1983	In superparasitised hosts (with <i>Asobara tabida</i> ): more successful at 20°C and 25°C	VAN STRIEN-VAN LIEMPT 1983
<b>Host habitat location</b>	Odours emitted by fermenting substrates; kairomones	VET <i>et al.</i> 1984	Odours emitted by fermenting substrates; kairomones;	VET <i>et al.</i> 1984; CARTON <i>et al.</i> 1986; NUFIO & PAPAJ 2001; WERTHEIM <i>et al.</i> 2003; DELPUECH <i>et al.</i> 2005
			aggregation pheromone cVA	WISKERKE <i>et al.</i> 1993; HEDLUND <i>et al.</i> 1996
<b>Host location on the patch</b>	Vibrotaxis; (ovipositor searching)	CARTON <i>et al.</i> 1986	Ovipositor searching	CARTON <i>et al.</i> 1986; KRAAIJEVELD & VAN ALPHEN 1995b
<b>Preferred host</b>	Those hosts are preferred, where survival probability of offspring is high; in general <i>Drosophila subobscura</i>	KRAAIJEVELD <i>et al.</i> 1995; VAN ALPHEN & JANSSEN 1981	no real preference; higher survival probability in <i>Drosophila simulans</i> compared to <i>Drosophila melanogaster</i>	RIZKI <i>et al.</i> 1990; JANSSEN 1989
<b>Detection of host quality</b>	Can distinguish unparasitised from parasitised host larvae	CARTON <i>et al.</i> 1986	Can distinguish unparasitised from parasitised host larvae and even how often the host has been parasitised	CARTON <i>et al.</i> 1986; NUFIO & PAPAJ 2001; VAN LENTEREN <i>et al.</i> 2007
			Can discriminate between hosts with different numbers of eggs already present in the host; prefers ovipositing into hosts containing fewer eggs	VAN ALPHEN & NELL 1982; BAKKER <i>et al.</i> 1990
	Internal examination	CARTON <i>et al.</i> 1986; GODFRAY 1994	<i>no information found</i>	-
	Internal marking	CARTON <i>et al.</i> 1986	<i>no information found</i>	-
	Superparasitism occurs	CARTON <i>et al.</i> 1986	Superparasitism occurs, but only very rarely	CARTON <i>et al.</i> 1986; VARALDI <i>et al.</i> 2005
<b>Suppression/evasion of host immune defences (virulence)</b>	<b>Passive evasion:</b> Eggs with 'sticky' chorion, adhering to host tissues and being embedded	ESLIN & PREVOST 2000, MOREAU <i>et al.</i> 2003; PREVOST <i>et al.</i> 2005	eggs are found floating freely in the larval haemolymph	SCHLENKE <i>et al.</i> 2007
	<b>Active suppression:</b> deleterious effect on host Phenoloxidase activity (mechanism unclear); aspartylglucosamidase-like protein	MOREAU <i>et al.</i> 2000; MOREAU <i>et al.</i> 2004	<b>Active suppression:</b> inject VLPs which destroy lamellocytes	RIZKI & RIZKI 1990, 1991, 1992; RIZKI <i>et al.</i> 1990; CHIU <i>et al.</i> 2006; SCHLENKE <i>et al.</i> 2007
			VLPs may cause apoptosis of prohaemocytes in the lymph gland	SCHLENKE <i>et al.</i> 2007

### 3.2 *Asobara tabida* & *Leptopilina heterotoma*

Table 1 (continued)

Characteristic	<i>Asobara tabida</i>	References	<i>Leptopilina heterotoma</i>	References
<i>virulence factor</i>	Degree of 'stickiness' of the eggs' chorion	KRAAIJEVELD & VAN ALPHEN 1994	VLPs	several, e.g. RIZKI & RIZKI 1990
<b>Resistance in <i>Drosophila melanogaster</i></b>	Encapsulation;		wild type <i>Drosophila</i> larvae are reported to only rarely encapsulate; encapsulation occurred in several studies	WALKER 1959, 1962; NAPPI & STREAMS 1969; RIZKI <i>et al.</i> 1990; FELLOWES <i>et al.</i> 1999a; MORALES <i>et al.</i> 2005
	probably race between the haemocytic reaction and the embedment of the egg of <i>Asobara tabida</i> within host tissues	ESLIN & PRÉVOST 2000	higher encapsulation rates may occur; parasitoid susceptibility to encapsulation is correlated with the amount of previous ovipositions	FELLOWES <i>et al.</i> 1999a; STREAMS 1968
	lines selected for increased resistance exhibited a significant increase in haemocyte number	KRAAIJEVELD <i>et al.</i> 2001	encapsulation rate of eggs increases with increasing age of the parasitoid female	STREAMS 1968; STREAMS & GREENBERG 1969; VASS & NAPPI 1998a; LABROSSE <i>et al.</i> 2003
			encapsulation ability appeared to be optimal between temperatures of 18°C to 20°C	WALKER 1959
<b>Geographic pattern of virulence</b>	Low in the north of Europe, high in the south	KRAAIJEVELD & GODFRAY 1999	Geographic variation found in Swiss populations	WALKER 1959, 1962
<b>Possible explanation for the geographic pattern of virulence</b>	Main host in the north: <i>Drosophila subobscura</i> (much rarer in the south, immunodeficient)	KRAAIJEVELD & VAN DER WEL 1994; KRAAIJEVELD & GODFRAY 1999	-	-
<b>Geographic pattern of resistance in <i>Drosophila melanogaster</i></b>	Resistance is strongest in central southern Europe, weaker in the north, in the Iberian Peninsula, and in the southeast	KRAAIJEVELD & VAN ALPHEN 1995a	<i>Drosophila melanogaster</i> is reported to be a weak encapsulator in general of <i>Leptopilina heterotoma</i> offspring	RIZKI <i>et al.</i> 1990
<b>Possible explanation for the geographic pattern of resistance in <i>Drosophila melanogaster</i></b>	<i>Drosophila melanogaster</i> resistance to <i>Asobara tabida</i> higher where virulent <i>Asobara tabida</i> strains occur	KRAAIJEVELD & GODFRAY 1999	-	-
	resistance is low in northern Europe where <i>Asobara tabida</i> preferably attacks <i>Drosophila subobscura</i>	KRAAIJEVELD & GODFRAY 1999	-	-
<b>Possible explanation for the geographic virulence-resistance pattern</b>	geographic differences in host-parasitoid community structure	KRAAIJEVELD & VAN ALPHEN 1994; KRAAIJEVELD & GODFRAY 1999	-	-
	positive geographic correlation between puparial thickness and encapsulation	KRAAIJEVELD 1994	-	-
	encapsulation is more effective at higher temperatures	KRAAIJEVELD & GODFRAY 1999	-	-
	virulence as well as resistance often seems to be species specific: no cross resistance found (with <i>Leptopilina boulandi</i> )	KRAAIJEVELD & GODFRAY 1999	-	-

### 3.3 The microbial environment

Microorganisms are living organisms that are too small to be seen by the human eye without the aid of a microscope. They include microscopic algae, bacteria, fungi, protozoa, viruses and even various parasitic worms.

*Yeasts* are microscopic fungi found among the two taxonomic classes Ascomycetes and Basidiomycetes. They do not form a taxonomic group of their own and there is no clear or generally binding definition of yeasts (KOCKOVÁ-KRATOCHVÍLOVÁ 1990; KURTZMAN 1994). The English name “yeast” (Old English “gist” meaning “foam”) is probably derived from the foam formed during fermentation (KOCKOVÁ-KRATOCHVÍLOVÁ 1990). Falsely, this physiological process of fermentation (anaerobic utilisation of various carbon sources) is often thought to be an ability possessed by all yeasts, probably due to the widely known and vigorously fermenting group of *Saccharomyces* species. In fact, however, about half of the yeast species known today lack the ability of fermenting and instead have to derive their energy from aerobic oxidation (DEAK 2006).

Among microorganisms capable of fermentation, yeasts are the most important ones exploited for economical purposes and have been used in bread-making and the production of alcoholic beverages since thousands of years, but are also used in the production of cheese, contributing to its flavour and texture, and fermented milk products such as kefir (ROMANO *et al.* 2006; FLEET 2007).

Mostly due to their physiological adaptability, yeasts are ubiquitous and occur in terrestrial (soil, plants, animals), aerial and aquatic environments (fresh water and marine water), and are even found in deep-sea environments and the Antarctic (DEAK 2006; NAGAHAMA 2006; RODRIGUES *et al.* 2006; VISHNIAC 2006b).

Though yeasts are predominantly unicellular, some form pseudohyphae (often branched strands of elongated buds without crosswalls) and true hyphae (with well developed crosswalls like in typical filamentous fungi) (KURTZMAN 1994, 2006; MORAIS *et al.* 2006). Most yeasts reproduce asexually via cytokinesis, either by budding or binary fission (BALASUBRAMANIAN *et al.* 2004). Budding yeasts form a new yeast cell by protruding part of the mother cell. This protrusion is then separated and relieved from the mother cell. Fission yeasts reproduce by splitting of the mother cell into two equally sized daughter cells. Yeasts may also exhibit sexual reproduction via the formation of haploid spores, such as ascospores in ascomycetous yeasts or basidiospores in basidiomycetous yeasts (KURTZMAN & FELL 2006). When both asexual and sexual reproduction occur in a fungal species, the reproductive stages are often referred to as Anamorph (asexual reproductive stage) and Teleomorph (sexual reproductive stage).

Yeasts are heterotrophs and thus dependent on other organisms (fungi, bacteria, animals and plants) for their existence. They predominantly occur on substrates rich in carbohy-

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drates (HAGLER 2006; MORAIS *et al.* 2006). Yeasts are known as specialised organisms with limited physiological characteristics and ecological studies have shown that the majority of yeast species and groups have specialised habitats (LACHANCE & STARMER 1986; PHAFF & STARMER 1980; PHAFF & STARMER 1987; PHAFF 1990; ROSA *et al.* 1995; MORAIS *et al.* 2006).

Due to different methodology of species identification and different species concepts (especially concerning fungal and bacterial diversity), it is extremely difficult to state an exact number of how many different yeast species actually exist. Based on different models for prediction, the number of yeast species in the world is assumed to range between 1500 and 15000 species (LACHANCE 2006). Estimates also indicate, that only 1% of the yeast species in nature have been described so far (KURTZMAN & FELL 2006).

Yeasts are a highly heterogeneous and complex metabolic group. Though the metabolic pathways of the central carbon metabolism are basically identical between different yeast species, the mechanisms for nutrient uptake, the number of isoenzymes and the regulation of fermentation and respiration differ substantially (FLORES *et al.* 2000). Yeasts are capable of utilising more than 50 different nitrogen sources, such as L- and D-amino acids, pyrimidines, purines, polyamines, amines, nitrates and nitrites (MESSENGUY *et al.* 2006).

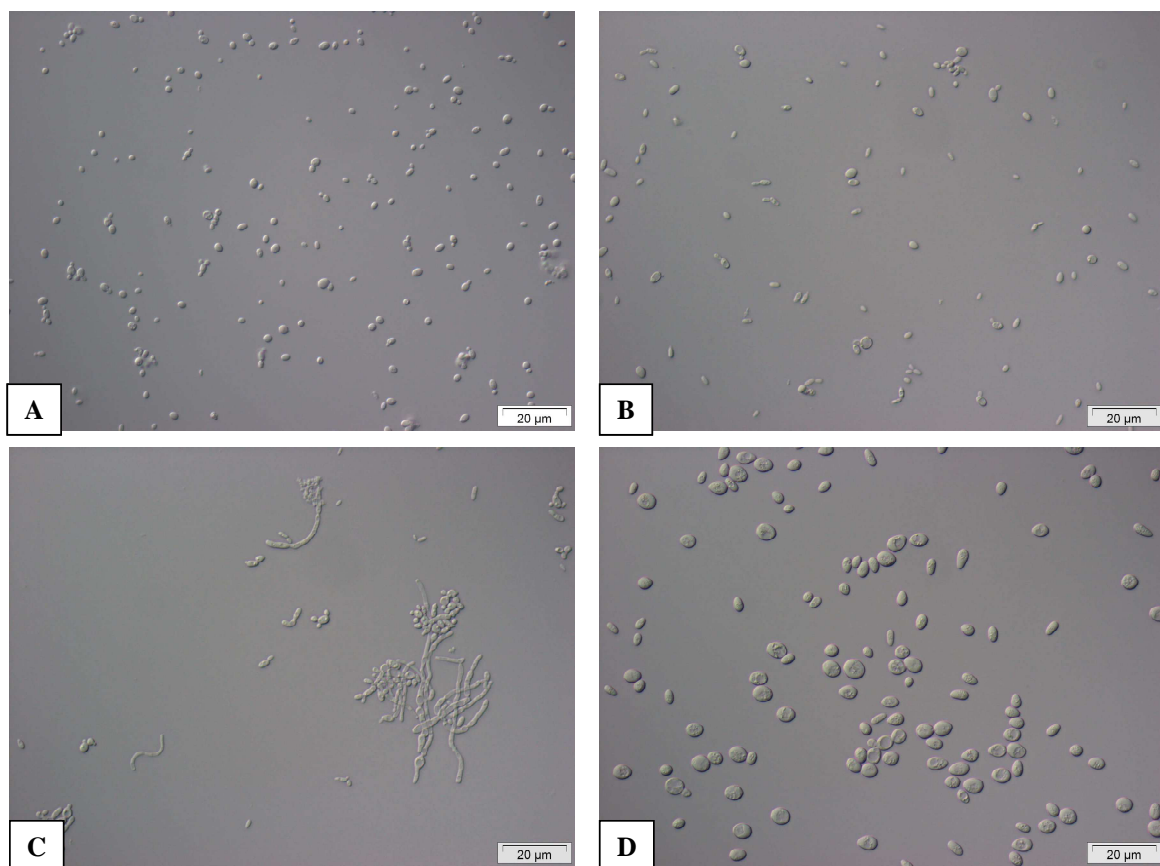
Since yeasts are immotile organisms, they depend on vectors, mainly insects, to be carried to new exploitable substrates (MORAIS *et al.* 2006). Various species of *Drosophila* have been shown to be important vectors for yeasts (GILBERT 1980; STARMER & FOGLEMAN 1986; GANTER 1988; STARMER *et al.* 1988; MORAIS *et al.* 1994; GANTER 2006). They carry yeasts picked up in slime fluxes, decaying fruits or vegetables. Though most yeasts are rapidly digested by the flies, some cells may survive the passage through the gut and thus be able to set up new colonies. Other yeasts are reported to be transmitted during oviposition (SPENCER *et al.* 1992; SPENCER & SPENCER 1997). Yeasts often provide nutrients needed by most insects such as various amino acids, sterols, several B vitamins and specific fatty acids, which are often not present in the natural nutritional substrates of these insects. Additionally, yeasts may also mediate both digestive and detoxifying reactions as well as pheromone production (SANG 1978; STARMER *et al.* 1986; VEGA & DOWD 2004). Many yeast species exhibit a more or less intense scent due to aroma-active volatiles (VAN LAERE *et al.* 2007). These may play a role in the attraction or repelling of *Drosophila* adults and larvae (see chapter 3.1) as well as in parasitoid host habitat location (see chapter 3.2).

For the present study, five exemplary yeasts were chosen, whereby mainly the last four of them were used in most of the experiments: *Cryptococcus albidus*, *Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae* (**Fig. 9**). These yeast species have been reported to occur in close association with *Drosophila melanogaster* (BEGON & SHORROCKS 1978; KEARNY 1982; LACHANCE 1995).

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*Cryptococcus albidus* belongs to the division Basidiomycota. The other four species are ascomycetous yeasts within the order Saccharomycetales, with *Metschnikowia pulcherrima* belonging to the family Metschnikowiaceae, *Pichia toletana* to the Pichiaceae, and *Kluyveromyces lactis* as well as *Saccharomyces cerevisiae* to the family Saccharomycetaceae.



**Fig. 9:** Four of the yeast species chosen to be used in the experiments. **A)** *Kluyveromyces lactis*. **B)** *Metschnikowia pulcherrima*. **C)** *Pichia toletana*. **D)** *Saccharomyces cerevisiae*. Pictures were taken via differential interference contrast microscopy (DIC) using an Axioskop (Zeiss, Jena) and a digital camera (Soft Imaging System). Pictures with courtesy of Monika Dorsch and Barabara Nixdorf-Bergweiler.

*Cryptococcus albidus*, and the genus *Cryptococcus* in general, has been found to predominate in most soils over a great geographical latitude, probably due to the fact that they possess a polysaccharide capsule which is particularly advantageous in arid soils (IKEDA *et al.* 1991; VISHNIAC 1995; VISHNIAC 2006a). The species has been found occurring in high Arctic glaciers (BUTINAR *et al.* 2007), in birch forests on podzolic soils (YURKOV *et al.* 2004), in permafrost soils of Siberia (DIMITRIEV *et al.* 1997), in fresh water (NAGAHAMA 2006), on various leaf litter types taking part in decomposition (SAMPAIO *et al.* 2007), in nectar and pollen and the gut and frass of bees (INGLIS *et al.* 1993; SPENCER & SPENCER 1997), on the surfaces of leaves of trees, shrubs, herbaceous plants and grasses (SPENCER

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& SPENCER 1997), on native fruits of tropical rain forests in Brazil (MORAIS *et al.* 2006) and on cacti (STARMER *et al.* 2006), as well as in house dust (PETROVA-NIKITINA *et al.* 2000) and black and white cinematographic film samples (ABRUSCI *et al.* 2005). It has also been isolated from cloacae of migratory birds (CAFARCHIA *et al.* 2006), as unwanted yeasts in ripening cheese (VILJOEN *et al.* 2003), from kefir grain and culture (SONG *et al.* 2007) and belongs to a wide variety of wine contaminating yeast species (ENRIQUE *et al.* 2007). Although *Cryptococcus albidus* has generally been regarded as saprobiont, infections of humans and animals due to these yeasts have occurred and partly increased, especially in immunosuppressed and immunodeficient patients (KHAWCHAROENPORN *et al.* 2007). It is able to produce and accumulate lipids even under carbon-limited growth conditions (HANSSON & DOSTÁLEK 1986). It has been reported capable of producing xylanases (BIELY & VRSANSKA 1988; COLLINS *et al.* 2005) and toxins active against other fungi (VADKERTIOVA & SLAVIKOVA 1995). Probably due to its potential killer activity against other fungi, *Cryptococcus albidus* is also applied as biocontrol agent (BCA) against various plant diseases and postharvest diseases of fruits (HELBIG 2002; CALVO *et al.* 2003; REELEDER 2004).

*Kluyveromyces lactis* is able to utilise lactose (it produces a  $\beta$ -galactosidase) as well as glucose (SPENCER *et al.* 2002; ORNELAS *et al.* 2008) and its ability to grow on lactose as sole carbon source distinguishes it from most other yeasts (SCHAFFRATH & BREUNIG 2000; DICKINSON & KRUCKEBERG 2006). It has been found in fermented milk such as kefir and kumiss, but also in some traditional cheeses (such as Camembert), although it had not been adopted for deliberate use (SEILER 2003; KUMURA *et al.* 2004; ROMANO *et al.* 2006). *Kluyveromyces lactis* has turned out to be a very promising candidate as a probiotic microorganism, since it showed high adhesive ability to human enterocyte-like Caco-2-cells, indicating adhesive ability to the intestinal mucosa which is considered important for exclusion of pathogens and undesirable bacteria (KUMURA *et al.* 2004). Since growth of *Kluyveromyces lactis* was moderate at 37°C, KUMURA *et al.* (2004) concluded that *Kluyveromyces lactis* could rather be expected to enhance immune modulation rather than improving the intestinal flora (presumably at least within organisms with a body temperature of approx. 37°C). *Kluyveromyces lactis* is capable of producing chitinase (DUO-CHUAN 2006) and toxins active against other fungi (STARK *et al.* 1990; MAGLIANI *et al.* 1997; GUYARD *et al.* 2000, 2001).

Various strains of the species *Metschnikowia pulcherrima* have been shown to exhibit antibiotic activity against other yeasts and fungal species and are therefore often used as biocontrol agents against fruit diseases and postharvest decay of various fruits (FARRIS *et al.* 1991; NGUYEN & PANON 1998; JANISIEWICZ *et al.* 2001; SPADARO & GULLINO 2004; SIPICZKI 2006). *Metschnikowia pulcherrima* is one predominant species found in agricul-

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tural soil (SLAVIKOVA & VADKERTIOVA 2003) as well as during the first phase of spontaneous and induced alcoholic fermentations (SCHUTZ & GAFNER 1993). *Metschnikowia pulcherrima* usually produces a red pigment (pulcherrimin) accumulating in the cells and in the medium near a colony (KLUYVER *et al.* 1953). Formation of this pigment is hypothesised to deplete free iron in the growth medium, which may lead to inhibition of sensitive microorganisms by pigmented *Metschnikowia* strains (SIPICZKI 2006).

*Pichia toletana* has often been thought to be identical with *Pichia xylosa*. The latter, however, turned out to be a distinct but closely related species (KURTZMAN 1992). The genus *Pichia* currently represents one of the largest yeast genera with regard to species number. DNA and RNA studies have indicated and confirmed the polyphyletic origin of the genus (VILLA-CARVAJAL *et al.* 2006). Yeasts of the genus *Pichia* are widely distributed. They occur in soil, freshwater, tree exudates, insects, plants and fruits and are also found as contaminants in a variety of foods and beverages (VILLA-CARVAJAL *et al.* 2006). *Pichia toletana* has also been isolated from yogurts (SURIYARACHCHI & FLEET 1981). The species is reported to be incapable of fermenting sucrose or lactose, but may utilise glucose and lactic acid (SURIYARACHCHI & FLEET 1981). *Pichia toletana* displays a morphological peculiarity compared to the other four yeast species, since it exhibits vegetative reproduction by multilateral budding and thus forms a pseudomycelium (PHAFF 1956).

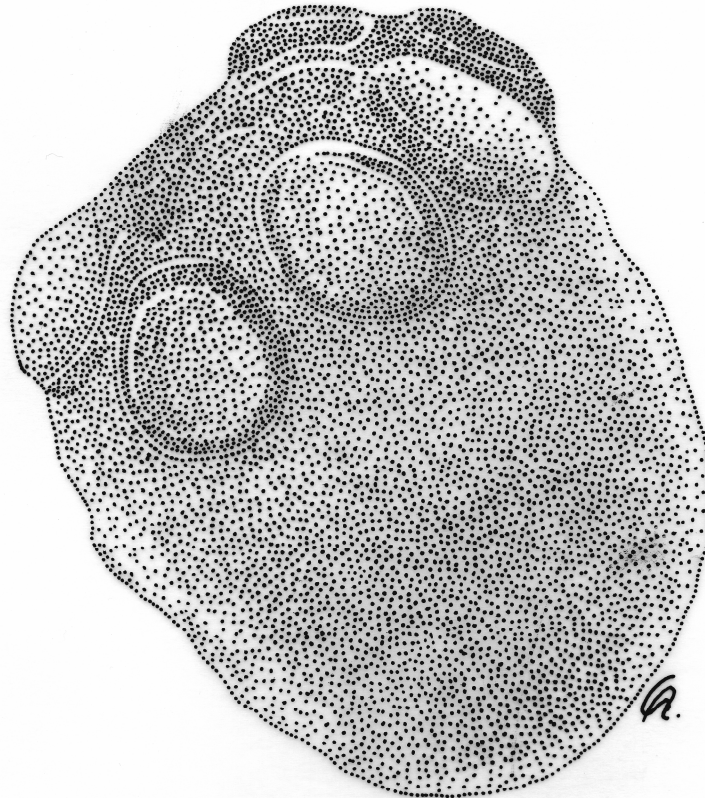
*Saccharomyces cerevisiae* is probably the best known yeast species and the term yeast is mostly equated with this species. Since ancient times *Saccharomyces cerevisiae* (often called ‘baker’s yeast’ or ‘brewer’s yeast’) has been used in baking and brewing and other branches in food industry. In cell biology and molecular biology it is a widely used and intensively studied eukaryotic so called ‘model organism’. They were the first microorganisms to be examined scientifically due to their large cells and their economic importance (BARNETT 2003). *Saccharomyces cerevisiae* is able to synthesise and degrade the polysaccharide glycogen and the disaccharide trehalose (LILLIE & PRINGLE 1980). As potential nitrogen sources it may utilise ammonium, urea and amino acids, as potential carbon sources glucose, lactate, pyruvate and fatty acids (KINGSBURY *et al.* 2006). *Saccharomyces cerevisiae* has been reported to have beneficial effects to other organisms such as promoting iron absorption, production of hydrosoluble vitamins with anti-scavenger effect, binding of mycotoxins, and improving intestinal conditions (MAI *et al.* 2002; MARTINS *et al.* 2005; SHETTY & JESPERSEN 2006; TEODOR *et al.* 2007). Its adhesive ability to Caco-2-cells, however, was negligible compared to *Kluyveromyces lactis* (KUMURA *et al.* 2004), though adhesion might not be a mandatory prerequisite for a probiotic effect (VAN DER AA KÜHLE *et al.* 2005). Not all strains of *Saccharomyces cerevisiae* may survive the adverse conditions in gastrointestinal tracts (AGRAWAL *et al.* 2000). The probiotic nature of *Saccharomyces cerevisiae* has not proved to match that of its already widely accepted and



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used conspecific *Saccharomyces boulardii* (FIETTO *et al.* 2004; EDWARDS-INGRAM *et al.* 2007). Additionally, *Saccharomyces cerevisiae* has been identified as a potential pathogen (ENACHE-ANGOULVANT & HENNEQUIN 2005; SKOVGAARD 2007). The natural habitats of *Saccharomyces cerevisiae* are supposed to comprise above all various fruits (BRAUS *et al.* 2003). LACHANCE (2003), however, expresses the difficulty of naming the exact natural habitat of this yeast species and argues that the best speculation would be that the wild forms are associated with an interface involving *Drosophila* spp., oaks and the surrounding soil.



*Saccharomyces cerevisiae* cell

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*I love fools' experiments; I am always making them [Charles Darwin].*

*The true worth of an experimenter consists in his pursuing not only what he seeks in his experiment, but also what he did not seek [Claude Bernard].*

*The true method of knowledge is experiment [William Blake].*

*No amount of experimentation can ever prove me right; a single experiment can prove me wrong [Albert Einstein].*

*To call in the statistician after the experiment is done may be no more than asking him to perform a post-mortem examination: he may be able to say what the experiment died of [Ronald Fisher].*

#### 4.1 Animal cultures

##### 4.1.1 *Drosophila melanogaster*

The first fly population originated from descendants of isofemale lines caught by Dr. Marko Rohlf in Kiel, Northern Germany, in September 2003. In summer 2006 an axenic culture was established. Flies were kept in transparent 22l plastic boxes (IKEA: 'Samla') furnished with various holes where 100ml plastic flasks (KAUTEX: 'Weithalsflaschen') containing food and water could be screwed on to the box (**Fig. 10A**). Remaining holes were closed with foam plugs for aeration of the box. Drinking water contained 30ml nipagin, 10ml chloramphenicol and 10ml sorbic acid (each with a concentration of 10%, dissolved in 100% ethanol) per litre tap water and was offered by inserting a piece of cotton wool into a filled flask. As food a mixture of sucrose and yeast hydrolysate (approx. 70:30) was offered. Flies were cultured at 24°C and an L/D cycle of 14:10. For culture maintenance flies were allowed to oviposit over night twice a month. Eggs were then dechorionated and transferred onto fresh culture medium and allowed to develop at 24°C and an L/D cycle of 14:10 until emergence. Freshly eclosed flies were then transferred into a new culture box.

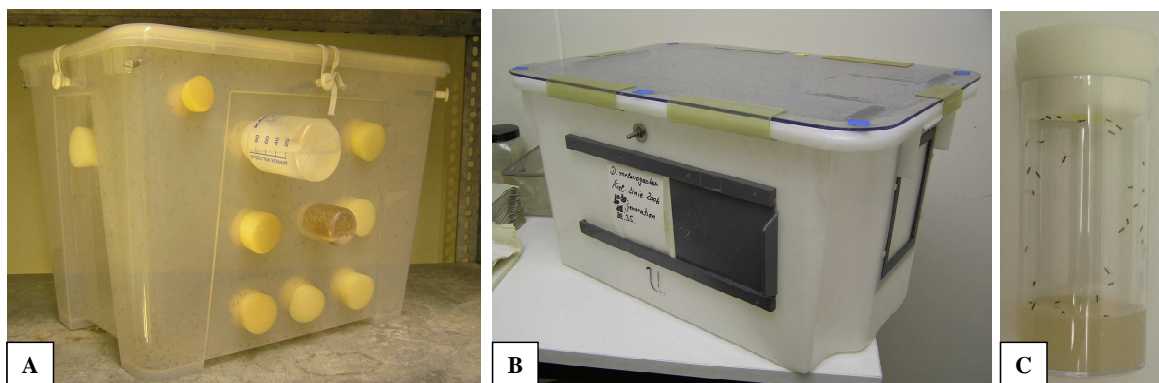
##### 4.1.2 *Asobara tabida*

The population of *Asobara tabida* stemmed from animals provided by University Leiden, NL, in 1998 and was cultured on *Drosophila melanogaster* at 20°C and an L/D cycle of 16:8. Flies used for culturing *Asobara tabida* stemmed from isofemale lines caught by Dr. Marko Rohlf in Kiel, Northern Germany, in September 2003 and were kept in big culture boxes (**Fig. 10B**) at 20°C and an L/D cycle of 16:8. Flies were fed with a 70:30 mixture of sucrose and yeast hydrolysate offered in a 90mm Petri dish. Drinking water was provided by filling a glass vial with tap water and putting it upside down on a 90mm Petri dish lined with absorbent paper. Twice a week two vials containing *Drosophila* basic culturing me-

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dium were placed inside the *Drosophila* culturing box for several hours for oviposition. The eggs were then incubated for two days at 20°C and an L/D cycle of 16:8. Several (up to ten) female and a few male (up to five) *Asobara tabida* individuals (approx. seven days old) were transferred into the flasks and left there for parasitisation. After several days, freshly emerged unparasitised flies or flies that had successfully fended off the parasitoid were removed from the vials. After approximately four weeks the first adult *Asobara tabida* adults eclosed and were transferred into culturing flasks containing Nipagin-agar (see chapter 4.3.1). The foam plug for closing the vials was supplied with a drop of honey (**Fig. 10C**). The adults were kept in the vials at 20°C and an L/D cycle of 16:8. Their life expectancy under these conditions was approx. four weeks. Adult female parasitoids used for the experiment were usually not older than 7 days depending on how many individuals were available. Sometimes older females had to be used in case a lot of individuals were needed or the culture experienced a slight depression. For the maintenance of the *Drosophila melanogaster* culture used for rearing *Asobara tabida*, about once a month flies were allowed to oviposit into *Drosophila* culturing flasks containing basic culturing medium. The freshly eclosed flies were transferred into a new culturing box. In irregular intervals the eggs were dechorionated prior to incubation.



**Fig. 10:** **A)** A *Drosophila* culturing box for culturing the *Drosophila melanogaster* population that was used in the experiments. **B)** A *Drosophila* culturing box for culturing the *Drosophila melanogaster* population that was used for the *Asobara tabida* and *Leptopilina heterotoma* cultures. **C)** Vial used for keeping adult parasitoids, filled with nipagin agar (see chapter 4.3.1) and covered with a foam plug provided with a drop of honey.

### 4.1.3 *Leptopilina heterotoma*

The population of *Leptopilina heterotoma* stemmed from animals caught by Dr. Marko Rohlf in Kiel, Northern Germany, in September 2003 and was cultured on *Drosophila melanogaster* at 20°C and an L/D cycle of 16:8. Flies used for culturing *Leptopilina heterotoma*, maintenance of the *Drosophila melanogaster* culture and rearing and storing of parasitoids was similar to that for *Asobara tabida* (see chapter 4.1.2).

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### 4.2 Microorganismal cultures

Microorganisms were purchased from the GERMAN COLLECTION OF MICROORGANISMS AND CELL CULTURES (DSMZ) in Braunschweig.

The following species strains were used: *Cryptococcus albidus* DSM 70215, *Kluyveromyces lactis* DSM 4909, *Metschnikowia pulcherrima* DSM 70321, *Pichia toletana* DSM 70390 and *Saccharomyces cerevisiae* DSM 70449.

*Pichia toletana* stems from a specimen isolated by PHAFF *et al.* (1956) from the crop of *Drosophila miranda*. At that time, this species of *Pichia* was named *Pichia xylosa*, a presumed synonym of *Pichia toletana*. KURTZMAN (1992), however, proved *Pichia xylosa* to be a distinct but closely related species to *Pichia toletana*. Additionally, the presumably new species (*Pichia xylosa*), isolated by PHAFF from *Drosophila miranda*, was thought to be identical with *Pichia rhodanensis* (MILLER *et al.* 1962). Thus, it is not certain, which species has actually been used in the present study and is held by DSMZ, respectively: *Pichia toletana*, *Pichia xylosa* or *Pichia rhodanensis*.

The yeast strains were obtained as colonies on agar in glass tubes. Shortly after receiving the specimens, glycerine stocks (15% glycerine in malt extract broth) were set up and stored in 2.2ml experimental tubes at -80°C. For each experiment new glycerine stocks were taken out of the freezer.

For the preparation of yeast suspensions with a defined yeast cell mass (actually measured as dry weight) in some of the experiments, calibration curves (see **Table 2**) were used which had been created by DORSCH (2007) for yeasts grown on malt extract agar (see chapter 4.3.3).

**Table 2:** Equations of the calibration curves and dry weight per yeast cell for the yeast species *Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae* after having grown on malt extract agar. Parameters in the equation:  $y \triangleq$  number of yeast cells per  $\mu\text{l}$ ;  $x \triangleq$  optical density at 600nm.

Yeast species	Equation	Dry weight per yeast cell [g] $\pm$ SE
<i>Kluyveromyces lactis</i>	$y = 23609x$	$7.044 \times 10^{-12} \pm 6.91086 \times 10^{-14}$
<i>Metschnikowia pulcherrima</i>	$y = 30638x$	$6.2 \times 10^{-12} \pm 3.15785 \times 10^{-13}$
<i>Pichia toletana</i>	$y = 46124x$	$3.664 \times 10^{-12} \pm 2.22117 \times 10^{-13}$
<i>Saccharomyces cerevisiae</i>	$y = 12105x$	$2.0528 \times 10^{-11} \pm 1.55769 \times 10^{-13}$

### 4.3 Media

#### 4.3.1 Basic culturing medium for *Drosophila melanogaster*, *Asobara tabida* and *Leptopilina heterotoma*

The axenic medium used for culturing *Drosophila* contained 62.5g each of sugar, corn flour and yeast extract, 12.5g agar and one litre tap water. After autoclaving and cooling down (to approx. 50°C) 30ml nipagin, 10ml sorbic acid, 10ml chloramphenicol (each with a concentration of 10%, dissolved in 100% ethanol) and 2ml nystatin dihydrate (0.05g dissolved in 1ml DMSO) were added. The medium was then poured into 100ml sterile culturing tubes.

The medium used for keeping eclosed *Asobara tabida* and *Leptopilina heterotoma* adults was prepared with 12.5g agar powder mixed with one litre tap water plus 30ml nipagin (with a concentration of 10%, dissolved in 100% ethanol) per litre autoclaved medium (**Fig. 10C**).

#### 4.3.2 Egg laying medium for *Drosophila melanogaster*

The medium used for egg laying prior to an experiment contained the same ingredients as the basic medium for culturing *Drosophila melanogaster* except of lacking nystatin dihydrate and being prepared with the double amount of agar (25g) since this made it much easier to wash the eggs off for dechoriation.

#### 4.3.3 Microbial media

Liquid cultures of microorganisms were prepared in *malt extract broth* containing 6g each of malt extract, glucose and maltose and 1.2g yeast extract (ROTH) in one litre broth. Additionally *malt extract agar* was used for culturing, containing 33.6g ready made powder (ROTH) in one litre agar.

#### 4.3.4 Experimental media

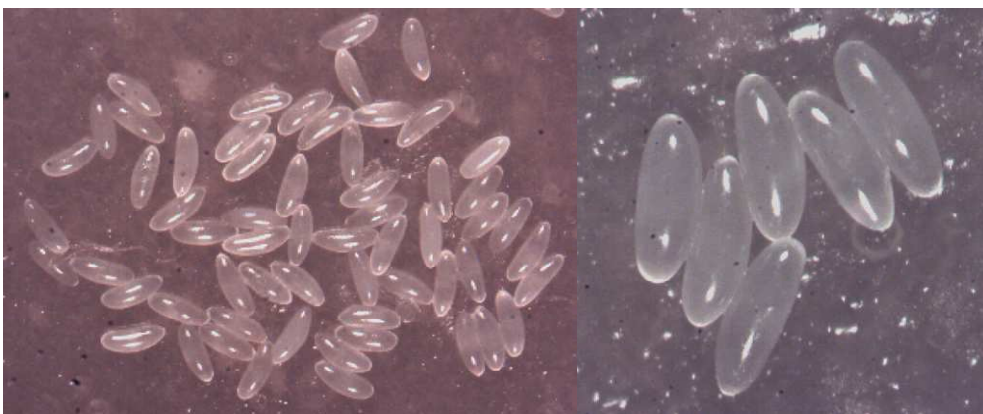
**Banana medium I:** Finely crushed banana mixed with the same amount of tap water and 12.5g agar per litre banana-water mixture. After autoclaving and cooling down (to approx. 50°C), 30ml nipagin, 10ml sorbic acid and 10ml chloramphenicol (each with a concentration 10%, dissolved in 100% ethanol) were added. **Banana medium II:** Same as Banana medium I without antibiotics. **Banana medium III:** Same as Banana medium I but with 25g agar per litre banana-water mixture. **Banana medium IV:** Same as Banana medium III without antibiotics. **Nipagin agar:** 20g agar mixed with one litre tap water plus 30ml nipagin per litre autoclaved medium.

### 4.4 Preparation of larvae for the experiments

48 hours prior to an experiment, axenically cultured flies were allowed to oviposit for approx. 14 hours into *Drosophila* culturing flasks containing axenic egg laying medium (see chapter 4.3.2). The eggs were then dechorionated with 50% sodium hypochlorite (ROTH, NaOCl in H<sub>2</sub>O, 12% Cl; diluted to 50% with tap water), washed onto a nylon gaze disc (mesh size 100µm, diameter 55mm) and were carefully transferred with a fine and sterile brush onto a Petri dish (diameter 55mm) containing Banana medium I (see chapter 4.3.4). For respiration the lid was provided with a small whole covered with nylon gaze (hole bored with a heated metal borer, nylon gaze glued to the lid with diethyl ether). A narrow parafilm strip was wrapped around the Petri dish to keep larvae from escaping. The Petri dish was put into a moist chamber (closable plastic box containing some distilled water). The eggs were incubated in a climate chamber (RUMED ® RUBARTH APPARATE GmbH) at 25°C and a 16:8 L/D cycle. The freshly hatched larvae were washed onto a small sterile nylon gaze sieve (mesh size 50µm) using tap water and could then be transferred with a fine brush.

### 4.5 Sterilisation and dechorionation of *Drosophila* eggs

When working with singular microorganisms, it is necessary to avoid contamination. Since *Drosophila* larvae feed on and develop in decaying plant material their whole outside body and their gut are covered and filled with a vast number of different microorganisms. Especially prior to an experiment it is therefore essential to gain sterilised eggs of *Drosophila* and transfer them onto axenic medium directly afterwards. Eggs can be sterilised using dilute solutions of Na or Ca hypochlorite which also leads to a chemical removal of the egg's chorion (**Fig. 11**).



**Fig. 11:** Dechorionated eggs of *Drosophila melanogaster*. The removal of the chorion also results in the absence of the two breathing tubes that usually characterise the eggs.

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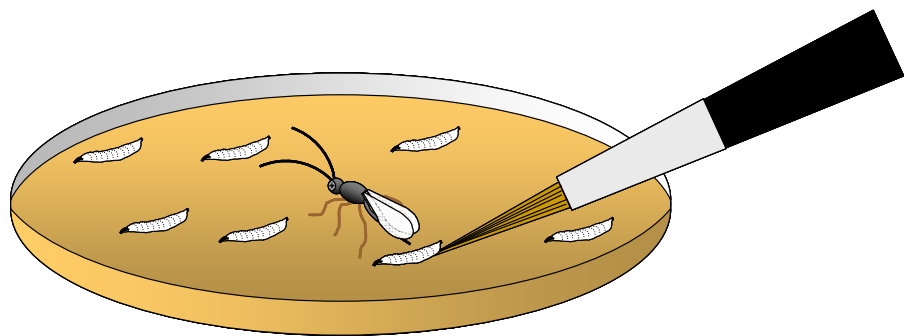
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The sterilisation and dechoriation of eggs were carried out as follows: After *Drosophila melanogaster* had been allowed to oviposit for approx. 14 hours onto egg laying medium, the eggs were washed off the medium using a soft brush and tap water and were collected on a fine sterile nylon gaze disc (mesh size 50µm, diameter 55mm) placed in a small funnel. The gaze disc bearing the eggs was then put for ten minutes into a beaker containing 50% sodium hypochlorite solution. Most of the eggs floated on the surface of the solution and were evenly spread by gently spinning the beaker. Hereafter, solution and eggs were filtered through a second sterile gaze disc. The eggs were rinsed thoroughly with tap water and transferred with a soft and sterile brush onto a Petri dish (diameter 55mm) containing Banana medium I.

### 4.6 Gaining of parasitised *Drosophila* larvae

#### 4.6.1 Observational

This method implied observing the parasitoid during oviposition. Several *Drosophila* larvae were transferred onto a Petri dish (Ø 35mm) filled with Banana medium III (see chapter 4.3.4) and given time to distribute. A female parasitoid was gently aspirated with a pooter and was allowed to walk onto the Petri dish. The lid was placed onto the dish and left there until the parasitoid showed interest in parasitising larvae. The lid was then removed and the parasitoid watched while ovipositing a larva (through a stereo microscope). If the parasitoid took more than 10 seconds to finish parasitisation the larva was assessed as being parasitised, removed from the dish with a fine brush and used for the experiment. Larvae that engaged the parasitoid for less than 10 seconds were also removed but not used in the experiment (**Fig. 12**).



**Fig. 12:** Illustration of the observational method used for gaining parasitised *Drosophila melanogaster* larvae. A female *Asobara tabida* and seven larvae of *Drosophila melanogaster* can be seen on the Petri dish with Banana medium III.

Since this method is rather time consuming it was not chosen for experiments where one had to stick to a tight time schedule or had to have many parasitised larvae at the same time, such as for the larval food choice experiment (chapter 7). The method was especially used to avoid frequent penetration by the parasitoid's ovipositor as well as superparasitisation. Frequent penetrations might challenge the immune system of *Drosophila* to a higher degree due to an



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increased number of wounds that have to be healed. When assessing encapsulation ability the observational method was therefore more adequate, since it provided larvae that had been punctured only once. Despite observing the parasitoids during oviposition, it still occurred rather frequently that larvae turned out not to be parasitised, presumably since the female parasitoids did not consider those larvae as suitable hosts for their offspring.

### 4.6.2 Non-observational

For this less time consuming method 20 freshly hatched *Drosophila melanogaster* larvae each were transferred onto a Petri dish ( $\varnothing$  35mm) filled with Banana medium III (see chapter 4.3.4). Several dishes were thus prepared depending on how many parasitised larvae were needed for an experiment. Usually, however, the double amount of larvae actually needed for an experiment was prepared for parasitisation, since not all larvae could be recovered afterwards, either because of not finding them or because they died before they could be used for the experiment. For parasitisation, two female parasitoids were transferred into each Petri dish. The parasitoids were gently aspirated with a pooter and allowed to walk onto the dish, which was immediately covered with its lid. The parasitoids were then allowed to oviposit for approx. one and a half hours. Hereafter, they were removed from the Petri dish and the larvae could be transferred with a fine brush into the desired conditions. Besides occasional superparasitisations it also occurred that a varying number of larvae were not parasitised. A considerable disadvantage of this method was that repeated puncturing of larvae by the parasitoid's ovipositor (plus potential superparasitism) could not be avoided in case it happened.

### 4.7 Experimental setup

Most experiments were carried out in lidless 2.2ml experimental tubes that were filled with 1ml of the desired experimental medium (usually Banana medium II, see chapter 4.3.4), covered with dental rolls (HARTMANN) and stacked in racks (**Fig. 13**). Prior to the experiments, the reaction tubes were filled with the medium and could be autoclaved together with the racks. After cooling down, 50 $\mu$ l of the desired yeast suspensions (50% yeast suspension or specific cell weight, respectively) were pipetted onto the substrate and the tubes were then covered with sterile dental rolls. The tubes were then ready for transferring of larvae.



**Fig. 13:** Most of the experiments were carried out in lidless 2.2ml experimental tubes covered with dental rolls. The experimental tubes were stacked in racks.



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After larvae had been transferred, the racks were put into large transparent boxes (Makrolon cage) (Fig. 14) that could be closed with a lid and incubated at 20°C or 25°C, respectively, and an L/D cycle of 16:8 in an incubator (RUMED ® RUBARTH APPARATE GmbH) or climate chamber.



**Fig. 14:** Transparent box (Makrolon cage) as it was used for storing the experimental racks in the incubator or climate chamber, respectively.

### 4.8 Measured fitness traits

In order to be able to assess the impact of the different dietary conditions on *Drosophila melanogaster* and its parasitoids *Asobara tabida* and *Leptopilina heterotoma*, certain organismal traits had to be chosen and measured, which may act as reliable and adequate ‘response variables’. An organism may exhibit a great variety of morphological, developmental, physiological, behavioural etc. traits, thus a whole spectrum of response variables chosen to be measured are thinkable. Nevertheless, it is helpful to try and choose among those traits that best characterise an organism’s *fitness* and which allow a reliable and preferably faultless and precise measurement. Which kind of traits fulfil these demands, may vary between the organisms and the situations studied. Several different traits may be affected by environmental changes (and the variables altered in the experiments), but among those only a few may have a crucial impact on the fitness of the organism, whereas other traits may only slightly if at all contribute to its fitness. It would actually be best, to choose traits that directly refer to an organism’s fitness, such as reproductive success (fecundity, mating success, etc). To measure these traits is, however, often more difficult and time consuming than the measurement of more distant fitness-related traits. The traits chosen in this study comprise those, which are reported to be crucial to the fitness of a whole range of organisms (*‘principal life history traits’*, STEARNS 1992), and especially to the fitness of the insect species this study is devoted to. To judge, if a particular specification of a trait may be favourable for the organism studied or not, nevertheless remains a difficulty. Usually, organisms exhibit a great variability within

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a certain trait (e.g. individuals of small or large body size), all of which may be adaptive depending on the environmental conditions and the pool of options that exist within a given population.

### 4.8.1 Survival

It is probably immediately evident, that the survival of an organism, especially until the stage in life where it may reproduce, is a crucial *sine qua non* for an organism's fitness. Thus, this parameter should be more or less self-explanatory.

Survival measured in this study was either survival of flies until pupariation, or survival of flies and parasitoids, respectively, until eclosion, and does not refer to longevity (life-span). Individuals of *Drosophila melanogaster*, *Asobara tabida* and *Leptopilina heterotoma* that were able to fulfil their development from egg or larva, respectively, until eclosion (emergence from the puparium) were assessed as having survived. In parasitised *Drosophila melanogaster*, those individuals were assessed as having survived, that were able to fulfil their development from larva until pupariation. Pupariation of parasitised *Drosophila melanogaster* is also a prerequisite for the survival of the parasitoid. Especially at an ambient temperature in the experiments of 25°C, it occurred that individuals of *Asobara tabida* entered diapause as late 3<sup>rd</sup> instar larvae (prepupal stage). In this case, the experiment was nevertheless terminated, and the breaking of the diapause and the (potential) development of parasitoids until eclosion was not awaited. The seemingly diapausing parasitoid larvae were assessed as having survived (larvae were intact) over those, that obviously had died inside the fly's puparium (cuticle not intact, larva partly exhibiting decay and melanisation). When calculating survival of parasitoids, those data were excluded where mortality of the parasitoid was due to successful encapsulation by the *Drosophila melanogaster* host larva.

### 4.8.2 Development time

The time it takes for an organism to fulfil its juvenile development and reach a mature stage where it can itself reproduce, is looked upon as a crucial life history trait (also called 'age at maturity'; strongly connected to 'size at maturity') (STEARNS 1992; ROFF 2000). An early maturation and shorter juvenile life span (short development time) may have the benefit for an organism of (i) shortening the sensitive juvenile phase where an organisms is prone to many threats, in the worst case pre-reproductive death, and of (ii) reaching the reproductive state at an earlier age (than e.g. other conspecifics), reproducing earlier, and thus enhancing fitness. Typically, there seems to be a positive correlation between development time and size at maturity, and there is also abundant data showing that body size (see 4.8.3) itself is positively correlated with fecundity (ROFF 2000). Especially in ectotherms, development time and body size are intimately connected (BOCHDANOVITS & DE JONG 2003). A shorter development time is often interconnected with a decrease in body weight and body size (see 4.8.3), and probably also body fat (see 4.8.4), since it usually takes time to increase biomass, i.e. to grow large in

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size or heavy in weight or store (fat) resources. The shape of the correlation between development time and body size may, however, vary according to the environmental variable. At a low food supply, for example, development time tends to be longer and body size smaller, whereas at lower temperatures, slower development time tends to go together with a larger body size (BOCHDANOVITS & DE JONG 2003). Especially for organisms whose larvae develop in ephemeral and fragmented resources, such as *Drosophila melanogaster* in decaying plant material, intraspecific competition as well as interspecific competition, e.g. with filamentous fungi (ROHLFS 2006), may cause a selective pressure on development time, whereby individuals with a shorter development time may be favoured (PRASAD *et al.* 2001). Shorter development time in *Drosophila melanogaster* may, however, not necessarily imply a greater competitive ability (as shown in lines selected for shorter development time), at least not in intraspecific competition (PRASAD & JOSHI 2003; SHAKARAD *et al.* 2005).

Despite this ambiguity concerning the importance of development time for the fitness of an organism, I will, in this study, treat a shorter development time in *Drosophila melanogaster* and its parasitoids as favourable over a longer development time.

Development time of *Drosophila melanogaster* was measured as the time a freshly hatched 1<sup>st</sup> instar larva needed to fulfil its development until eclosion as adult. The experimental tubes harbouring the single larvae were checked once daily at approximately the same point in time, and the day of eclosion was noted for each emerging adult. In *Asobara tabida* and *Leptopilina heterotoma*, development time was measured as the time a freshly laid parasitoid egg needed to fulfil its development until the eclosion of the adult parasitoid.

### 4.8.3 Body size and body weight

Unfortunately, in *Drosophila* literature, body size and body weight are often treated as equivalents, on the assumption that a relative change in body size corresponds to the same relative change in body weight. It is, however, thinkable, that flies small in size and heavy in weight may exist, as well as flies large in size, but with a relatively low weight. The fact that both traits are often seen as equivalents, stems from the fact that both the linear measurement of a specific size-related body part (especially done in small insects), as well as body weight, are thought to be an estimation of the body size of an organism. In insects (and also many other animals), body size itself is thought to be positively correlated with reproductive success (ROFF 2000), so that – if not constrained by other factors and trade-offs – it should be favourable for an insect to grow as large as possible. Increased body size may hereby be achieved by an increased development time (see 4.8.2) or an increased rate of growth. If there is an increase in growth rate (i.e. the utilisation of resources is enhanced and thus the degree of growth per time unit), no correlation between development time and body size (as mentioned above in 4.8.2) may result (ROFF 2000).

Since insects do not grow as adults (and may increase biomass after eclosion only to a certain extent and as far as their exoskeleton allows), the final weight/size of adults is a product of

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their growth rate during the larval phases and the duration of the growth period (EDGAR 2006). In holometabolous insects a (genetically determined) target weight/size is supposed to exist, which is the commitment to metamorphosis. This target weight/size occurs, when the growing larva has reached a certain threshold (*'minimum viable weight/size'*), at which larvae can develop into adults if food is completely withdrawn (EDGAR 2006). This should be distinguished from the more biologically relevant (and also mainly genetically determined) *'critical weight/size'*, being the weight/size, after which feeding no longer affects the time course to pupation (the two terms have, however, often been used interchangeably in the literature). Thus, if food were withdrawn just before minimum viable weight/size was reached, pupation would be delayed (resulting in elongated development time), meaning that it occurs a few hours earlier and is less than critical weight/size. Critical weight/size is assumed not to be significantly affected by the larval diet, though this is not clear yet (EDGAR 2006). Adult weight/size in *Drosophila melanogaster* is largely fixed by the weight/size at which 3<sup>rd</sup> instar larvae undergo pupariation, thus, the larval stage is important to these life history traits (body weight & size) and to adult fitness (PRASAD & JOSHI 2003). Dry weight reaches its maximum about 84 hours after hatching and 10-12hrs after the attainment of wet weight maximum (at 25°C) (PRASAD & JOSHI 2003). Increases in dry body weight may occur after eclosion, especially in females. In *Drosophila melanogaster*, the time from hatching until attainment of the critical weight/size (maybe the authors here referred to *'minimal viable weight/size'*) is sensitive to nutritional levels and can be lengthened considerably by a suboptimal diet (PRASAD & JOSHI 2003). When minimum viable weight/size has been attained despite suboptimal nutritional conditions, an adult fly may still finally emerge, though mostly of a very low weight and a very small size (EDGAR 2006). After critical weight/size has been reached, the larva may still continue to feed and grow until critical weight/size is physiologically sensed and metamorphosis is initiated (this time span is also called *'interval to cessation of growth'*). The timing when *Drosophila melanogaster* 3<sup>rd</sup> instar larvae cease to feed and metamorphosis is initiated is partly controlled by a hormonal network.

Changes in cell size are supposed to account for a significant amount of the variation in body size seen in *Drosophila melanogaster*, both in wild and laboratory populations. The final number of cells in each organ is supposed to be developmentally programmed, so that nutrition has a very limited effect on cell number, but profound effects on cell size (occurring when cell growth rate is increased without a simultaneous increase in cell division) (EDGAR 2006). Cell size in adult insects (consisting almost completely of non-proliferating cells) is mostly determined by the relative rates of synthesis, storage and turnover of macromolecules, metabolites and water. Nevertheless, variation in cell numbers exists, between species of *Drosophila* as well as more divergent species, where the differences in cell number are vastly greater than those in cell size. Thus, from an evolutionary standpoint, cell number is a more important determinant of body size than cell size (EDGAR 2006).

At temperatures below 25°C, the growth rate of *Drosophila melanogaster* is supposed to be reduced (both larval and pupal stage are affected), the weight of the mature 3<sup>rd</sup> instar larva is

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increased, as well as the weight of the subsequent adult and the size of its thorax (mesothorax), wing and leg (FRENCH *et al.* 1998).

Especially in invertebrates, increased **body size** is often correlated with increased **female fecundity** and increased **male mating success**, being two major fitness traits. There is, however, evidence, that the type and degree of correlation of body size with these fitness traits is affected by the type of causes that lead to a phenotypic variation in body size, and the correlation may additionally be affected by gene-by-environment interactions (JOSHI 2004).

In *Drosophila melanogaster*, body size is often estimated by the linear measurement of size-related traits, such as wing, thorax, or leg length. Body weight is thought to be far more variable than linear dimensions, and body weight is also variable according to feeding conditions and the fly's age. Male body weight, however, is reported to be insensitive to age and not very sensitive to ambient temperature. Females show a higher age-and temperature-related variability in body weight (KARAN *et al.* 1998). All in all, it is difficult to tell, which trait – the linear measurement of a size-related trait or body weight – is a better estimate for body size.

Especially in solitary parasitoids, body size is strongly influenced by the body size of their host (GODFRAY 1994). How big in size a host will grow, may to a large extent not be assessed by the female parasitoid, despite it being able to get some information on host quality at the time of oviposition. Thus, (not only) the body size of parasitoid offspring is at the mercy of the conditions (e.g. nutritional conditions) which shape the body size of their host. In *Asobara tabida*, it could be shown, that fitness strongly increases with size (ELLERS *et al.* 1998).

Due to the fact that the positive correlation between body size and reproductive success seems to be one of the few general patterns discernable (ROFF 2000), I will in this study, treat a larger body size and a heavier body weight in *Drosophila melanogaster* and its parasitoids, as favourable over a smaller body size and a low body weight, both in males and females.

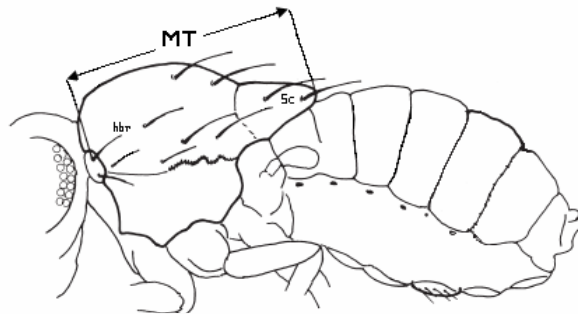
In this study, mostly **body weight** was chosen as an estimate for body size and was measured as **dry weight**. For the measurement of dry weight, freshly eclosed adult flies were transferred into 1.5ml experimental tubes (furnished with a lid) and stored in the freezer at -20°C. Prior to weighing, the tubes (lid opened) with flies were put into a drying cupboard and were dried at 70°C for 24hrs. After cooling down (lid of tube closed), the dried flies were weighed individually with the help of an ultra-microbalance (SC2, SARTORIUS, Göttingen) with an accuracy of 0.001mg.

**Body size** in *Drosophila melanogaster* was measured as the **mesothorax length**, according to FRENCH *et al.* (1998). After the flies had eclosed, they were usually killed and stored in the freezer at -20°C for subsequent measurements. To assess mesothorax length, the thawed adult flies were put under a stereo microscope fitted with an ocular micrometer and laid on one side. Mesothorax length was then measured from the base of the most anterior humeral bristle to the posterior tip of the scutellum (**Fig. 15**). If necessary, the wing turned to the viewer was gently lifted with fine forceps in order to expose the scutellum. The ocular micrometer was

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calibrated by using a slide micrometer (WILD, HEERBRUGG, Switzerland). The maximum accuracy of measurement possible was 0.029mm.



**Fig. 15:** Drawing of an adult *Drosophila melanogaster* (lying on its right side, and with wings removed) to illustrate, how the length of the mesothorax (MT) was measured. hbr: humeral bristle; Sc: scutellum. Picture source: FRENCH *et al.* (1998), slightly modified.

Body size in *Asobara tabida* and *Leptopilina heterotoma* were measured as length of the hind tibia. Body weight was, just as in *Drosophila melanogaster*, measured as dry weight.

### 4.8.4 Body fat & egg load

**(a) Body fat:** Fat or adipose tissue is a tissue where metabolic energy is stored in the form of fat, usually as triacylglycerol (TAG), which can be broken down to meet an organism's energetic needs. Fat as energy storage has the advantage, that an organism is not forced to store high amounts of water at the same time, as this would for example be necessary with glucose. In many insects, dietary fatty acids as well as lipids synthesised from carbohydrates, are primarily converted into TAG and stored in the fat body during the larval stages (lipogenesis). These accumulated resources are needed and used up during metamorphosis and the adult stage (lipolysis) (ARRESE *et al.* 2001; CANAVOSO *et al.* 2001; ZIEGLER & VAN ANTWERPEN 2006; GRÖNKE *et al.* 2007). In insects, fat may be used for body maintenance, as an energy source during flight, and as a resource for oogenesis (ELLERS *et al.* 1998). The most metabolically active, and thus energy-consuming tissue in adult winged insects, are the flight muscles (CANASOVO *et al.* 2001). In female adults, a great amount of storage fat is transferred to oocytes, and approx. 30%-40% of an oocyte's dry weight consists of TAG, phospholipids and cholesterol (ZIEGLER & VAN ANTWERPEN 2006). These lipids are the main source of energy for the developing embryo.

The main sites of resource allocation related to reproduction and survival in parasitoid wasps are the ovaries (as lipids and proteins in the yolk of oocytes, in the later stages of their development) and the fat body (as lipid, protein and glycogen) (JERVIS *et al.* 2001). Female *Asobara tabida* have been shown to use fat reserves as a resource for both reproduction and survival (life span/longevity) (ELLERS & VAN ALPHEN 1997). Fat reserves may be used to produce eggs which stay in the ovaries until oviposition (*egg load*), or for the female's own me-

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tabolism and survival (stored in the fat body). Resources are reported not to shift back from the eggs to survival (no 'oosorption' occurs), and fat reserves may also not be increased by feeding (ELLERS 1996; ELLERS *et al.* 1998). The allocation of fat resources either to survival or egg load may vary in response to environmental conditions, such as the availability of oviposition sites (hosts).

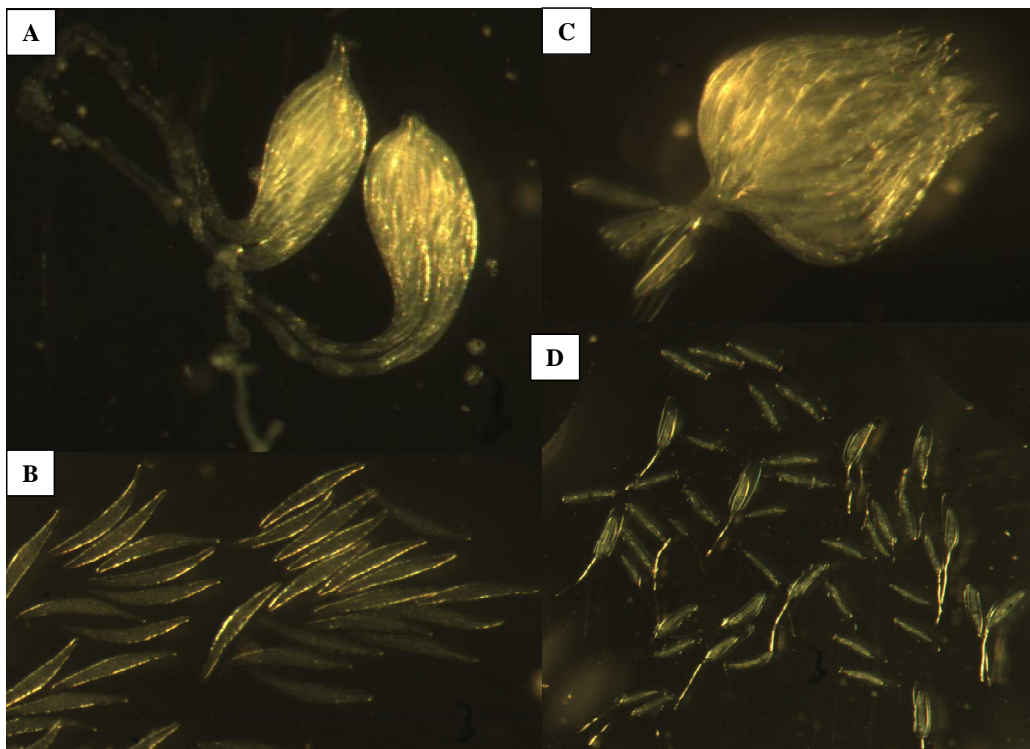
**(b) Egg load:** The egg load is defined by the total number of mature oocytes carried in the ovaries by a female at a given moment in her lifetime. It has been identified as a significant fitness variable in insects, together with the ovigeny index, defined as the proportion of the potential lifetime complement of eggs that is mature upon female emergence (PAPAJ 2000; JERVIS & FERNS 2004). Most information on these two parameters has been obtained for parasitoid wasps. In reference to egg load, parasitoid wasps are thought to face either *egg limitation* (female egg supply is depleted before oviposition opportunities are exhausted) or *time limitation* (females die or otherwise lose reproductive competence before all mature eggs have been laid) (PAPAJ 2000). Insects in general, are capable of maturing eggs rapidly (ECKELBARGER 1994), which permits them to adjust their rate of egg maturation to variations in the availability of oviposition sites, hosts and host quality, and resource availability for egg production (PAPAJ 2000). Thus, egg load may not be the limiting factor for a female parasitoid, unless she is strictly proovigenic. Egg load may rather be a good predictor of the variation in behaviour in many host-specific insects, rather than a predictor of reproductive success (PAPAJ 2000).

Individuals of *Asobara tabida* with a higher egg load at emergence are thought to have an advantage over conspecifics with a lower egg load. The higher egg load may enable them to lay eggs earlier, i.e. reproduce earlier than their conspecifics (ELLERS & VAN ALPHEN 1997). This advantage may, however, depend on the availability of host larvae and how patchy they are distributed. Sometimes, parasitoids may have to disperse and travel far distances to reach the next host patch, consuming both time and energy, in the form of fat reserves. The amount of fat reserves (as well as egg load), has been shown to be positively correlated with body size. ELLERS *et al.* (1998) assume, that individuals with a larger body size and thus higher fat reserves (and a higher egg load), may have a fitness advantage over conspecifics with a lower body size and lower fat reserves, in terms of a higher dispersal ability.

In this study, body fat and egg load were measured in the parasitoids *Asobara tabida* and *Leptopilina heterotoma*, according to the method described in ELLERS (1996) (description see below). The body size (as length of hind tibia), the number of eggs present upon emergence and the fat content of the whole female parasitoid (including eggs) had to be correlated to one another using multiple regression, since there is supposed to be a positive correlation between body size (as hind tibia length), body fat and egg load. The regression should allow to suggest how much of the fat reserves present in a female parasitoid have been allocated to reproduction already upon emergence. The method, however, implied the presumption, that oocytes contain approx. the same amount of fat.

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After parasitoids had emerged from the various experimental conditions, they were sexed and killed by transferring them into the freezer at a temperature of  $-20^{\circ}\text{C}$ . After thawing, the female parasitoid was put under a stereo microscope, and first, the length of the hind tibia was measured (see 4.8.3). Both hind tibiae - left and right - were measured, in case of fluctuating asymmetry. The maximum accuracy of measurement possible was 0.014mm. The parasitoid was then put onto a round coverslip of known weight (diameter of coverslips used: 15mm, 18mm and 20mm). The abdomen was then dissected in a drop of distilled water and the ovaries were carefully spread out to singularise the eggs (**Fig. 16**). The eggs were immediately counted and their number noted. Only fully matured eggs were counted. The coverslips with the dissected parasitoids were put into small plastic Petri dishes ( $\varnothing$  35mm), dried in a drying cupboard at  $60^{\circ}\text{C}$  for 24hrs and weighed. Calculating the difference between this value and the weight of the coverslip alone, gave the dry weight of the parasitoid. The coverslips with parasitoids were then transferred into closable vials containing 5ml diethyl ether for fat extraction, and were left there for 24 hrs. After rinsing with fresh diethyl ether, the coverslips were again dried and weighed. The difference of the already obtained dry weight of the parasitoid and the dry weight of the parasitoid after fat extraction finally yielded the absolute fat content of the parasitoid. In order to be able to compare the fat content of parasitoid females of different body size, a ratio between the absolute fat content and the dry weight of an individual parasitoid was calculated, yielding the relative fat content.



**Fig. 16** : Eggs spread out for counting, of *Asobara tabida* and *Leptopilina heterotoma*, after dissection of the abdomen and disrupting of the ovaries. **A**) Ovaries and **B**) Eggs of *Asobara tabida*. **C**) One ovary and **D**) Eggs of *Leptopilina heterotoma*.



### 4.8.5 Food choice behaviour

The success of an individual at surviving and reproducing may critically depend on its behaviour. In natural selection, those animals will tend to be favoured, that perform more efficient behavioural patterns (efficient foraging, efficient predator avoidance, efficient parasite avoidance or elimination, efficient mating, etc.) than their conspecifics (KREBS & DAVIES 1993). What kind of behaviour may be more efficient than another strongly depends on the prevalent environmental conditions and the pool of behavioural options that exists in a given population. In a wide array of taxa, there is evidence for a genetically based geographical variation in behaviour (FOSTER 1999). Additionally, individual differences in animal behaviour ('personality differences') are apparently common and also adaptive (DALL *et al.* 2004). Parasites and pathogens are supposed to pose major forces shaping behaviour of an organism, including behavioural patterns to avoid, control and eliminate parasites (HART 1990). These behavioural changes in infected individuals have, however, not always proven to be adaptive and lead to a fitness gain in either the host or the parasite or pathogen (POULIN 1995b).

The ability of an organism to sense its metabolic nutritious needs and exhibit a feeding behaviour, enabling it to adjust for potential nutritious deficiencies and imbalances, seems to be highly adaptive over a whole range of organisms and environmental situations. Dietary self-selection is reported to be ubiquitous across kingdoms and beneficial to an organism (an organism's fitness), provided it enhances crucial fitness traits, such as survival. It may increase the efficiency in food utilisation, which may be an essential factor in conditions, where food is scarce or scattered (WALDBAUER & FREIDMAN 1991).

It has been shown, that larvae of *Drosophila melanogaster* prefer particular species of yeast when given the choice, and that these preferred yeast species are the ones that better support their larval development (LINDSAY 1958; COOPER 1960; DORSCH 2007). Thus, the ability to self-select their diet, if given the opportunity, seems to be adaptive also in *Drosophila melanogaster*.

In this study, those *Drosophila melanogaster* larvae were assessed as having an enhanced fitness that preferred the yeast species, which had been shown to best support particular fitness traits of *Drosophila melanogaster* larvae, such as enhanced survival probability and encapsulation ability, shorter development time, larger body size and heavier body weight.

To assess behavioural responses, the behaviour of a group of *Drosophila melanogaster* larvae (20 larvae) was measured, not that of single larvae. This may imply the problem that, although larvae may still behave as individuals, the strength of the behavioural responses of individual larvae may be affected by the presence of other larvae (KAISER & COBB 2008). This loss was, nevertheless, accepted, in order to decrease the number of food

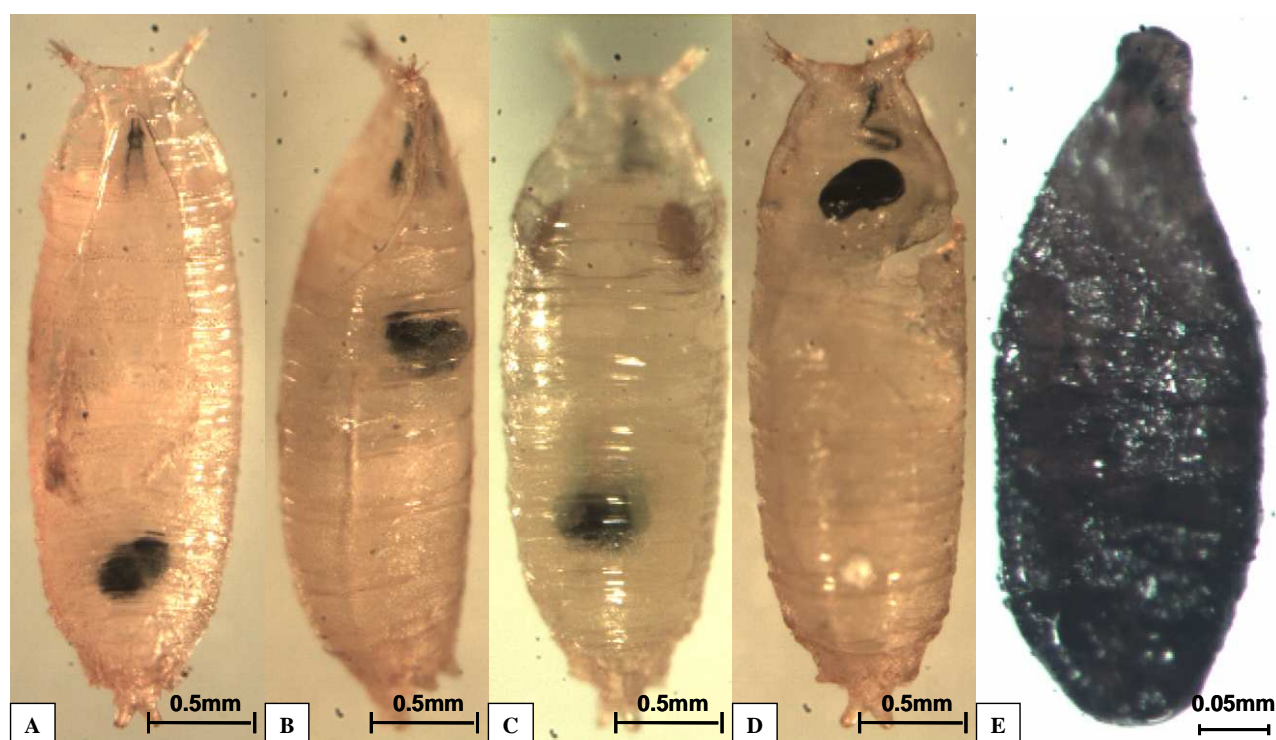
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choice arenas necessary, as well as to gain more data points simultaneously. For more details on the edition of the data on food choice see chapter 7.

### 4.8.6 Encapsulation ability

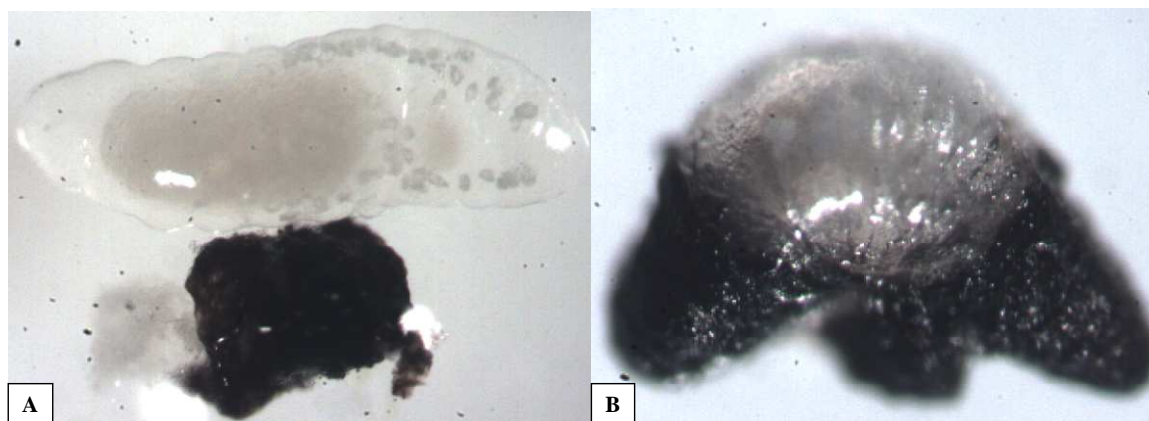
The ability of an insect to encapsulate a parasitoid egg or larva and thus fend off the invader may be a crucial trait deciding over the life or death of an organism, especially when the risk of potential parasitism is high. Being able to fend off the parasitoid offspring, is a prerequisite for a parasitised larva for its survival and future reproduction, and poses an advantage over those individuals which do not succeed in fending off the parasitoid.

In this study, encapsulation ability was measured as the proportion of parasitised larvae that harboured a capsule (**Fig. 17**) and no live and developing parasitoid. For this, either approx. 2 day old pupae were dissected or freshly emerged adult flies.



**Fig. 17:** Capsules formed around eggs or larvae of *Asobara tabida* as they were found in *Drosophila melanogaster*. **A**) & **B**): Approx. 1-2 day old pupae of *Drosophila melanogaster* with a capsule shining through the puparium. **C**) Approx. 3 day old pupa (with metamorphosis nearly finished) with capsule. **D**) Approx. 1-2 day old pupa which has been ripped open to reveal the melanised capsule. **E**) Encapsulated, completely melanised parasitoid larva.

In some cases, parasitoid larvae succeeded in breaking out of a capsule (**Fig. 18**) and could fulfil their development, resulting in the death of the *Drosophila melanogaster* larva. These fly larvae were assessed as not having exhibited a successful encapsulation.



**Fig. 18:** Parasitoid larvae of *Asobara tabida* that had (nearly) succeeded in breaking out of a capsule formed by the *Drosophila melanogaster* host larva. **A)** Complete escape from the melanised capsule. **B)** The parasitoid larva is still struggling to get free.

### 4.9 Statistical analysis

All statistical analyses were carried out with R 2.5.0. Prior to each analysis, (especially) continuous data (e.g. data on development time, dry weight, mesothorax length, hind tibia length, relative fat content, number of eggs and haemocyte number, delta value in food choice experiments) were tested for normal distribution with the Shapiro-Wilk normality test. Homogeneity of variances was tested with Levene's test. If data were both normally distributed and showed equal variances across samples, General linear models (GLMs) with family: gaussian and function: identity, were used for data analysis. Not normally distributed data were analysed with GLMs, for which a poisson distribution (or in order to correct for underdispersion or overdispersion, respectively, also quasipoisson) was specified, since it is especially suitable for not normally distributed and continuous data. Proportional data such as data on survival and encapsulation ability were outright analysed with GLMs (family: binomial or quasibinomial, function: logit), since they were never normally distributed due to their characteristic of comprising merely two values, zero and one. Thus, a binomial or quasibinomial distribution was specified here. When GLMs yielded a significant difference between treatments, post hoc tests (Tukey) were carried out to find significances between specific treatment pairs. ANCOVAs were carried out when more than one factor (e.g. yeast species and initial yeast cell mass) had to be considered. On data of mesothorax length and dry weight of *Drosophila melanogaster* a correlation (Pearson's coefficient) was carried out. On data of hind tibia length, dry weight, absolute fat content and number of eggs of female *Asobara tabida* as well as *Leptopilina heterotoma*, linear regressions were carried out, with model assumptions (homoscedascity and normal distribution of residuals as well as linearity) being tested for validity.

## **5 Influence of dietary microorganisms on various fitness traits of *Drosophila melanogaster* and *Asobara tabida***

### **5.1 General Introduction**

One crucial question absorbing evolutionary ecology with regard to immune defence in hosts is to explain, why the observed immune responses vary widely across species and situations (SCHMID-HEMPEL 2003). The hypotheses generated as plausible explanation comprise e.g. that immune defence is a trait whose costs in terms of energetic/nutritious demands or fitness costs (evolutionary, maintenance and deployment costs) are traded off against other fitness components, such as somatic growth, duration of development or reproduction (SHELDON & VERHULST 1996). It has long been recognised that there is a close relationship between the host's nutritious state and its ability to overcome infection (CHANDRA 1996). Besides the more obvious detrimental consequences on immune responsiveness resulting from starvation or lack of food (CHANDRA 1996, 1997, 1999, 2002; BROWN *et al.* 2000; HOUSTON *et al.* 2007; CAMPERO *et al.* 2008), caterpillars of *Spodoptera littoralis*, e.g., challenged with an entomopathogenic virus, displayed a higher resistance against the pathogen when fed additional dietary protein, and were even observed to self-compose their diet according to the resources required (LEE *et al.* 2006). MACEY & COYNE (2005) could show that diet supplemented with specific probiotics improved disease resistance in farmed *Haliothis midae*, in the way that the number and phagocytic activity of circulating haemocytes was significantly higher in probiotic treated animals compared to non-treated animals, following challenge with the pathogenic bacterium *Vibrio anguillarum*. A study on *Penaeus vannamei* reared on experimental diets containing different yeast species even showed differences between the yeast species in their influence on immune response (clearing bacteria from the haemolymph) and other fitness traits (survival, wet weight) (SCHOLZ *et al.* 1999).

Larvae of *Drosophila melanogaster* develop in injured or decaying plant material (see chapter 3.1). Usually these substrates are rich in microorganisms such as bacteria and fungi, especially yeasts, and it is mainly those microorganisms which act as actual nutrient resource, processing the plant raw material into important dietary factors (STARMER 1981), and providing additional nitrogen for the developing larvae, since often the plant material itself is poor in nitrogen compounds. Yeasts are considered as a major food source for the majority of species of *Drosophila* in both adult and larval stages (BEGON 1982). Various habitats of *Drosophila* exhibit characteristic yeast communities, and each fly species may have a distinct yeast community associated with it (STARMER 1981). The presence of yeast itself, irregardless of a particular species, is crucial for the fly's successful development

## 5 Influence of dietary microorganisms on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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and also turned out to enhance the encapsulation ability of larvae parasitised by *Leptopilina boulardi* (VASS & NAPPI 1998).

KEARNY (1982) investigated the impact of selected yeast species, collected from natural substrates of *Drosophila* spp., on various life-history traits, such as survival and development time, and found a marked effect of both yeast species and substrate type on fitness. With regard to the ubiquitous threat parasitoids may pose to virtually all insects, the ability to fend off such invaders is another highly important fitness trait. *Drosophila melanogaster* larvae possess the ability to fend off parasitoid eggs or larvae deposited in their haemocoel, with the help of a cellular innate immune defence (*encapsulation*, see chapter 2.2.2). Does the main food source of *Drosophila melanogaster*, in the form of different microbial species, influence the ability of *Drosophila melanogaster* to fend off parasite offspring? The aim of the experiments described in this study was to find out, whether particular dietary yeast species fed to *Drosophila melanogaster* larvae may have a different impact on the immunological ability of *Drosophila melanogaster* to fend off parasitoid offspring of the braconid wasp *Asobara tabida*, and may thus provide another potential explanation for the observed variation in immune defences. Since host nutrition is also a decisive factor for the development, survival and reproduction of the parasite (COOP & HOLMES 1996; COOP & KYRIAZAKIS 1999; THOMPSON *et al.* 2005) - especially in koinobiont endoparasitoids, such as *Asobara tabida* and *Leptopilina heterotoma* - the impact of the particular dietary yeast species on parasitoid offspring and the role of dietary microorganisms for the interaction between a saprophagous host and its parasitoids, were also of major interest in this study.

To investigate this, parasitised larvae were fed with different yeast species reported to have been isolated from *Drosophila* spp. and various breeding sites (BEGON 1982; KEARNY 1982) (for choice of yeast species see chapter 3.3) and the larvae's encapsulation ability was assessed. Additionally, several other fitness traits in both parasitised and unparasitised larvae were recorded, as well as fitness traits of the successfully developing parasitoid offspring.

In chapter 5.2, yeasts were offered at *ad libitum* conditions directly after larvae had been parasitised. In chapter 5.3, yeasts were also offered at *ad libitum* conditions, but after a delay of three, six and 24 hours after parasitisation. This was mainly done to complement the experiment on larval food choice (see chapter 7), where larvae were kept on yeast free substrate for three, six and 24 hours after parasitisation. In chapter 5.4 larvae were offered the particular dietary yeast species only at limited conditions (specific initial yeast cell mass). Here, also the development of the parasitoid offspring was observed until maturity, in order to investigate the impact of yeast species in the host's diet on specific life history traits of *Asobara tabida*. In chapter 5.5 larvae were offered a 'mixed diet' composed of two or more yeast species simultaneously.

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

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### 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

#### 5.2.1 Introduction

Decaying plant material, such as fallen fruits, may usually harbour a kaleidoscope of microorganisms. As soon as the fruit leaves its mother plant, and even before that, soil microorganisms and microorganisms waivering in the air, may settle on this ephemeral resource and start to feed. Besides various microbes, larger organisms, such as *Drosophila melanogaster*, accept this welcoming, temporally available resource as a food source for their offspring. Thus, larvae of *Drosophila melanogaster* may encounter a whole range of microorganisms, which they may potentially feed from. Especially of *Drosophila* species that develop on cacti, a variety of yeast species – the assumedly preferred microbial diet of *Drosophila* – have been found (STARMER *et al.* 1986; BARKER *et al.* 1994; ROSA *et al.* 1995; MORAIS *et al.* 1996; FOGLEMAN AND DANIELSON 2001; STARMER *et al.* 2003), though also in connection with other habitats (LACHANCE *et al.* 1995). Despite the overwhelming variety of yeast species growing on decaying plant material, *Drosophila* larvae were found to prefer consuming specific yeast species over others, and not randomly and indiscriminately devour any yeast present (FOGLEMAN *et al.* 1981). The prevailing yeast species used in studies on the evolutionary ecology of *Drosophila melanogaster* is *Saccharomyces cerevisiae*, usually in the form of Baker's yeast. All studies dealing with the immune defence of *Drosophila melanogaster* (*encapsulation ability*) against its larval parasitoids *Asobara tabida* and *Leptopilina heterotoma* have been carried out using Baker's yeast as microbial food source. The use of a single dietary yeast species may, however, obscure a potential variability in immune defences, due to the impact of nutrition on immunity, especially with regard to the fact, that different microorganismal species may highly vary in their physiology and thus in their role for the nutrition of *Drosophila melanogaster* larvae.

The following study was performed to primarily find out, whether different yeast species in the diet of *Drosophila melanogaster* larvae may have a differing influence on their ability to defend themselves against parasitoid offspring, and thus provide an explanation for the observed variation in immune responses.

In view of the vast number of yeast species in general, and those found in association with *Drosophila* spp., it was unavoidable to choose a few yeast species and specific species strains as 'model yeasts' for this study (see chapter 3.3).

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

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### 5.2.2 Material and methods

#### (a) *Experimental setup*

The experiment comprised five treatments in accordance to the number of different yeast species in the larval diet (each yeast species as one treatment) (*Cryptococcus albidus*, *Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae*). Parasitised as well as unparasitised larvae were exposed to the different treatments.

#### (b) *Fly and parasitoid cultures*

See chapter 4.1.

#### (c) *Preparation of media:*

See chapter 4.3.

#### (d) *Preparation of larvae*

See chapter 4.4 and 4.5.

#### (e) *Gaining of parasitised larvae*

For this experiment the observational method described in chapter 4.6.1 was used. One parasitoid each was allowed to parasitise approx. 10 larvae, of which two larvae each were transferred into each of the five yeast treatments, directly after the parasitoid had finished the 10 parasitisations.

#### (f) *Preparation of microorganisms*

For this experiment *ad libitum* food conditions were prepared with 50% yeast suspensions (see chapter 4.2).

#### (g) *Experimental proceeding*

50µl each of the 50% yeast suspensions were pipetted into the prepared test tubes filled with Banana medium II. Hereupon one larva each was transferred into the tubes with a fine brush and the tubes covered with dental rolls (HARTMANN). Test tubes containing one parasitised larva each as well as tubes with one unparasitised larva each (control tubes) were prepared. The tubes were incubated in a climate chamber at 25°C and a 16:8 L/D cycle. The development of unparasitised larvae was followed until eclosion. Adult flies were dried and weighed.

#### (h) *Determination of encapsulation ability*

Approx. two days after pupariation of parasitised larvae, puparia were collected from the test tubes and dissected under a stereo microscope. The absence or presence of developing flies, parasitoids and capsules were noted. Only those individuals were included in the analysis that either contained a live unencapsulated parasitoid larva (parasitised, unsuccessful defence) or a capsule (parasitised, successful defence/encapsulation). Very often

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

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parasitised flies contained a live parasitoid larva and rests of a former capsule, which the parasitoid had succeeded in breaking out of. These flies were assigned as being parasitised and not exhibiting successful defence/encapsulation. Unparasitised flies (neither containing parasitoid larva nor capsule) and superparasitised flies (inhabiting more than one parasitoid larva, a parasitoid larva and a capsule or more than one capsule) were excluded from the analysis.

### (i) Statistical analysis

Proportional data (survival and encapsulation ability) and data on development time and dry weight (response variables) were tested using generalised linear models (GLMs) (family: binomial or quasibinomial, function: logit for proportional data; family: poisson or quasipoisson, function: identity for continuous data, respectively), with yeast species or parasitisation status (yes/no) as linear predictor.

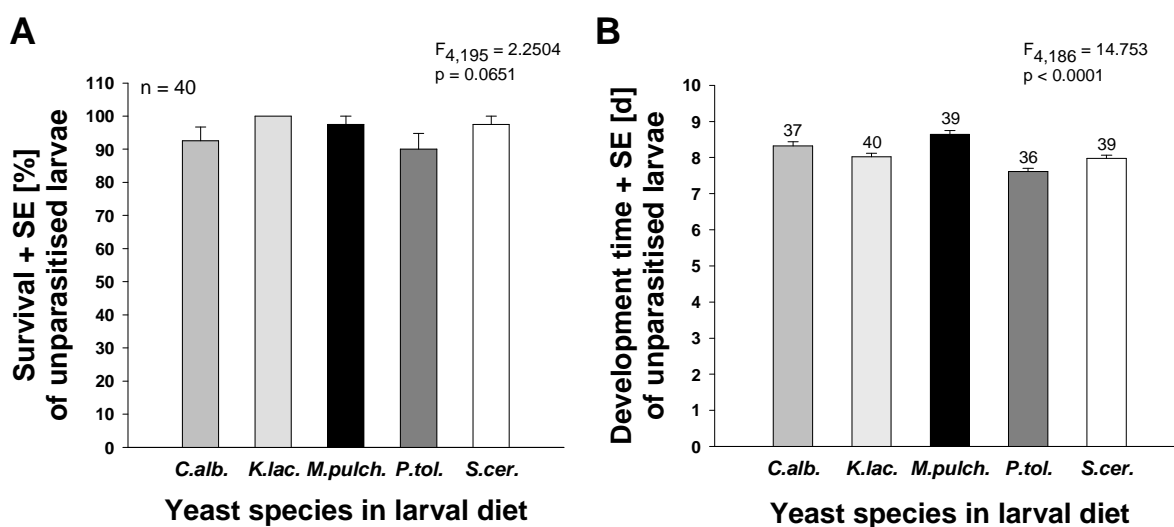
### 5.2.3 Results

(a) *Unparasitised larvae*: In unparasitised larvae, the different microbial species in the larval diet did not significantly influence *survival until eclosion* ( $F_{4,195} = 2.2504$ ,  $p = 0.0651$ ). Survival was rather high, irregardless which yeast species larvae had fed on. Those fed on *Kluyveromyces lactis* exhibited the highest survival ( $100.0 \pm 0.00\%$ ), followed by *Metschnikowia pulcherrima* ( $97.5 \pm 2.50\%$ ) and *Saccharomyces cerevisiae* ( $97.5 \pm 2.50\%$ ), and finally by those fed on *Cryptococcus albidus* ( $92.5 \pm 4.22\%$ ) and *Pichia toletana* ( $90.0 \pm 4.80\%$ ) (**Fig. 19A**).

There was a significant effect of the dietary yeast species on *development time* of unparasitised larvae ( $F_{4,186} = 14.753$ ,  $p < 0.0001$ ). Larvae fed on *Pichia toletana* eclosed earliest ( $7.6 \pm 0.1d$ ), followed by those fed on *Saccharomyces cerevisiae* ( $8.0 \pm 0.1d$ ), *Kluyveromyces lactis* ( $8.0 \pm 0.1d$ ), *Cryptococcus albidus* ( $8.3 \pm 0.1d$ ), and finally *Metschnikowia pulcherrima* ( $8.6 \pm 0.1d$ ) (**Fig. 19B**).



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**Fig. 19:** A) *Survival* of unparasitised *Drosophila melanogaster* larvae (in %) until eclosion and B) *Development time* of unparasitised *Drosophila melanogaster* larvae (in days), depending on the yeast species in the larval diet. Shown are mean values + standard error (SE). n and numbers above columns = number of replicates. Abbreviations of yeast species: **C.alb.** = *Cryptococcus albidus*, **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **P.tol.** = *Pichia toletana*, **S.cer.** = *Saccharomyces cerevisiae*.

The dietary yeast species had a significant effect on adult **dry weight** of female flies ( $F_{4,91} = 12.672$ ,  $p < 0.001$ ). Male dry weight was unaffected ( $F_{4,90} = 1.8204$ ,  $p < 0.1318$ ). Female larvae fed on *Saccharomyces cerevisiae* eclosed as the heaviest flies ( $0.4137 \pm 0.0137\text{mg}$ ), followed by those fed on *Kluyveromyces lactis* ( $0.3750 \pm 0.0085\text{mg}$ ), *Cryptococcus albidus* ( $0.3592 \pm 0.0091\text{mg}$ ), *Metschnikowia pulcherrima* ( $0.3285 \pm 0.0097\text{mg}$ ), and *Pichia toletana* ( $0.3252 \pm 0.0066\text{mg}$ ). In male adults those were heaviest, that had as larvae fed on *Kluyveromyces lactis* ( $0.2723 \pm 0.0051\text{mg}$ ), followed by those fed on *Cryptococcus albidus* ( $0.2690 \pm 0.0064\text{mg}$ ), *Saccharomyces cerevisiae* ( $0.2687 \pm 0.0053\text{mg}$ ), *Metschnikowia pulcherrima* ( $0.2558 \pm 0.0076\text{mg}$ ), and *Pichia toletana* ( $0.2539 \pm 0.0062\text{mg}$ ) (**Fig. 21A**).

The influence of dietary yeast species and sex (factors) as well as the interaction between the two factors on dry weight of adult *Drosophila melanogaster* (response variable) can be seen in **Table 3**. The results of the ANCOVA show a global significant influence of yeast species on adult dry weight. Adult dry weight differed significantly between female and male flies. Female flies were generally heavier than male flies. Indicated by the significant influence of the interaction between yeast and sex on adult dry weight, the influence of the dietary yeast species on female dry weight differed significantly from male dry weight. The relative difference in dry weight of female and male flies that had as larvae fed on

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

*Metschnikowia pulcherrima*, for example, was much smaller than of flies that had as larvae fed on *Saccharomyces cerevisiae* (**Fig. 21A**)

**Table 3:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence dry weight of adult *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Dry weight of adult <i>Drosophila melanogaster</i>	Yeast	$F_{4,186} = 24.5206$	$p < 0.0001$
	Sex	$F_{1,185} = 316.7778$	$p < 0.0001$
	Yeast*Sex	$F_{4,181} = 6.1852$	$p = 0.0001095$

**(b) Parasitised larvae:** No significant effect was found on the proportion of parasitised larvae *surviving until pupariation* ( $F_{4,419} = 1.7334$ ,  $p = 0.1416$ ). Those having fed on *Cryptococcus albidus* exhibited the highest survival ( $94.9 \pm 2.23\%$ ), followed by those having fed on *Pichia toletana* ( $88.6 \pm 3.40\%$ ), *Kluyveromyces lactis* ( $87.1429 \pm 4.03\%$ ), *Saccharomyces cerevisiae* ( $86.5 \pm 3.64\%$ ), and finally *Metschnikowia pulcherrima* ( $83.5 \pm 4.20\%$ ) (**Fig. 20**).

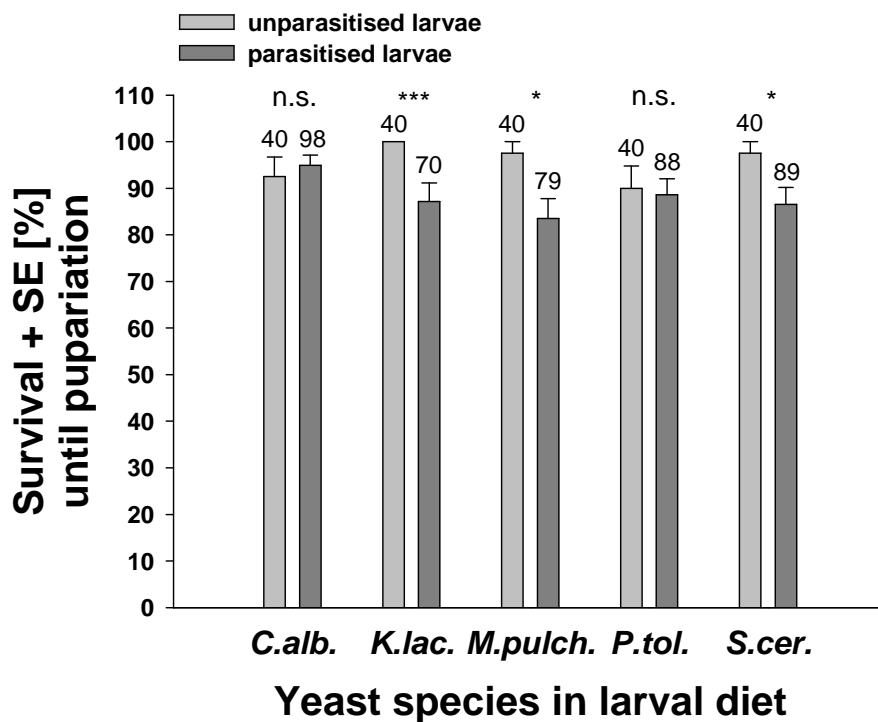
When comparing *survival until pupariation* of unparasitised as well as parasitised larvae, a significant difference was only found in larvae fed on *Kluyveromyces lactis*, *Metschnikowia pulcherrima*, and *Saccharomyces cerevisiae* (*C.alb.*:  $F_{1,136} = 0.2813$ ,  $p = 0.5967$ ; *K.lac.*:  $F_{1,108} = 13.251$ ,  $p = 0.0004198$ ; *M.pulch.*:  $F_{1,117} = 6.0996$ ,  $p = 0.0149629$ ; *P.tol.*:  $F_{1,126} = 0.0524$ ,  $p = 0.8193$ ; *S.cer.*:  $F_{1,127} = 4.4932$ ,  $p = 0.03598$ ). Survival until pupariation in unparasitised larvae was similar to survival until eclosion (see **Fig. 19A**), since all larvae that pupariated also eclosed and mortality only occurred prior to pupariation (**Fig. 20**).

The influence of dietary yeast species and parasitisation status (no, yes) (factors) as well as the interaction between the two factors on survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 4**. The results of the ANCOVA show that survival of unparasitised larvae differed significantly from survival of parasitised ones. Indicated by the significant influence of the interaction between yeast and parasitisation status on survival, the effect of the dietary yeast species on the survival differed between unparasitised and parasitised larvae. When larvae had fed on *Cryptococcus albidus* and *Pichia toletana*, for example, the relative difference in survival between unparasitised and parasitised larvae was smaller than in larvae that had fed on the other three yeast species (**Fig. 20**).

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

**Table 4:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

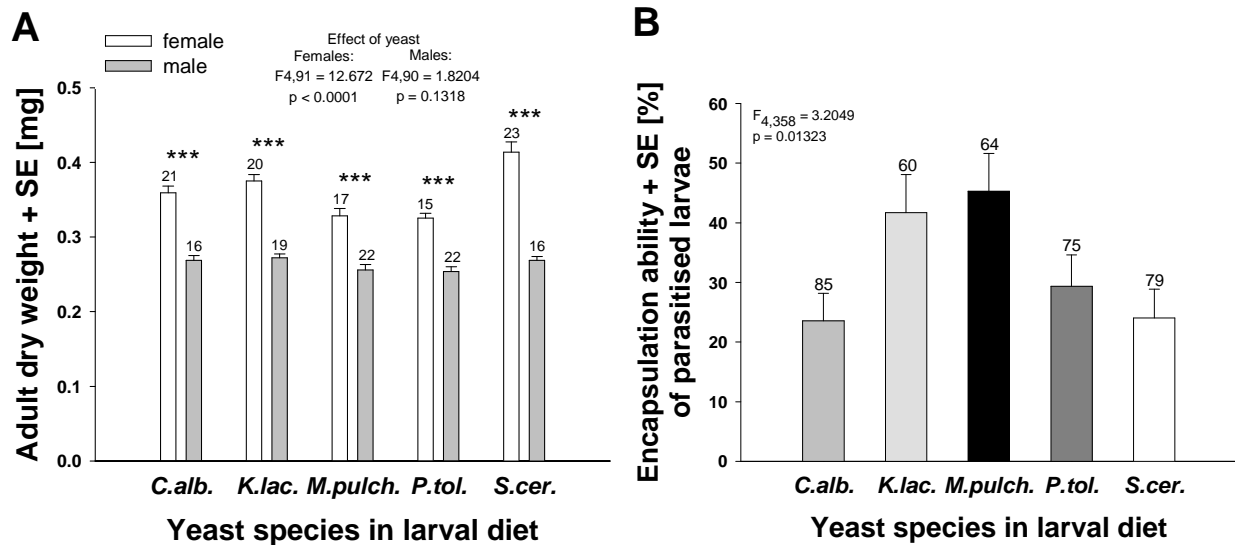
Measured fitness trait	Factors and interactions	F-value	p-value
Survival until pupariation of <i>Drosophila melanogaster</i>	Yeast	$F_{4,619} = 0.9813$	$p = 0.417059$
	Parasitisation	$F_{1,618} = 9.7616$	$p = 0.001866$
	Yeast*Parasitisation	$F_{4,614} = 2.7364$	$p = 0.028104$



**Fig. 20:** *Survival until pupariation* (in %) of unparasitised and parasitised *Drosophila melanogaster* larvae, depending on the yeast species in the larval diet. Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *C.alb.* = *Cryptococcus albidus*, *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

There was a significant effect ( $F_{4,358} = 3.2049$ ,  $p = 0.01323$ ) of the microbial diet fed to parasitised larvae shortly after parasitisation on their **encapsulation ability** against offspring of *Asobara tabida*. Larvae that had fed on *Metschnikowia pulcherrima* and *Kluyveromyces lactis* were most successful in encapsulating *Asobara tabida* eggs or larvae (*M.pulch.*:  $45.3 \pm 6.3\%$ ; *K.lac.*:  $41.7 \pm 6.4\%$ ). Those larvae having fed on *Pichia toletana* still exhibited a comparably high encapsulation ability ( $29.3 \pm 5.3\%$ ), followed by *Saccharomyces cerevisiae* ( $24.1 \pm 4.8\%$ ) and *Cryptococcus albidus* ( $23.5 \pm 4.6\%$ ) (**Fig. 21B**).

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*



**Fig. 21:** **A)** *Dry weight* (in mg) of adult *Drosophila melanogaster* emerging from unparasitised larvae and **B)** *Encapsulation ability* (in %) of parasitised *Drosophila melanogaster* larvae, depending on the yeast species in the larval diet. Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *C.alb.* = *Cryptococcus albidus*, *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: \*\*\*  $p < 0.001$ .

### 5.2.4 Discussion

The results suggest that specific dietary compounds may act as an influential factor explaining the observed variation in immune responses of parasitised hosts. The dietary yeast species chosen in this study were able to act as significant modulators of cellular innate immune defence in *Drosophila melanogaster* against its parasitoid *Asobara tabida*.

Yeasts make up a highly heterogeneous and complex metabolic group with basically identical metabolic pathways of the central carbon metabolism, but substantially differing in their mechanisms for nutrient uptake, the number of different isoenzymes, and the regulation of fermentation and respiration (RODRIGUES *et al.* 2006). Some yeast species also produce secondary metabolites which may have an antagonistic activity against filamentous fungi, other yeasts and bacteria (SIPICZKI 2006). The biochemical constitution of yeasts and their by-products, together with the yeast-substrate combinations, are supposed to affect the development and survival of *Drosophila* larvae (BEGON 1982). The nutritional qualities exhibited by a specific yeast species might hereby not necessarily be the same for unparasitised and parasitised larvae, since their nutritional needs may differ considerably. The nutritional requirements of unparasitised and parasitised *Drosophila melanogaster* larvae may, for example, differ especially in respect to micronutrients, and these requirements may only be met by specific yeast species, as e.g. *Kluyveromyces lactis*. According

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

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to FEKETE & KELLEMS (2007), there is increasing evidence that the concentration of trace elements required for healthy animals are often below what is required for animals experiencing an immunological challenge. Harmonious functioning of the immune system, especially the cell mediated immune response, is supposed to require the presence of several macro- and microelements. So has the lack of calcium, magnesium, iron, zinc, copper, iodine and selenium in experimental animals been associated with signs of immunodeficiency (RIVERA *et al.* 2003; FEKETE & KELLEMS 2007). Additionally, feeding and nutrient intake may differ markedly between unparasitised and parasitised larvae and depend on the ratio of digestible protein and carbohydrate, as could be shown in *Manduca sexta* parasitised by *Cotesia congregata* (THOMPSON *et al.* 2005). Parasitised larvae that were offered a choice of diets with variable nutrient content could even be shown to select a different ratio of nutrients from that preferred by unparasitised larvae (THOMPSON *et al.* 2001).

The results of this experiment indicate, that even though unparasitised larvae exhibited a high survival, a short and highly synchronous development time and a high adult dry weight (especially in females) when fed with *Saccharomyces cerevisiae*, the successful defence of parasitised larvae against *Asobara tabida* was significantly lower than e.g. with *Metschnikowia pulcherrima* or *Kluyveromyces lactis*. Yet, *Metschnikowia pulcherrima*, that obviously did not have a favourable impact on various fitness traits of unparasitised larvae (e.g. long development time, low dry weight in female flies), as already shown by DORSCH (2007) and confirmed by the results in this experiment, was able to enhance the encapsulation ability of parasitised larvae.

Larval mortality in parasitised larvae that had fed on *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* was enhanced compared to unparasitised larvae. Unfortunately, it was not possible to find out for all of these larvae, whether they had carried a capsule or not, nor whether they had actually been parasitised at all. Larvae usually died as 1<sup>st</sup> or early 2<sup>nd</sup> instar, and a post mortem dissection did only rarely reveal the presence of a parasitoid egg or larva or a capsule, probably due to a beginning disintegration of the larval carcass. Sometimes, larvae were even overgrown by the yeast species and could not be found again for further dissection. Due to using the observational method in order to gain parasitised larvae, these larvae had definitely been pricked by the parasitoid's ovipositor, which is by no means sterile, so that parasitised larvae may have additionally been challenged by the wound inflicted on them. A higher mortality of larvae parasitised by *Asobara tabida* may, however, rather have been caused by the venom injected into the host during oviposition. MOREAU *et al.* (2002) reported that artificially injected venoms led to higher mortality rates in *Drosophila melanogaster* larvae. Significant differences in survival until pupariation between parasitised and unparasitised larvae among the different yeast treatments suggests, that the dietary microorganisms may also modulate virulence factors (venom) of the parasitoid.

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

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Which mechanisms may lie behind the different influence of dietary yeast species on the encapsulation ability of *Drosophila melanogaster*? ESLIN & PRÉVOST (1998) found a high correlation between the number of circulating haemocytes in a parasitised host and its ability to form a haemocytic capsule around the parasitoid egg. Regarding this, the different dietary yeast species may be thought to have influenced haemocyte proliferation and in doing so, have modulated the ability of *Drosophila melanogaster* to encapsulate *Asobara tabida* offspring. It could be shown in the farmed mollusc *Haliotis midae* that a probiotic-supplemented diet did not only improve growth rate and survival, but also increased the number and phagocytic activity of circulating haemocytes (MACEY & COYNE 2005). A higher haemocyte count could also be found after a probiotic treatment of *Pennaeus vannamei* juveniles (RODRÍGUEZ *et al.* 2007). This eventuality of a beneficial impact of dietary yeast species on the number of circulating haemocytes in parasitised *Drosophila melanogaster* larvae was tested in the experiment that will be presented in chapter 6.

Yet, besides the potential influence on haemocyte load, a whole range of other mechanisms are thinkable that might provide an explanation for the differences in encapsulation ability, induced by the different dietary yeast species. Even though haemocytes are reported to play the major role in the encapsulation process, also other features of the immune system of *Drosophila melanogaster* are involved, such as the Phenoloxidase cascade (CERENIUS *et al.* 2008), which may just as well have experienced a modulation by the larval dietary yeast species. It would also be possible, that some microbial species in the larval diet may stimulate components of the immune system in a way to make it more alert and ready for an actual challenge by a serious pathogen or parasite (FREITAK *et al.* 2007). So called probiotic microorganisms are reported to possess the ability of modulating the host's immune response, but also of interfering with the ability of another, potentially pathogenic microorganism, to colonise the host (REID *et al.* 2001). By using non viable dietary components (so called prebiotics) that fortify certain components of the intestinal flora, immune stimulation can also be achieved (GIBSON 1999). Larvae having fed on *Kluyveromyces lactis* and *Metschnikowia pulcherrima* exhibited an enhanced encapsulation ability compared to larvae that had fed on the other yeast species. At least *Kluyveromyces lactis* could be shown to possess potentially probiotic qualities, due to its ability to adhere to enterocyte-like Caco-2 cells (KUMURA *et al.* 2004). Whether and how this ability may be linked to a beneficial impact on the cellular immunity of *Drosophila melanogaster* is an open question.

Instead of influencing the host's immune defence and thus its resistance against the parasitoid, the different yeast species may also have interfered with the virulence factors of *Asobara tabida*, such as the capacity of the eggs to adhere to specific tissues of *Drosophila melanogaster*, a means by which an egg may escape a complete encapsulation by the host's haemocytes (PRÉVOST *et al.* 2005). Other virulence factors, such as the one found to reduce Phenoloxidase activity (MOREAU *et al.* 2000), may also have been impaired.

## 5.2 Influence of microbial species (*ad libitum* conditions) on fitness traits of *Drosophila melanogaster*

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Although it is not known, in which qualitative and quantitative composition yeast species actually occur on potential breeding substrates of *Drosophila melanogaster*, particularly in Northern Germany, and whether the insects do find themselves in the position to choose eating particular yeast species - even though this has been observed in other studies (FOGLEMAN *et al.* 1981) - the results of this experiment may add a small explanatory piece to the puzzle of observed variation in immune defences.

## 5.3 Influence of microbial species on fitness traits of *Drosophila melanogaster* after delayed addition of microorganisms

### 5.3.1 Introduction

This experiment was performed to find out, whether a certain period of N-starvation (no yeast on a basic substrate of banana), after parasitisation of 1<sup>st</sup> instar *Drosophila melanogaster* larvae, may have an impact on the encapsulation ability of these larvae. The experiment was actually carried out as a complement to the larval food choice experiment dealt with in chapter 7. Since it was not known, at which point in time exactly parasitism would show an effect on the physiology and the behaviour of 1<sup>st</sup> instar *Drosophila melanogaster* larvae parasitised by *Asobara tabida*, I decided to carry out food choice experiments at three different time spans after parasitisation, at three, six and 24hrs. During these three different time spans, larvae should preferably not have any contact to the yeast species used in the food choice experiments, in order to avoid potential biases larvae might develop while feeding on them. Since there is no real alternative other than yeast as an N-source for larvae (they are not able to utilise peptone), I decided to completely abstain from adding any N-source to the basic substrate of banana. This, however, implied, that larvae would suffer from N-starvation during the three time spans. Yet, *Drosophila melanogaster* is only able to develop successfully in the presence of yeast. Additionally, N-starvation may have an effect on the encapsulation ability of *Drosophila melanogaster* larvae, presumably a negative one, since it is reported - at least for *Leptopilina boulardi* and *Leptopilina heterotoma* - that parasitoids may develop more successfully on starved hosts, primarily due to a reduced encapsulation ability in starved host larvae (WAJNBERG *et al.* 1985, BOULÉTREAU 1986; SIVA-JOTHY & THOMPSON 2002). This may, however, not necessarily be the case, since the parasitoid offspring are themselves dependent on their host's nutritional status and have to derive the resources for their own development from the host. With regard to the potentially beneficial impact of particularly *Kluyveromyces lactis* on encapsulation ability of *Drosophila melanogaster* larvae (see chapter 5.2), it was desirable to find out, whether a food choice would still make sense for the parasitised larvae after

### ***5.3 Influence of microbial species on fitness traits of *Drosophila melanogaster* after delayed addition of microorganisms***

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having been N-starved for a certain time period after parasitisation. As second yeast species, *Saccharomyces cerevisiae* was chosen, which did not turn out to yield a comparably high encapsulation ability as *Kluyveromyces lactis*, but which had a comparably beneficial or even more favourable influence on other fitness traits of *Drosophila melanogaster* larvae, such as survival and development time.

Besides its reference to the larval food choice in chapter 7, the experiment described here, may also allow an implication to conditions in the wild. Although female *Drosophila melanogaster* flies usually prefer to oviposit on substrates already colonised by microorganisms (BARKER *et al.* 1994; MORAIS *et al.* 1994) and are also supposed to actively inoculate their breeding substrates during oviposition (RÖHLFS & HOFFMEISTER 2005), N-starvation of larvae may also occur in their natural habitat. The yeast source may e.g. be rapidly depleted at high larval densities, or filamentous fungi may disturb the growth of yeasts (FLEET 2003). This alternative reference will also be discussed.

#### **5.3.2 Material and Methods**

##### **(a) *Experimental setup***

The bifactorial experiment comprised two yeast species (*Kluyveromyces lactis* and *Saccharomyces cerevisiae*) as first factor, and the time spans (three, six and 24 hours) after parasitisation of parasitised larvae, after which the yeast suspensions were added to the basic banana substrate, as second factor, adding up to six treatments. Only parasitised larvae were exposed to the different treatments.

##### **(b) *Fly and parasitoid cultures***

See chapter 4.1.

##### **(c) *Preparation of media:***

See chapter 4.3.

##### **(d) *Preparation of larvae***

See chapter 4.4 and 4.5.

##### **(e) *Gaining of parasitised larvae***

For this experiment the observational method described in chapter 4.6.1 was used. One parasitoid each was allowed to parasitise approx. 12 larvae, of which two larvae each were transferred into each of the six treatments, directly after the parasitoid had finished the 12 parasitisations. The starting point from which the three different time spans after parasitisation were calculated, was that point in time, when each parasitoid had finished parasitising six of the twelve larvae.



### 5.3 Influence of microbial species on fitness traits of *Drosophila melanogaster* after delayed addition of microorganisms

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#### (f) Preparation of microorganisms

For this experiment *ad libitum* food conditions were prepared with 50% yeast suspensions (see chapter 4.2).

#### (g) Experimental proceeding

One larva each was transferred into the tubes with a fine brush and the tubes were covered with dental rolls (HARTMANN). The tubes were incubated in a climate chamber at 25°C and a 16:8 L/D cycle. After the time spans (three, six and 24 hours) after parasitisation had elapsed 50µl of the 50% yeast suspensions were pipetted into each tube and the tubes were again incubated.

#### (h) Determination of encapsulation ability

See chapter 5.2.1 (h).

#### (i) Statistical analysis

Data on survival and on encapsulation ability of parasitised larvae (response variables) was tested using GLMs (family: quasibinomial, function: logit) with yeast species and time span as linear predictor (and covariate, respectively).

### 5.3.3 Results

Neither the dietary yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae* nor the time span of three, six and 24 hours after parasitisation, that the yeast suspensions had been added, had a significant effect on the *survival* of parasitised larvae until pupariation (Yeast effect: 3hrs:  $F_{1,37} = 1.2071$ ,  $p = 0.279$ ; 6hrs:  $F_{1,47} = 0.0526$ ,  $p = 0.8196$ ; 24hrs:  $F_{1,46} = 0.7426$ ,  $p = 0.3933$ ; effect of time: *K.lac.*:  $F_{2,69} = 0.6359$ ,  $p = 0.5325$ ; *S.cer.*:  $F_{2,61} = 0.0191$ ,  $p = 0.981$ ) (**Fig. 22A**). The survival of larvae was rather high at all three time spans after parasitisation and irregardless of whether they had fed on *Kluyveromyces lactis* or *Saccharomyces cerevisiae*, though survival of larvae fed on *Kluyveromyces lactis* was slightly (not significantly) increased (*K.lac.*: 3hrs:  $96.0 \pm 4.0\%$ , 6hrs:  $87.0 \pm 7.2\%$ , 24hrs:  $91.7 \pm 5.8\%$ ; *S.cer.*: 3hrs:  $85.7 \pm 9.7\%$ , 6hrs:  $84.6 \pm 7.2\%$ , 24hrs:  $83.3 \pm 7.8\%$ ).

The influence of dietary yeast species and time span (3hrs, 6hrs, 24hrs) (factors) as well as the interaction between the two factors on survival until pupariation of parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 5**. The results of the ANCOVA show that none of the factors had a significant influence on survival.

### 5.3 Influence of microbial species on fitness traits of *Drosophila melanogaster* after delayed addition of microorganisms

**Table 5:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until pupariation of parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until pupariation of <i>Drosophila melanogaster</i>	Yeast	$F_{1,134} = 1.6908$	$p = 0.1958$
	Time span	$F_{1,133} = 0.0552$	$p = 0.8146$
	Yeast*Time span	$F_{1,132} = 7.753 \times 10^{-6}$	$p = 0.9978$

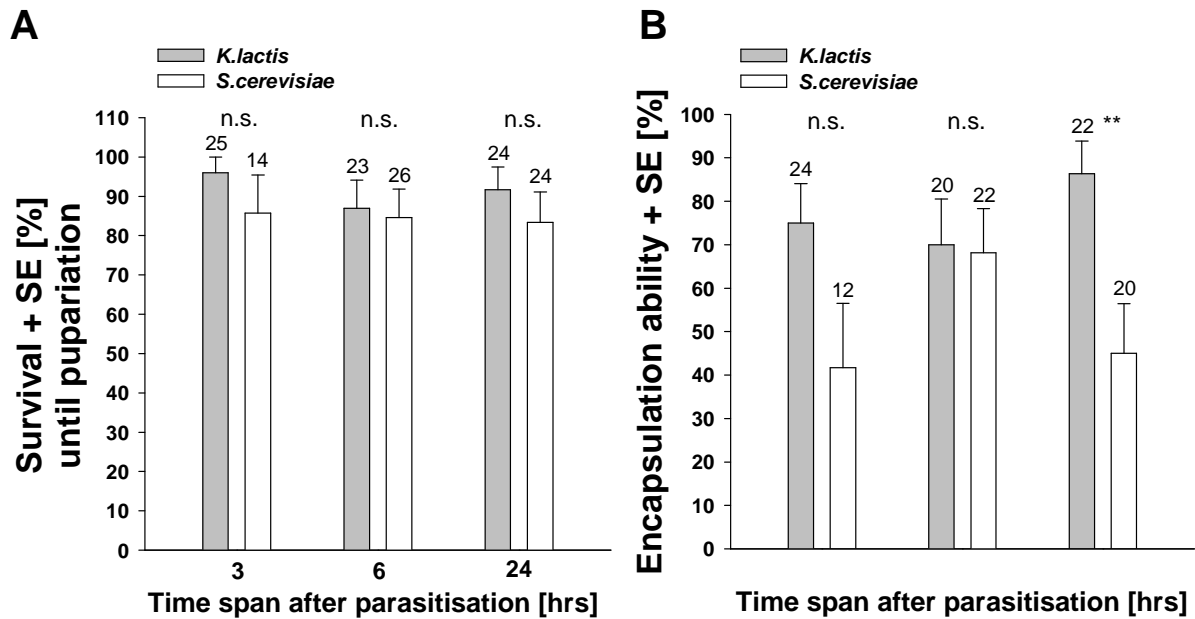
**Encapsulation ability** of larvae fed with *Kluyveromyces lactis* as well as of larvae fed with *Saccharomyces cerevisiae* was unaffected by the time span (effect of time: *K.lac.*:  $F_{2,63} = 0.8565$ ,  $p = 0.4295$ ; *S.cer.*:  $F_{2,51} = 1.5184$ ,  $p = 0.2288$ ). There was, however, a significant effect of the yeast species on encapsulation ability of larvae, though merely at the time span of 24hrs after parasitisation (Yeast effect: 3hrs:  $F_{1,34} = 3.5881$ ,  $p = 0.06673$ ; 6hrs:  $F_{1,40} = 0.0154$ ,  $p = 0.9017$ ; 24hrs:  $F_{1,40} = 8.0154$ ,  $p = 0.007224$ ) (**Fig. 22B**). At all three time spans the yeast suspension had been added after parasitisation, larvae fed on *Kluyveromyces lactis* were very successful in encapsulating *Asobara tabida* offspring (3hrs:  $75.0 \pm 9.0\%$ , 6hrs:  $70.0 \pm 10.5\%$ , 24hrs:  $86.4 \pm 7.5\%$ ). Larvae that had fed on *Saccharomyces cerevisiae* were less successful, though encapsulation ability was still rather high, and surprisingly also higher at 6hrs after parasitisation ( $68.2 \pm 10.2\%$ ) than at 3hrs ( $41.7 \pm 14.9\%$ ) or 24hrs ( $45.0 \pm 11.4\%$ ) after parasitisation, that the yeast suspension had been added.

The influence of dietary yeast species and time span (3hrs, 6hrs, 24hrs) (factors) as well as the interaction between the two factors on the encapsulation ability of parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 6**. The results of the ANCOVA show that the dietary yeast species had a significant influence on encapsulation ability.

**Table 6:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the encapsulation ability of parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Encapsulation ability of <i>Drosophila melanogaster</i>	Yeast	$F_{1,118} = 7.2120$	$p = 0.008304$
	Time span	$F_{1,117} = 0.0507$	$p = 0.822223$
	Yeast*Time span	$F_{1,116} = 1.9956$	$p = 0.160430$

### 5.3 Influence of microbial species on fitness traits of *Drosophila melanogaster* after delayed addition of microorganisms



**Fig. 22:** **A**) *Survival* (in %) of parasitised larvae until pupariation and **B**) *Encapsulation ability* (in %) of parasitised larvae depending on the dietary yeast species (*Kluyveromyces lactis* & *Saccharomyces cerevisiae*) and the time span after parasitisation (3hrs, 6hrs & 24hrs) after which the yeast suspensions had been added. Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lactis* = *Kluyveromyces lactis*, *S.cerevisiae* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*\*  $p < 0.01$ .

#### 5.3.4 Discussion

The results of this experiment suggest that it would still make sense for parasitised larvae to choose a particular yeast species rather than the other, assuming that the different yeast species may have a differing impact on the immune defence mechanism of the parasitised larvae, and that larvae behave according to their own nutritional needs (and not that of the parasitoid within them). Thus, it was still appropriate to carry out the food choice experiments described in chapter 7 after the chosen three time spans after parasitisation.

The beneficial impact of *Kluyveromyces lactis* compared to *Saccharomyces cerevisiae* on the encapsulation ability of parasitised larvae, as indicated by the results in chapter 5.2, was only partly confirmed by the results achieved in this experiment. Especially the high encapsulation ability of larvae having fed on *Saccharomyces cerevisiae* after the time span of six hours is rather surprising. This as well as the only partly significant effect on encapsulation ability between larvae having fed on *Kluyveromyces lactis* and on *Saccharomyces cerevisiae* may be due to the lower number of replicates in this experiment compared to that described in chapter 5.2. Studies using the isofemale technique have shown that there is a substantial within-population variation in encapsulation ability against parasitoids,

### 5.3 Influence of microbial species on fitness traits of *Drosophila melanogaster* after delayed addition of microorganisms

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*Asobara tabida* as well as *Leptopilina boulardi*, often due to a single gene locus (VASS *et al.* 1993; CARTON & NAPPI 1997; ORR & IRVING 1997; BENASSI *et al.* 1998; KRAAIJEVELD *et al.* 1998; HITA *et al.* 1999; FELLOWES & GODFRAY 2000). It has also been shown, that there is a difference in encapsulation ability of *Drosophila melanogaster* larvae parasitised by *Asobara tabida* between female and male larvae, with female larvae being able to defend themselves more strongly against *Asobara tabida* than males (KRAAIJEVELD *et al.* 2008). Thus, depending on how many female or male larvae were exposed to a particular treatment in the experiment, the within-treatment variation may have been as high as to level out potential differences between the treatments. Unfortunately, I did not determine the sex of the particular larvae so as to be able to check for potential differences in encapsulation ability between the sexes. As will be described in chapter 7, there also seems to be a high inter-individual variability in the influence of the specific yeast species on the encapsulation ability (and other fitness traits) of *Drosophila melanogaster* larvae. Thus, potential sex-determined differences in encapsulation ability as well as a high within-population variation and inter-individual variation in encapsulation ability, may be an explanation for the differing results of this experiment and that described in chapter 5.2.

Additionally, one may have to consider the fact that the 'diet' fed to the larvae in the experiments is not only made up of dead organic matter (such as the basic banana substrate), but above all of live microorganisms. Since microorganisms themselves may show a high intraspecific variation (RODRIGUEZ-VALERA 2002), these living dietary yeast cell populations themselves may distinctly vary in their physiology and thus their nutritional quality for *Drosophila melanogaster* larvae, so that their influence on the encapsulation ability of parasitised larvae (or other fitness traits) may not always be identical, even though the particular yeast species stemmed from the same glycerine stock.

The fact that encapsulation ability was hardly affected by the different periods of N-starvation (except of the weird outlier at 6 hrs after parasitisation with *Saccharomyces cerevisiae* as diet), is also rather surprising, since immune functions are reported to be hampered by a lack of food or down-regulated during nutritional deprivation (CALDER & JACKSON 2000; MORET & SCHMID-HEMPEL 2000; SIVA-JOTHY & THOMPSON 2002). On the other hand, organisms may rapidly compensate for the energy losses they suffered from during food deprivation, once they again get access to food, and up-regulate their immune effector system to pre-deprivation levels (SIVA-JOTHY & THOMPSON 2002). Yet, this ability depends on the length of time organisms have to suffer from food deprivation, with host resistance being less profoundly reduced when food is deprived immediately after infection, compared to e.g. a food deprivation after more than 24hrs post infection (OARADA *et al.* 2002). According to this, the chosen time spans of three, six and 24hrs after parasitisation without a nitrogen source, may have still been within the frame of time, at which parasitised *Drosophila melanogaster* larvae were yet able to compensate for nutritional losses.

### ***5.3 Influence of microbial species on fitness traits of *Drosophila melanogaster* after delayed addition of microorganisms***

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In their natural habitat, larvae may also be able to bridge a certain time of food deprivation after having been parasitised, without suffering from an impairment of their ability to encapsulate the parasitoid egg or larva.

The period of food deprivation may not only concern the parasitised larva, but may also bear on the parasitoid offspring within the host's haemocoel. Hosts in poor nutritional conditions may also provide fewer resources for the parasitoid, leading to reduced growth and survival, and the effects of host nutrition may even outweigh those of the host's immune defence (SEPPÄLÄ *et al.* 2008). Since the host's food deprivation was only temporary, this may merely have led to a temporary interruption of the parasitoid's development (physiological arrest). This, however, remains speculative, since in this experiment the development of parasitoids was not followed until maturity.

### **5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida***

#### **5.4.1 Introduction**

Food availability and the presence of enemies are major characteristics that determine habitat quality (SCHMID-HEMPEL & SCHMID-HEMPEL 1998). Food availability usually varies over time (WHITE 2008), and organisms experience the occurrence of resource pulses, i.e. episodes of increased availability that combine low frequency (rarity), large magnitude (intensity), and short duration (YANG *et al.* 2008). This is also the case for *Drosophila melanogaster* and other drosophilid species, developing on ephemeral, often patchily distributed resources, mostly in the form of fallen and decaying fruits. Even if breeding site density is very high, females of many *Drosophila* species significantly aggregate their eggs (HOFFMEISTER & ROHLFS 2001), not necessarily due to resource limitation, but Allee-effects that can be more important than competition at lower larval densities (ROHLFS & HOFFMEISTER 2003, 2004). Drosophilid female flies have been shown to transfer yeasts to their oviposition sites, presumably in order to positively affect offspring development, whereby the beneficial effect may depend on the nutritional and reproductive status of the female (ROHLFS & HOFFMEISTER 2005). Depending on these maternal effects, but also on climatic, and especially microclimatic conditions, as well as microbial growth conditions, *Drosophila melanogaster* larvae may experience substantial differences in food availability, both as food quality (which microbial species are present on the ephemeral fruit resource) and food quantity.

The nutritional status of host animals is reported to have an important impact on the dynamics of host-parasite interactions. Yet, too little is known to predict general patterns in the relationship between host nutrition and parasite success (LOGAN *et al.* 2005). The

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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host's nutritional status may either have a negative or a positive effect on parasite development, or none at all. Nutrition may influence the development and consequences of parasitism by (i) increasing the ability of the host to cope with the adverse consequences of parasitism (metabolic disturbances and pathophysiology), (ii) by improving the ability of the host to overcome parasitism (to mount an effective (immune) response against parasite establishment and/or development and to induce parasite rejection) (COOP & KYRIAZAKIS 2001), (iii) by malnutrition of the parasite (LOGAN *et al.* 2005), (iv) or even by direct negative (intake of antiparasitic compounds) or positive influences of the host's food on parasite development (e.g. fats that appear to have immunosuppressive properties) (CHANDRA 1993; COOP & KYRIAZAKIS 2001).

Life history theory suggests that an organism must allocate limited resources to a range of tasks - including immune defence - each contributing to its fitness, that each task is costly in terms of needed resources, and that tasks may be traded off among each other (SCHMID-HEMPEL 2005). HOUSTON *et al.* (2007) showed in an analytical model that it is optimal to increase the allocation to immune defence as reserves increase. When food changes over time, the optimal response depends on the frequency of changes. As the rate of environmental change increases, immune defence becomes less dependent on food availability (HOUSTON *et al.* 2007). Consuming part of a host's resources is one cause of a parasite's virulence, i.e. part of the fitness cost imposed on the host by the parasite, which can affect the host simply by reducing the resources available for growth or maintenance (BEDHOMME *et al.* 2004). With regard to immune defence being energetically costly, parasites are often assumed to perform better in hosts in poor nutritional condition, due to a reduced immune function of the host (SEPPÄLÄ *et al.* 2008). Immunosuppression may hereby be a result of either resource allocation to other metabolic needs, and/or avoidance of immunopathology caused by food stress via neuroendocrine mechanisms (RÅBERG *et al.* 1998; KRASNOV *et al.* 2003). In several studies on specific host-parasite interactions, food shortage in the host's environment, and thus a poor nutritional host condition, has been shown to increase host susceptibility to (parasitic) infections and/or reduce the host's ability to resist parasite within-host growth, development and reproduction (WAINBERG *et al.* 1985, 1990; FEDER *et al.* 1997; SIVA-JOTHY & THOMPSON 2002; KRASNOV *et al.* 2005). Parasites may, on the other hand, perform worse in hosts in poor nutritional condition, since these hosts provide fewer resources and may exhibit an increased mortality (leading to increased mortality of the parasite) due to pathophysiological effects of parasitism. Accordingly, several other studies showed that hosts in poor nutritional condition were less suitable for parasite development than hosts in good nutritional condition (JOKELA *et al.* 1999; BROWN *et al.* 2000; BEDHOMME *et al.* 2004; KRIST *et al.* 2004; PULKKINEN & EBERT 2004; SEPPÄLÄ *et al.* 2008). From the evolutionary perspective, the strategy of either para-

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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sitising energy deprived or energy rich hosts would depend on relative fitness awards from exploiting these hosts (KRASNOV *et al.* 2005).

It is often assumed that (endo) parasites inhabit predictable, homeostatically maintained internal environments within their hosts. Yet, parasite environments may also contain many levels of heterogeneity, namely the host itself (the parasite's immediate environment), that can be sensitive or resistant, well or poorly fed, etc., as well as the host's habitat, which can be rich or poor in resources, in predators or parasitic species (THOMAS *et al.* 2002). Parasites have been shown to be capable of recognising the physiological and biochemical condition of their host that are of selective importance, and also perceive (by direct or indirect cues) the external environment of their host and coordinate their adaptive responses accordingly (THOMAS *et al.* 2002).

Parasites, and especially koinobiont endoparasites, need to keep their host sufficiently viable, so that it can effectively feed, avoid predation etc., while the parasite matures. This presumably requires parasites to minimise or limit their impact on host physiology, behaviour and energy budgets. Yet effects on host physiology and metabolism are clearly evident during parasitism (ALLEYNE *et al.* 1997). Many insect parasitoids actually induce dramatic alterations in host physiology and behaviour before killing their hosts, and successful infection is often characterised by profound reprogramming of the host's endocrine and nervous system, thus, ensuring parasitoid survival, growth, and development (COLE *et al.* 2002 and references therein). The parasite may adjust its host exploitation rate to match changes in the host's environment (as e.g. food availability), but environmental variation may alternatively also lead to non adaptive levels of host exploitation (JOKELA *et al.* 1999).

In *Drosophila melanogaster* only few studies have investigated the influence of the host's nutritional status on developmental success of its parasitoids. This was mainly done by either decreasing the amount of yeast (baker's yeast) offered to host larvae, or by increasing larval density, with the intention to decrease food supply per larva (exploitation competition). The availability of nutritional resources both reduced the host's encapsulation ability, and also reduced fitness traits of the parasitoid. Starved hosts parasitised by *Leptopilina boulardi* were unable to encapsulate eggs of the parasitoid (WAJNBERG *et al.* 1985; VASS & NAPPI 1998b). Crowded (and thus poorly fed) *Drosophila melanogaster* hosts turned out to be more suitable to *Leptopilina boulardi* than well fed hosts (WAJNBERG *et al.* 1985, 1990), due to the reduction in encapsulation ability. *Leptopilina heterotoma* was also found to survive better in starved hosts (BOULÉTREAU 1986), but compared to *Leptopilina boulardi* the development of *Leptopilina heterotoma* was better in well fed hosts (BOULÉTREAU & WAJNBERG 1986). Adult *Leptopilina boulardi* wasps emerging from poorly nourished hosts, however, were smaller in size (WAJNBERG *et al.* 1990).

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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As could already be shown in chapter 5.2, besides nutritional quantity, also nutritional quality (in the form of different yeast species) may have a substantial influence on the encapsulation ability of *Drosophila melanogaster*. With regard to the fact that the host's nutritional status may not only influence host-parasite interaction via the host and its immunocompetence, but also life history traits of the parasite, the aim of this experiment was to try and answer the following questions:

- (i) Does nutritional quality (different dietary yeast species) as well as nutritional quantity (different initial yeast cell masses) influence preadult developmental success and adult fitness traits of unparasitised and parasitised *Drosophila melanogaster*?
- (ii) Does nutritional quality (different dietary yeast species) as well as nutritional quantity (different initial yeast cell masses) influence developmental success and adult fitness traits of *Asobara tabida*?

### 5.4.2 Material and Methods

#### (a) Experimental setup

The bifactorial experiment comprised four yeast species (*Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae*) as first factor (treatment) and two different initial cell masses ( $10^{-10}$ g and  $10^{-8}$ g) of each of the yeast species as second factor (treatment), adding up to overall eight different treatments. Parasitised as well as unparasitised larvae were exposed to the different treatments. According to DORSCH (2007), all four yeast species yielded a comparably good larval development, even when only very low initial yeast cell masses had been added to the banana substrate. I decided to choose the lowest initial yeast cell mass tested by DORSCH (2007) ( $10^{-10}$ g), roughly posing limited conditions for *Drosophila melanogaster* larvae, as well as a cell mass posing intermediate conditions ( $10^{-8}$ g).

#### (b) Fly and parasitoid cultures

See chapter 4.1.

#### (c) Preparation of media:

See chapter 4.3.

#### (d) Preparation of larvae

See chapter 4.4 and 4.5.

#### (e) Gaining of parasitised larvae

For this experiment the observational method described in chapter 4.6.1 was used. One parasitoid each was allowed to parasitise approx. 16 larvae, of which two larvae each were



## **5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida***

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transferred into each of the eight yeast treatments, directly after the parasitoid had finished the 16 parasitisations.

### **(f) Preparation of microorganisms**

For this experiment limited food conditions were prepared with  $10^{-10}$ g and  $10^{-8}$ g initial yeast cell mass per experimental tube and thus larva. These two different cell masses were chosen after DORSCH (2007), who had tested fitness traits of unparasitised larvae for initial yeast cell masses ranging from  $10^{-10}$ g till  $10^{-2}$ g.

### **(g) Experimental proceeding**

50 $\mu$ l each of the yeast suspensions containing a yeast cell mass of  $10^{-10}$ g and  $10^{-8}$ g respectively, were pipetted into the prepared test tubes filled with Banana medium II. Hereupon one larva each was transferred into the tubes with a fine brush and the tubes covered with dental rolls (HARTMANN). Test tubes containing one parasitised larva each as well as tubes with one unparasitised larva each (control tubes) were prepared. The tubes were incubated in a climate chamber at 25°C and a 16:8 L/D cycle. The development of unparasitised as well as parasitised larvae was followed until eclosion. Adult flies emerging from unparasitised larvae were dried and weighed. Adult flies emerging from parasitised larvae were dissected to look for the presence of a capsule. Female eclosed *Asobara tabida* adults were dissected to determine egg load, were dried and weighed, and the fat content was measured.

### **(h) Determination of encapsulation ability**

After eclosion of adult flies emerging from parasitised larvae, the adults were killed in the freezer at a temperature of -20°C and stored there until dissection. When adults were dissected, the absence or presence of capsules was noted. Only those individuals were included in the analysis that contained a capsule (parasitised, successful defence/encapsulation). Unparasitised flies (not containing a capsule) and superparasitised flies (inhabiting more than one capsule) were excluded from the analysis.

### **(i) Determination of egg load and fat content in *Asobara tabida***

See chapter 4.8.4.

### **(j) Statistical analysis**

See chapter 4.9. The analysis was carried out with yeast species, initial yeast cell mass as well as parasitisation status (no, yes) and sex (female, male) as linear predictor.

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

### 5.4.3 Results

(a) *Unparasitised Drosophila melanogaster larvae*: The dietary yeast species had a significant effect on the *survival until eclosion*, at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,76} = 7.6463$ ,  $p < 0.001$ ;  $10^{-8}$ g:  $F_{3,76} = 19.046$ ,  $p < 0.0001$ ). Larvae that had fed on *Kluyveromyces lactis* survived best at both initial yeast cell masses ( $10^{-10}$ g:  $100.0 \pm 0.0\%$ ,  $10^{-8}$ g:  $95.0 \pm 5.0\%$ ). The survival of larvae fed on *Saccharomyces cerevisiae* was also comparably high, though they experienced a significant decrease at the lower initial yeast cell mass ( $10^{-10}$ g:  $80.0 \pm 9.2\%$ ,  $10^{-8}$ g:  $100.0 \pm 0.0\%$ ). When fed on *Pichia toletana*, larvae survived just as well at both initial yeast cell masses ( $10^{-10}$ g:  $85.0 \pm 8.2\%$ ,  $10^{-8}$ g:  $85.0 \pm 8.2\%$ ). Merely those larvae that had fed on *Metschnikowia pulcherrima* were not able to survive well, and surprisingly, survival at the higher initial yeast cell mass ( $10^{-8}$ g) was even lower than at the initial yeast cell mass of  $10^{-10}$ g ( $10^{-10}$ g:  $50.0 \pm 11.5\%$ ,  $10^{-8}$ g:  $20.0 \pm 9.2\%$ ) (**Fig. 23A**). The initial yeast cell mass did not have a significant effect on survival, when larvae had fed on *Kluyveromyces lactis* ( $F_{1,38} = 2.6827$ ,  $p = 0.1097$ ), *Metschnikowia pulcherrima* ( $F_{1,38} = 3.8511$ ,  $p = 0.05707$ ) and *Pichia toletana* ( $F_{1,38} = 0$ ,  $p = ;1.0$ ). Those larvae having fed on *Saccharomyces cerevisiae*, however, experienced a significant effect on their survival ( $F_{1,38} = 11.382$ ,  $p = 0.001718$ ). The survival until pupariation of unparasitised larvae only marginally differed from survival until eclosion, and is shown in comparison to survival until pupariation of parasitised larvae in **Fig. 25**.

The influence of dietary yeast species and initial yeast cell mass (factors) as well as the interaction between the two factors on survival until eclosion of *Drosophila melanogaster* larvae (response variable) can be seen in **Table 7**. The results of the ANCOVA show that the dietary yeast species had a global significant influence on survival. As indicated by the significant influence of the interaction between yeast and initial cell mass on survival until eclosion, the influence of the initial cell mass on survival differed significantly among the different dietary yeast species. The relative difference in survival of larvae that had fed on *Kluyveromyces lactis*, for example, was smaller than that of larvae that had fed on *Metschnikowia pulcherrima* (**Fig. 23A**).

**Table 7:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until eclosion of unparasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until eclosion of <i>Drosophila melanogaster</i>	Yeast	$F_{3,156} = 21.9888$	$p < 0.0001$
	Initial cell mass	$F_{1,155} = 0.6085$	$p = 0.436561$
	Yeast*Initial cell mass	$F_{3,152} = 4.6342$	$p = 0.003944$

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

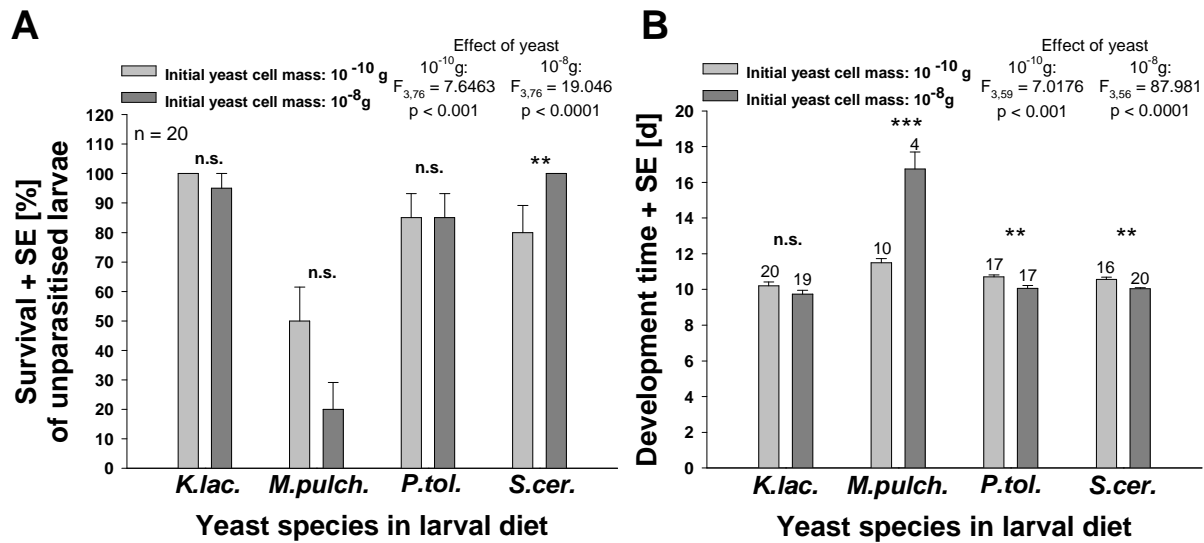
The dietary yeast species significantly affected *development time*, again at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,59} = 7.0176$ ,  $p < 0.001$ ;  $10^{-8}$ g:  $F_{3,56} = 87.981$ ,  $p < 0.0001$ ). Larvae that had fed on *Kluyveromyces lactis* showed a short development time that only slightly differed between the initial yeast cell masses ( $10^{-10}$ g:  $10.2 \pm 0.2$ d,  $10^{-8}$ g:  $9.7 \pm 0.2$ d). Those that had fed on *Saccharomyces cerevisiae* and *Pichia toletana*, developed comparably fast, though each developed faster at the higher yeast cell mass (*P.tol.*:  $10^{-10}$ g:  $10.7 \pm 0.1$ d,  $10^{-8}$ g:  $10.1 \pm 0.2$ d; *S.cer.*:  $10^{-10}$ g:  $10.6 \pm 0.1$ d,  $10^{-8}$ g:  $10.1 \pm 0.1$ d). Feeding on *Metschnikowia pulcherrima* caused the longest larval development time, and surprisingly, larvae took longer to reach adulthood at the higher of the two initial yeast cell masses ( $10^{-10}$ g:  $11.5 \pm 0.2$ d,  $10^{-8}$ g:  $16.8 \pm 0.9$ d) (**Fig. 23B**). The effect of the initial yeast cell mass on development time was significant, when larvae had fed on *Metschnikowia pulcherrima* ( $F_{1,12} = 66.948$ ,  $p < 0.0001$ ), *Pichia toletana* ( $F_{1,32} = 10.758$ ,  $p = 0.002509$ ) and *Saccharomyces cerevisiae* ( $F_{1,34} = 16.499$ ,  $p < 0.001$ ), but not when they had fed on *Kluyveromyces lactis* ( $F_{1,37} = 2.2207$ ,  $p = 0.1446$ ).

The influence of dietary yeast species and initial yeast cell mass (factors) as well as the interaction between the two factors on development time of unparasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 8**. The results of the ANCOVA show that the dietary yeast species had a global significant influence on development time. Additionally, the influence of the initial cell mass on development time differed significantly among the different dietary yeast species, as indicated by the significant influence of the interaction between yeast and initial cell mass on development time. The relative difference in development time of larvae that had fed on *Metschnikowia pulcherrima*, for example, was greater than that of larvae that had fed on the other three yeast species (**Fig. 23B**).

**Table 8:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence development time of unparasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Development time of <i>Drosophila melanogaster</i>	Yeast	$F_{3,119} = 57.3439$	$p < 0.0001$
	Initial cell mass	$F_{1,118} = 0.4842$	$p = 0.4879$
	Yeast*Initial cell mass	$F_{3,115} = 41.5478$	$p < 0.0001$

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 23:** A) *Survival* (in %) until eclosion and B) *Development time* (in days) of unparasitised *Drosophila melanogaster* larvae, depending on the dietary yeast species and the initial cell mass of each yeast species ( $10^{-10}$  g and  $10^{-8}$  g). Shown are mean values + standard error (SE). n = number of replicates. Numbers above columns in B) = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*\*  $p < 0.01$ , \*\*\*  $p < 0.0001$ .

The dietary yeast species also had a significant effect on *dry weight of female adult flies*, again at both initial yeast cell masses ( $10^{-10}$  g:  $F_{3,32} = 9.9034$ ,  $p < 0.0001$ ;  $10^{-8}$  g:  $F_{3,25} = 34.252$ ,  $p < 0.0001$ ; *M.pulch.* excluded:  $F_{2,24} = 8.0971$ ,  $p = 0.002054$ ). Female dry weight was highest for those adults that had emerged from larvae having fed on *Saccharomyces cerevisiae*, even at the lower of the two initial yeast cell masses ( $10^{-10}$  g:  $0.3858 \pm 0.0163$  mg,  $10^{-8}$  g:  $0.4120 \pm 0.0102$  mg). Females that as larvae had fed on *Pichia toletana* were nearly as heavy, whereby their weight hardly differed at all between the two initial yeast cell masses ( $10^{-10}$  g:  $0.3784 \pm 0.0146$  mg,  $10^{-8}$  g:  $0.3791 \pm 0.0138$  mg). Larvae that had fed on *Kluyveromyces lactis* still eclosed as comparably heavy adults, but surprisingly, their dry weight was lower at the higher of the two initial yeast cell masses ( $10^{-10}$  g:  $0.3739 \pm 0.0089$  mg,  $10^{-8}$  g:  $0.3400 \pm 0.0115$  mg). Females emerging from larvae that had fed on *Metschnikowia pulcherrima* were lightest and again showed the rather puzzling paradox of being far lighter at the initial yeast cell mass of  $10^{-8}$  g ( $10^{-10}$  g:  $0.2768 \pm 0.0122$  mg,  $10^{-8}$  g:  $0.1426 \pm 0.0134$  mg) (**Fig. 24A**). When larvae had fed on *Kluyveromyces lactis*, initial yeast cell mass had a significant effect on female dry weight (*K.lac.*:  $F_{1,13} = 5.5605$ ,  $p = 0.0347$ ). There was no effect on larvae that fed on *Pichia toletana* ( $F_{1,19} = 0.0011$ ,  $p = 0.9737$ ) and on *Saccharomyces cerevisiae* ( $F_{1,19} = 2.0511$ ,  $p = 0.1683$ ). No analysis was

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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carried out on data for larvae that had fed on *Metschnikowia pulcherrima*, since there were only two values at the higher yeast cell mass.

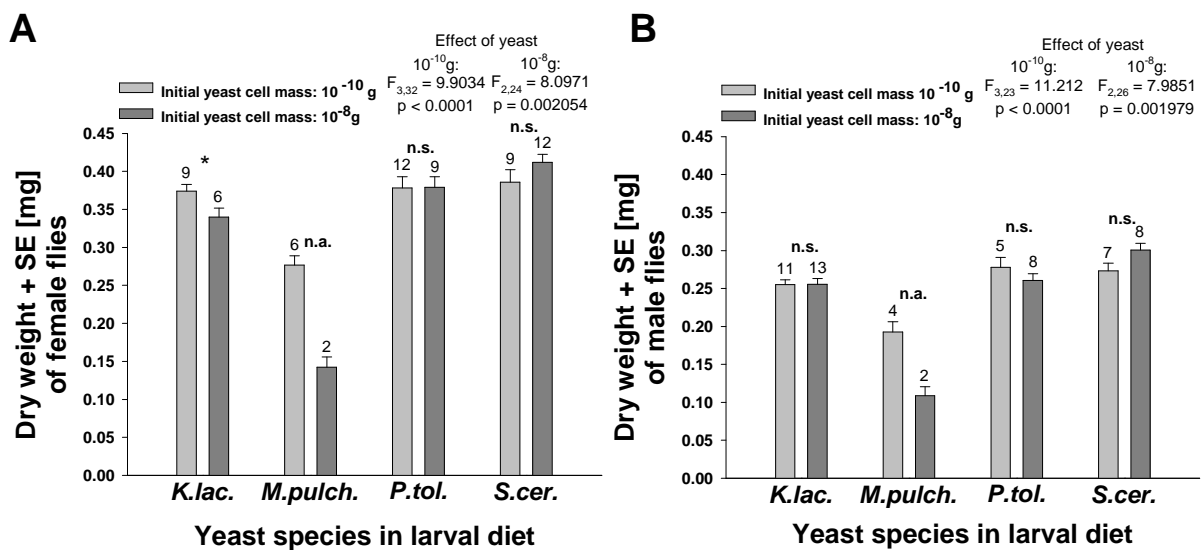
**Dry weight of male adult flies** was also significantly affected by the dietary yeast species, and again at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,23} = 11.212$ ,  $p < 0.0001$ ;  $10^{-8}$ g:  $F_{3,27} = 29.644$ ,  $p < 0.0001$ ; *M.pulch.* excluded:  $F_{2,26} = 7.9851$ ,  $p = 0.001979$ ). Male adults showed a somewhat similar pattern in their response in dry weight to the different yeast species as female adults. Males emerging from larvae that had fed on *Saccharomyces cerevisiae*, *Pichia toletana* and *Kluyveromyces lactis* were heaviest, and here, their dry weight did not differ significantly between the two initial yeast cell masses (*K.lac.*:  $10^{-10}$ g:  $0.2552 \pm 0.0063$ mg,  $10^{-8}$ g:  $0.2556 \pm 0.0073$ mg; *P.tol.*:  $10^{-10}$ g:  $0.2778 \pm 0.0130$ mg,  $10^{-8}$ g:  $0.2603 \pm 0.0091$ mg; *S.cer.*:  $10^{-10}$ g:  $0.2732 \pm 0.0100$ mg,  $10^{-8}$ g:  $0.3005 \pm 0.0091$ mg). Larvae that had fed on *Metschnikowia pulcherrima* emerged as adults with a very low dry weight, and again exhibited the phenomenon of being even lighter at the higher of the two initial yeast cell masses ( $10^{-10}$ g:  $0.1928 \pm 0.0131$ mg,  $10^{-8}$ g:  $0.1088 \pm 0.0116$ mg) (**Fig. 24B**). Initial yeast cell mass did not have a significant effect on dry weight of adults that as larvae had fed on *Kluyveromyces lactis* ( $F_{1,22} = 0.0022$ ,  $p = 0.9632$ ), *Pichia toletana* ( $F_{1,11} = 1.3038$ ,  $p = 0.2778$ ) and *Saccharomyces cerevisiae* ( $F_{1,13} = 4.0955$ ,  $p = 0.06405$ ). No analysis was carried out on data for larvae that had fed on *Metschnikowia pulcherrima*, since there were only two values at the higher yeast cell mass.

The influence of dietary yeast species, initial yeast cell mass and sex (factors), as well as the interaction between the three factors on adult dry weight of *Drosophila melanogaster* (response variable) can be seen in **Table 9**. The results of the ANCOVA show that the dietary yeast species, the initial yeast cell mass as well as sex had a global significant influence on adult dry weight. Additionally, the influence of the initial yeast cell mass on adult dry weight differed significantly among the dietary yeast species, as indicated by the significant influence of the interaction between yeast and initial cell mass. Both in males and females, the relative difference in adult dry weight between the two initial yeast cell masses was, for example, greater when larvae had fed on *Metschnikowia pulcherrima* than when they had fed on the other three yeast species (**Fig. 24**).

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

**Table 9:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence adult dry weight of *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Dry weight of adult <i>Drosophila melanogaster</i>	Yeast	$F_{3,119} = 70.3687$	$p < 0.0001$
	Initial cell mass	$F_{1,118} = 8.4634$	$p = 0.004408$
	Sex	$F_{1,117} = 286.5013$	$p < 0.0001$
	Yeast*Initial cell mass	$F_{3,114} = 11.5162$	$p < 0.0001$
	Yeast*Sex	$F_{3,111} = 1.4783$	$p = 0.224598$
	Initial cell mass*Sex	$F_{1,110} = 0.7994$	$p = 0.373273$
	Yeast*Initial cell mass*Sex	$F_{3,107} = 1.2292$	$p = 0.302785$



**Fig. 24:** Dry weight (in mg) of adult *Drosophila melanogaster* emerging from unparasitised larvae. **A)** Dry weight of female flies **B)** Dry weight of male flies, depending on the dietary yeast species and the initial cell mass of each yeast species ( $10^{-10}$  g and  $10^{-8}$  g) in the larval diet. Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*\*  $p < 0.01$ , \*\*\*  $p < 0.0001$ , n.a. = not analysed.

**(b) Parasitised *Drosophila melanogaster* larvae:** The dietary yeast species significantly affected *survival until pupariation*, both at the initial yeast cell mass of  $10^{-10}$  g and of  $10^{-8}$  g ( $10^{-10}$  g:  $F_{3,185} = 4.713$ ,  $p = 0.003405$ ;  $10^{-8}$  g:  $F_{3,176} = 26.603$ ,  $p < 0.0001$ ). Merely larvae fed on *Kluyveromyces lactis* and *Pichia toletana* exhibited a comparably high survival at both initial yeast cell masses (*K.lac.*:  $10^{-10}$  g:  $72.0 \pm 6.4\%$ ,  $10^{-8}$  g:  $82.6 \pm 5.7\%$ ; *P.tol.*:  $10^{-10}$  g:  $80.4 \pm 5.9\%$ ,  $10^{-8}$  g:  $78.0 \pm 6.5\%$ ). Of larvae having fed on *Saccharomyces cerevisiae*, only about 65% survived until pupariation ( $10^{-10}$  g:  $65.2 \pm 7.1\%$ ,  $10^{-8}$  g:  $65.9 \pm 7.2\%$ ). Only few larvae that had fed on *Metschnikowia pulcherrima* pupariated at all, and at the higher of the two initial yeast cell masses survival was extremely low ( $10^{-10}$  g:  $44.7 \pm 7.3\%$ ,  $10^{-8}$  g:  $6.1 \pm$

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

3.5%) (**Fig. 25**). Only larvae fed on *Metschnikowia pulcherrima* were significantly affected in their survival until pupariation by the initial yeast cell mass ( $F_{1,94} = 20.341$ ,  $p < 0.0001$ ). Survival of larvae fed on *Kluyveromyces lactis* ( $F_{1,94} = 1.5124$ ,  $p = 0.2218$ ), *Pichia toletana* ( $F_{1,85} = 0.0734$ ,  $p = 0.7871$ ) and *Saccharomyces cerevisiae* ( $F_{1,88} = 0.0047$ ,  $p = 0.829$ ) was not affected significantly.

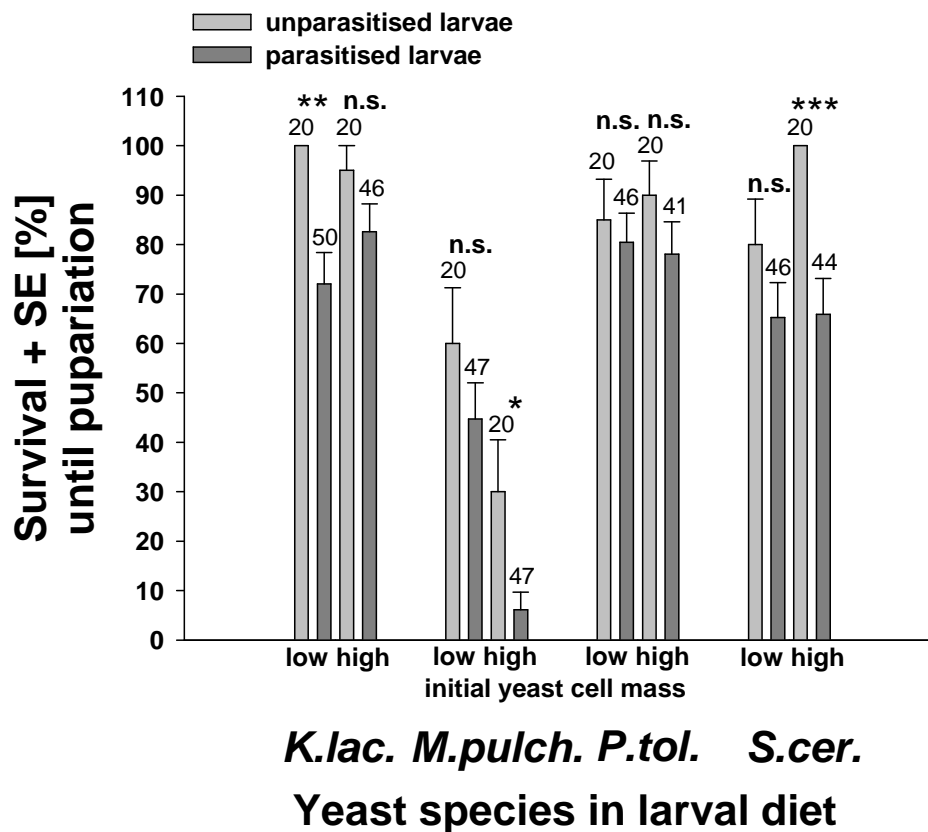
When comparing *survival until pupariation of unparasitised as well as parasitised larvae* at an initial yeast cell mass of  $10^{-10}$ g, a significant difference was only found, when larvae had fed on *Kluyveromyces lactis* (*K.lac.*:  $F_{1,68} = 14.635$ ,  $p = 0.0002861$ ; *M.pulch.*:  $F_{1,65} = 1.2839$ ,  $p = 0.2613$ ; *P.tol.*:  $F_{1,64} = 0.1949$ ,  $p = 0.6604$ ; *S.cer.*:  $F_{1,64} = 1.4678$ ,  $p = 0.2301$ ). Yet, at an initial yeast cell mass of  $10^{-8}$ g, larvae fed on *Metschnikowia pulcherrima* as well as *Saccharomyces cerevisiae* exhibited a significant difference in their survival until pupariation (*K.lac.*:  $F_{1,64} = 2.064$ ,  $p = 0.1557$ ; *M.pulch.*:  $F_{1,67} = 6.2426$ ,  $p = 0.01493$ ; *P.tol.*:  $F_{1,59} = 1.3652$ ,  $p = 0.2473$ ; *S.cer.*:  $F_{1,62} = 18.647$ ,  $p < 0.0001$ ).

The influence of dietary yeast species, initial yeast cell mass and parasitisation status (no, yes) (factors), as well as the interaction between the three factors on survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 10**. The results of the ANCOVA show that the dietary yeast species and the parasitisation status had a global significant influence on survival. Survival of parasitised larvae was, for example, always lower than that of unparasitised larvae, regardless of yeast species and initial yeast cell mass. Additionally, the influence of the initial yeast cell mass on survival differed significantly among the dietary yeast species, as indicated by the significant influence of the interaction between yeast and initial cell mass. The relative differences in survival between the two initial yeast cell masses were, for example, smaller when larvae had fed on *Pichia toletana* than when they had fed on *Saccharomyces cerevisiae* (**Fig. 25**). This holds true both for unparasitised as well as parasitised larvae.

**Table 10:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until pupariation of unparasitised and parasitised <i>Drosophila melanogaster</i>	Yeast	$F_{3,525} = 41.6410$	$p < 0.0001$
	Initial cell mass	$F_{1,524} = 3.2088$	$p = 0.07383$
	Parasitisation	$F_{1,523} = 16.5239$	$p < 0.0001$
	Yeast*Initial cell mass	$F_{3,520} = 7.9156$	$p < 0.0001$
	Yeast*Parasitisation	$F_{3,517} = 2.1760$	$p = 0.08994$
	Initial cell mass* Parasitisation	$F_{1,516} = 2.0666$	$p = 0.15117$
	Yeast*Initial cell mass* Parasitisation	$F_{3,513} = 2.3201$	$p = 0.07448$

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 25:** *Survival* (in %) until pupariation of unparasitised as well as parasitised *Drosophila melanogaster* larvae, depending on the dietary yeast species and the initial cell mass of each yeast species (**low**: initial yeast cell mass =  $10^{-10}$ g; **high**: initial yeast cell mass =  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

There was no significant effect of yeast species ( $10^{-10}$ g:  $F_{3,123} = 2.0355$ ,  $p = 0.1124$ ;  $10^{-8}$ g:  $F_{3,102} = 1.6391$ ,  $p = 0.185$ ) or initial yeast cell mass on **encapsulation ability**, irregardless which yeast species larvae had fed on (*K.lac.*:  $F_{1,73} = 0.08$ ,  $p = 0.7782$ ; *M.pulch.*:  $F_{1,25} = 2.3315$ ,  $p = 0.1393$ ; *P.tol.*:  $F_{1,67} = 3.4075$ ,  $p = 0.06932$ ; *S.cer.*:  $F_{1,60} = 0.3452$ ,  $p = 0.559$ ). Encapsulation ability was in general comparably low, irregardless of the yeast species and the initial yeast cell mass. When larvae had fed on *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*, encapsulation ability was higher at an initial yeast cell mass of  $10^{-8}$ g (*K.lac.*:  $10^{-10}$ g:  $8.3 \pm 4.7\%$ ,  $10^{-8}$ g:  $10.3 \pm 4.9\%$ ; *M.pulch.*:  $10^{-10}$ g:  $13.0 \pm 7.2\%$ ,  $10^{-8}$ g:  $50.0 \pm 28.9\%$ ; *S.cer.*:  $10^{-10}$ g:  $3.2 \pm 3.2\%$ ,  $10^{-8}$ g:  $6.5 \pm 4.5\%$ ). This was not the case for larvae fed on *Pichia toletana*. Here, larvae exhibited a higher encapsulation ability at an initial yeast cell mass of  $10^{-10}$ g than at  $10^{-8}$ g ( $10^{-10}$ g:  $21.6 \pm 6.9\%$ ,  $10^{-8}$ g:  $6.3 \pm 4.3\%$ ) (**Fig 26**).

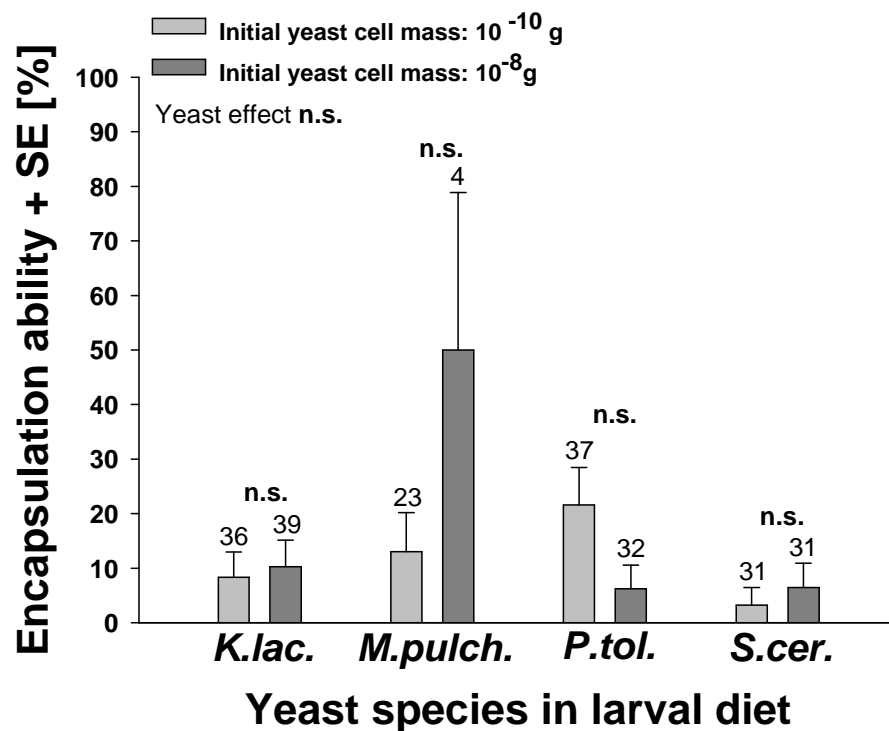


## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

The influence of dietary yeast species and initial yeast cell mass (factors), as well as the interaction between the two factors on the encapsulation ability of parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 11**. The results of the ANCOVA show that none of the factors influenced encapsulation ability significantly.

**Table 11:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the encapsulation ability of parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Encapsulation ability of parasitised <i>Drosophila melanogaster</i>	Yeast	$F_{3,229} = 1.7037$	$p = 0.1671$
	Initial cell mass	$F_{1,228} = 0.0527$	$p = 0.8187$
	Yeast*Initial cell mass	$F_{3,225} = 2.0638$	$p = 0.1059$



**Fig. 26:** Encapsulation ability (in %) of parasitised *Drosophila melanogaster* larvae, depending on the dietary yeast species and the initial cell mass of each yeast species ( $10^{-10}$ g and  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant.

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

(c) *Asobara tabida*: The dietary yeast species fed to *Drosophila melanogaster* larvae after parasitisation had a significant effect on **survival** of *Asobara tabida*, both at an initial yeast cell mass of  $10^{-10}$  g ( $F_{3,123} = 3.2422$ ,  $p = 0.02443$ ) and of  $10^{-8}$  g ( $F_{3,102} = 7.9546$ ,  $p < 0.0001$ ). Those *Asobara tabida* survived best, whose host larvae had fed on *Kluyveromyces lactis*, whereby more parasitoids reached adulthood at the initial yeast cell mass of  $10^{-8}$  g ( $10^{-10}$  g:  $61.1 \pm 8.2\%$ ,  $10^{-8}$  g:  $69.2 \pm 7.5\%$ ). When host larvae had fed on *Pichia toletana*, survival of *Asobara tabida* was still comparably high, but rather at the lower initial yeast cell mass ( $10^{-10}$  g:  $40.5 \pm 8.2\%$ ,  $10^{-8}$  g:  $28.1 \pm 8.1\%$ ). With *Saccharomyces cerevisiae* as dietary yeast species of host larvae, *Asobara tabida* was able to fulfil its development in only about a fourth of parasitised *Drosophila melanogaster* larvae ( $10^{-10}$  g:  $29.0 \pm 8.3\%$ ,  $10^{-8}$  g:  $22.6 \pm 7.6\%$ ). When host larvae had fed on *Metschnikowia pulcherrima*, survival of *Asobara tabida* was similarly low as that on *Saccharomyces cerevisiae*. At the initial yeast cell mass of  $10^{-8}$  g no adult parasitoid emerged at all ( $10^{-10}$  g:  $26.1 \pm 9.4\%$ ,  $10^{-8}$  g:  $0 \pm 0\%$ ) (**Fig 27A**). Initial yeast cell mass itself did not have a significant effect on survival of *Asobara tabida* (*K.lac.*:  $F_{1,73} = 0.5305$ ,  $p = 0.4687$ , *M.pulch.*:  $F_{1,25} = 2.3932$ ,  $p = 0.1344$ , *P.tol.*:  $F_{1,67} = 1.1415$ ,  $p = 0.2892$ , *S.cer.*:  $F_{1,60} = 0.3267$ ,  $p = 0.5697$ ).

The influence of dietary yeast species and initial yeast cell mass (factors), as well as the interaction between the two factors on survival of *Asobara tabida* (response variable) can be seen in **Table 12**. The results of the ANCOVA show that the dietary yeast species of *Drosophila melanogaster* host larvae had a global significant influence on survival of *Asobara tabida*.

**Table 12:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the survival of *Asobara tabida*. Interactions between factors are indicated by \*.

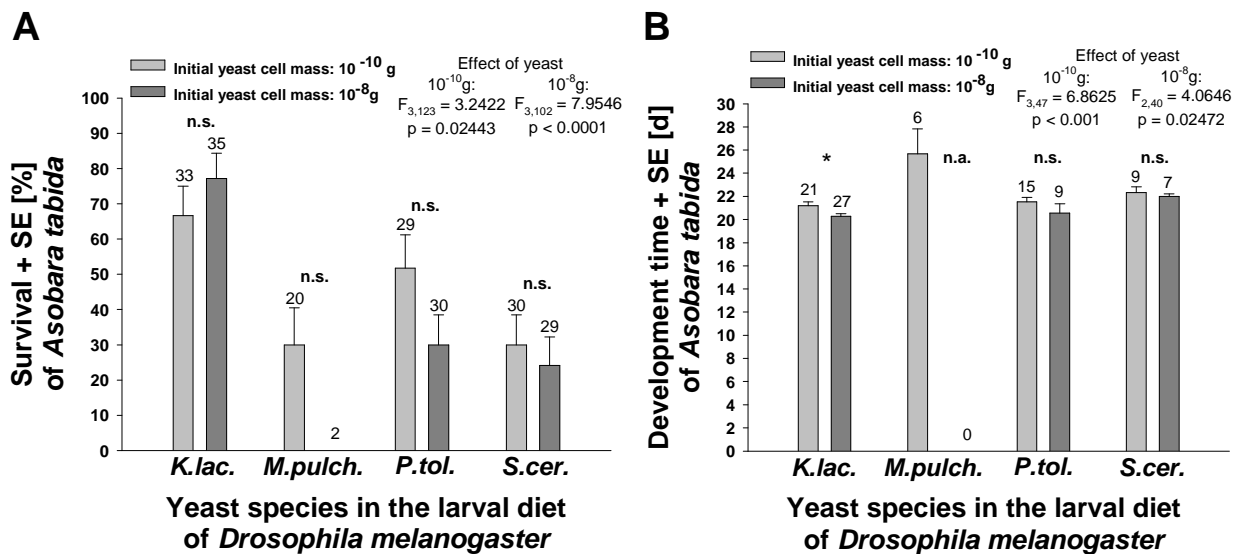
Measured fitness trait	Factors and interactions	F-value	p-value
Survival of <i>Asobara tabida</i>	Yeast	$F_{3,204} = 10.2750$	$p < 0.0001$
	Initial cell mass	$F_{1,203} = 0.7626$	$p = 0.3836$
	Yeast*Initial cell mass	$F_{3,200} = 1.5066$	$p = 0.2140$

**Development time** of surviving *Asobara tabida* was significantly influenced by the dietary yeast species fed to the *Drosophila melanogaster* larvae after parasitisation, both at an initial yeast cell mass of  $10^{-10}$  g ( $F_{3,47} = 6.8625$ ,  $p < 0.001$ ) and of  $10^{-8}$  g ( $F_{2,40} = 4.0646$ ,  $p = 0.02472$ ). The development of *Asobara tabida* was shortest when host larvae had fed on *Kluyveromyces lactis* and *Pichia toletana*, whereby parasitoids in both cases developed faster at the initial yeast cell mass of  $10^{-8}$  g (*K.lac.*:  $10^{-10}$  g:  $21.2 \pm 0.3$ d,  $10^{-8}$  g:  $20.3 \pm 0.2$ d; *P.tol.*:  $10^{-10}$  g:  $21.5 \pm 0.4$ d,  $10^{-8}$  g:  $20.6 \pm 0.8$ d). With *Saccharomyces cerevisiae* as dietary

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

yeast species of host larvae, development time of *Asobara tabida* took about as long at an initial yeast cell mass of  $10^{-10}$ g as of  $10^{-8}$ g ( $10^{-10}$ g:  $22.3 \pm 0.5$ d,  $10^{-8}$ g:  $22.0 \pm 0.2$ d). Development lasted longest, when host larvae had fed on *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $25.7 \pm 2.2$ d) (**Fig 27B**). Only when parasitised larvae were fed on *Kluyveromyces lactis* did initial yeast cell mass have a significant effect on the development time of *Asobara tabida* ( $F_{1,46} = 5.683$ ,  $p = 0.02131$ ). There was no significant effect when larvae had fed on *Pichia toletana* ( $F_{1,22} = 1.5867$ ,  $p = 0.221$ ) and *Saccharomyces cerevisiae* ( $F_{1,14} = 0.308$ ,  $p = 0.5877$ ). The data for larvae fed on *Metschnikowia pulcherrima* could not be analysed since there were no values for development time at the initial yeast cell mass of  $10^{-8}$ g.

The influence of dietary yeast species and initial yeast cell mass (factors), as well as the interaction between the two factors on development time of *Asobara tabida* (response variable) can be seen in **Table 13**. The results of the ANCOVA show that the dietary yeast species as well as the initial yeast cell mass fed to *Drosophila melanogaster* host larvae had a global significant influence on development time of *Asobara tabida*.



**Fig. 27:** A) *Survival* (in %) and B) *Development time* (in days) of *Asobara tabida*, depending on the dietary yeast species and the initial cell mass of each yeast species ( $10^{-10}$ g and  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ , n.a. = not analysed.

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

**Table 13:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the development time of *Asobara tabida*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Development time of <i>Asobara tabida</i>	Yeast	$F_{3,90} = 13.7557$	$p < 0.0001$
	Initial cell mass	$F_{1,89} = 4.3316$	$p = 0.04035$
	Yeast*Initial cell mass	$F_{2,87} = 0.1659$	$p = 0.84737$

The body size of female *Asobara tabida*, measured as **hind tibia length**, was significantly influenced by the dietary yeast species at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,30} = 7.54$ ,  $p = 0.0006674$ ;  $10^{-8}$ g:  $F_{2,21} = 4.6407$ ,  $p = 0.02143$ , *P.tol.* excluded:  $F_{1,20} = 4.3491$ ,  $p = 0.05005$ ). The initial yeast cell mass only had a significant effect on hind tibia length when host larvae had fed on *Kluyveromyces lactis* ( $F_{1,29} = 5.3273$ ,  $p = 0.02832$ ), not when they had fed on *Saccharomyces cerevisiae* ( $F_{1,8} = 0.9451$ ,  $p = 0.3594$ ). The data of parasitoids whose larvae had fed on *Metschnikowia pulcherrima* and *Pichia toletana* could not be tested, since there were not enough values for a statistical analysis (no survivors on *M.pulch.* at  $10^{-8}$ g, only two females on *P.tol.* at  $10^{-8}$ g). *Asobara tabida* females, whose host larvae had fed on *Kluyveromyces lactis*, had the longest hind tibiae ( $10^{-10}$ g:  $0.6547 \pm 0.0050$ mm;  $10^{-8}$ g:  $0.6680 \pm 0.0034$ mm), followed by those, whose host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $0.6419 \pm 0.0058$ mm;  $10^{-8}$ g:  $0.6521 \pm 0.0087$ mm), *Pichia toletana* ( $10^{-10}$ g:  $0.6309 \pm 0.0072$ mm;  $10^{-8}$ g:  $0.6398 \pm 0.0108$ ), and finally *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $0.5982 \pm 0.0176$ mm) (**Fig. 28A**). Hind tibia length was always greater when host larvae had fed on the higher of the two initial yeast cell masses.

The influence of dietary yeast species and initial yeast cell mass (factors), as well as the interaction between the two factors on hind tibia length of female *Asobara tabida* (response variable) can be seen in **Table 14**. The results of the ANCOVA show that the dietary yeast species as well as the initial yeast cell mass fed to *Drosophila melanogaster* host larvae had a global significant influence on hind tibia length of female *Asobara tabida*.

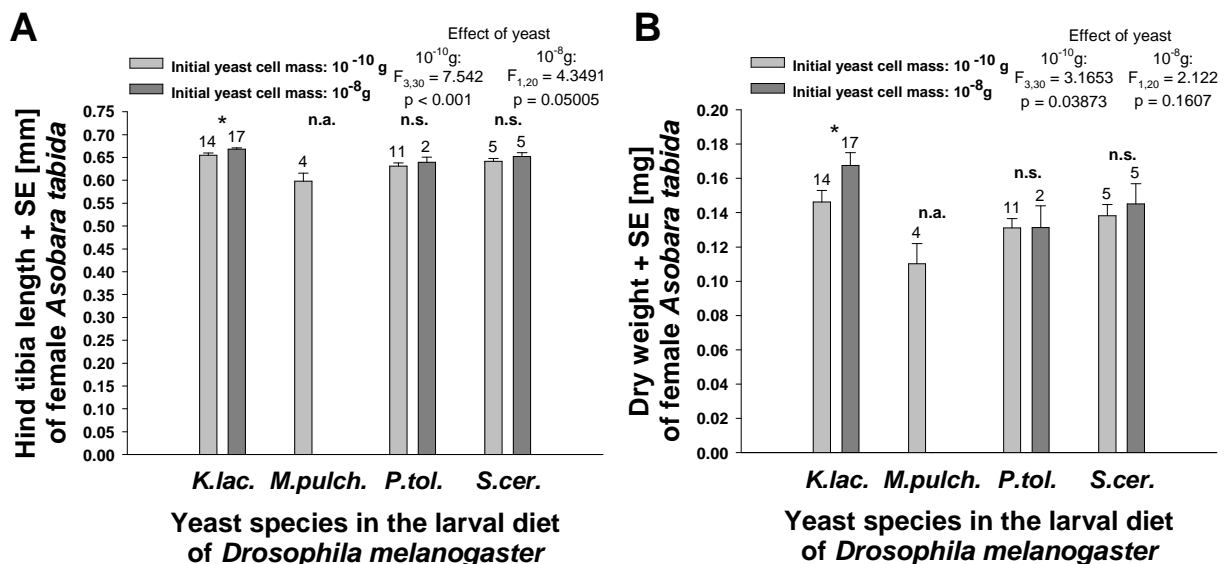
**Table 14:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence hind tibia length of female *Asobara tabida*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Hind tibia length of female <i>Asobara tabida</i>	Yeast	$F_{3,54} = 17.0144$	$p < 0.0001$
	Initial cell mass	$F_{1,53} = 4.4112$	$p = 0.04067$
	Yeast*Initial cell mass	$F_{2,51} = 0.0503$	$p = 0.95102$

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

The body size of female *Asobara tabida*, measured as **dry weight**, was also significantly influenced by the dietary yeast species of host larvae, though only at an initial yeast cell mass of  $10^{-10}$  g ( $10^{-10}$  g:  $F_{3,30} = 3.1653$ ,  $p = 0.03873$ ;  $10^{-8}$  g:  $F_{2,21} = 2.1011$ ,  $p = 0.1473$ , *P.tol.* excluded:  $F_{1,20} = 2.122$ ,  $p = 0.1607$ ). Just as in hind tibia length, initial yeast cell mass only had a significant effect on dry weight when host larvae had fed on *Kluyveromyces lactis* ( $F_{1,29} = 4.2722$ ,  $p = 0.04777$ ), not when they had fed on *Saccharomyces cerevisiae* ( $F_{1,8} = 0.2642$ ,  $p = 0.6212$ ). Again, dry weight of female *Asobara tabida* was highest when host larvae had fed on *Kluyveromyces lactis* ( $10^{-10}$  g:  $0.1462 \pm 0.0067$  mg;  $10^{-8}$  g:  $0.1675 \pm 0.0076$  mg), followed by those whose host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$  g:  $0.1382 \pm 0.0065$  mg;  $10^{-8}$  g:  $0.1451 \pm 0.0118$  mg), *Pichia toletana* ( $10^{-10}$  g:  $0.1311 \pm 0.0053$  mg;  $10^{-8}$  g:  $0.1313 \pm 0.0127$  mg), and finally *Metschnikowia pulcherrima* ( $10^{-10}$  g:  $0.1103 \pm 0.0116$  mg) (**Fig. 28B**) Dry weight of female *Asobara tabida* was usually higher when host larvae had fed on an initial yeast cell mass of  $10^{-8}$  g, with the exception of *Pichia toletana*, where dry weight was nearly similar at both initial yeast cell masses.

The influence of dietary yeast species and initial yeast cell mass (factors), as well as the interaction between the two factors on dry weight of female *Asobara tabida* (response variable) can be seen in **Table 15**. The results of the ANCOVA show that the dietary yeast species as well as the initial yeast cell mass fed to *Drosophila melanogaster* host larvae had a global significant influence on dry weight of female *Asobara tabida*.



**Fig. 28:** A) *Hind tibia length* (in mm) and B) *Dry weight* (in mg) of *Asobara tabida*, depending on the dietary yeast species and the initial cell mass of each yeast species ( $10^{-10}$  g and  $10^{-8}$  g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: ***K.lac.*** = *Kluyveromyces lactis*, ***M.pulch.*** = *Metschnikowia pulcherrima*, ***P.tol.*** = *Pichia toletana*, ***S.cer.*** = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ , n.a. = not analysed.

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

**Table 15:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence dry weight of female *Asobara tabida*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Dry weight of female <i>Asobara tabida</i>	Yeast	$F_{3,54} = 6.5690$	$p = 0.0007695$
	Initial cell mass	$F_{1,53} = 4.3614$	$p = 0.0417731$
	Yeast*Initial cell mass	$F_{2,51} = 0.6658$	$p = 0.5182858$

A linear regression between the dry weight of female *Asobara tabida* (dependent variable) and hind tibia length (independent variable), carried out over all yeast species and both initial yeast cell masses, yielded a significant correlation between the two traits ( $R^2 = 0.4301$ ,  $F_{1,56} = 44.02$ ,  $t = 6.635$ ,  $p < 0.0001$ ), indicating, that both measured traits may be equal in representing body size in *Asobara tabida*.

The different yeast species in the diet of host larvae did not significantly influence the **relative fat content** of female *Asobara tabida* ( $10^{-10}$ g:  $F_{3,30} = 0.5731$ ,  $p = 0.6371$ ;  $10^{-8}$ g:  $F_{2,21} = 3.1357$ ,  $p = 0.06432$ ). Only with the data for *Pichia toletana* as dietary yeast species excluded from the analysis at the initial yeast cell mass of  $10^{-8}$ g (only two values existed), a significant effect resulted ( $F_{1,20} = 6.1856$ ,  $p = 0.02183$ ). Similarly, initial yeast cell mass did not have an effect on relative fat content when host larvae had fed on *Kluyveromyces lactis* ( $F_{1,29} = 2.4586$ ,  $p = 0.1277$ ) or *Saccharomyces cerevisiae* ( $F_{1,8} = 0.0939$ ,  $p = 0.7671$ ). Relative fat content was highest in *Asobara tabida* females, whose host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $0.2995 \pm 0.0196$ mg/mg;  $10^{-8}$ g:  $0.3115 \pm 0.0341$ mg/mg), followed by those, whose host larvae had fed on *Pichia toletana* ( $10^{-10}$ g:  $0.2818 \pm 0.0465$ mg/mg;  $10^{-8}$ g:  $0.2805 \pm 0.0419$ mg/mg), *Kluyveromyces lactis* ( $10^{-10}$ g:  $0.2687 \pm 0.0068$ mg/mg;  $10^{-8}$ g:  $0.2493 \pm 0.0096$ mg/mg) and *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $0.2230 \pm 0.0353$ mg/mg) (**Fig. 29A**).

The influence of dietary yeast species and initial yeast cell mass (factors), as well as the interaction between the two factors on the relative fat content of female *Asobara tabida* (response variable) can be seen in **Table 16**. The results of the ANCOVA show that none of the factors had a significant influence on the relative fat content of female *Asobara tabida*.

**Table 16:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the relative fat content of female *Asobara tabida*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Relative fat content of female <i>Asobara tabida</i>	Yeast	$F_{3,54} = 1.5058$	$p = 0.2242$
	Initial cell mass	$F_{1,53} = 0.2370$	$p = 0.6285$
	Yeast*Initial cell mass	$F_{2,51} = 0.1549$	$p = 0.8569$

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

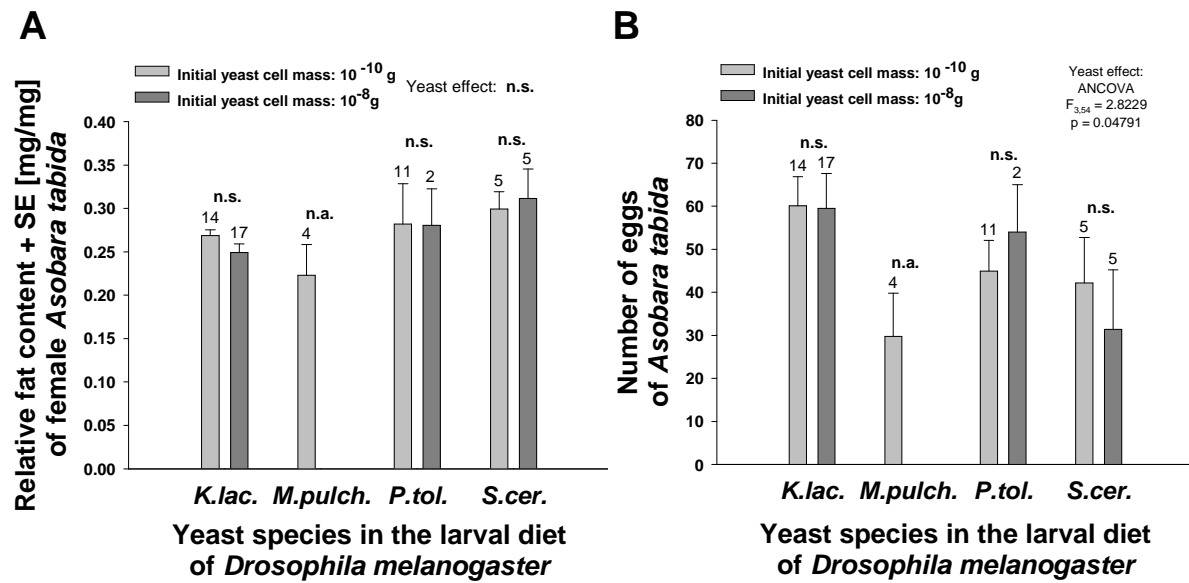
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The absolute *number of eggs* carried by freshly eclosed virgin *Asobara tabida* females was neither significantly influenced by the dietary yeast species of host larvae ( $10^{-10}$ g:  $F_{3,30} = 2.0912$ ,  $p = 0.1223$ ;  $10^{-8}$ g:  $F_{2,21} = 1.4722$ ,  $p = 0.2521$ ; *P.tol.* excluded:  $F_{1,20} = 2.8344$ ,  $p = 0.1078$ ) nor the initial yeast cell mass offered to host larvae (*K.lac.*:  $F_{1,29} = 0.0032$ ,  $p = 0.9551$ ; *S.cer.*:  $F_{1,8} = 0.3857$ ,  $p = 0.5518$ ) (but see results of the ANCOVA presented later in the text). *Asobara tabida* females whose host larvae had fed on *Kluyveromyces lactis* ( $10^{-10}$ g:  $60.1 \pm 6.73$ ;  $10^{-8}$ g:  $59.5 \pm 8.08$ ), carried the highest number of eggs, followed by *Pichia toletana* ( $10^{-10}$ g:  $44.9 \pm 7.20$ ;  $10^{-8}$ g:  $54.0 \pm 11.0$ ), *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $42.2 \pm 10.54$ ;  $10^{-8}$ g:  $31.4 \pm 13.83$ ) and *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $29.8 \pm 10.04$ ) (**Fig. 29B**).

The influence of dietary yeast species and initial yeast cell mass (factors), as well as the interaction between the two factors on the absolute number of eggs of female *Asobara tabida* (response variable) can be seen in **Table 17**. The results of the ANCOVA show that only the dietary yeast species of *Drosophila melanogaster* host larvae had a very weak significant influence on the number of eggs of female *Asobara tabida*. This result differs from that yielded by the GLM-analysis with only one factor, namely yeast as linear predictor. By adding the covariate ‘initial yeast cell mass’ into the model, the variation in number of eggs due to the covariate is removed, thus, providing a more precise analysis.

Even though a linear regression did not yield any significant correlation between the number of eggs and the size parameters hind tibia length and dry weight (see later in the text & **Fig. 30**), the absolute number of eggs was corrected for a potential influence of size parameters which may have been concealed by the different yeast treatments. For this, the absolute number of eggs of each *Asobara tabida* female was divided by the hind tibia length of the same female on the one hand and by the dry weight of the individual female on the other hand. The graphs very much resembled that of the absolute number of eggs and are therefore not shown here. The results of the ANCOVAs (see **Table 17**), however, were different in the way, that the (weak) significant influence of the dietary yeast species on the number of eggs disappeared.

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 29:** A) *Relative fat content* (in mg per mg dry weight) and B) *Number of eggs* of female *Asobara tabida* depending on the dietary yeast species and the initial cell mass of each yeast species ( $10^{-10}$  g and  $10^{-8}$  g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, n.a. = not analysed.

**Table 17:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the number of eggs of female *Asobara tabida*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Absolute number of eggs of female <i>Asobara tabida</i>	Yeast	$F_{3,54} = 2.8229$	$p = 0.04791$
	Initial cell mass	$F_{1,53} = 0.0292$	$p = 0.86497$
	Yeast*Initial cell mass	$F_{2,51} = 0.2679$	$p = 0.76602$
Number of eggs per hind tibia length	Yeast	$F_{3,54} = 2.4095$	$p = 0.0777$
	Initial cell mass	$F_{1,53} = 0.0860$	$p = 0.7705$
	Yeast*Initial cell mass	$F_{2,51} = 0.2571$	$p = 0.7743$
Number of eggs per dry weight	Yeast	$F_{3,54} = 1.3533$	$p = 0.2676$
	Initial cell mass	$F_{1,53} = 0.1681$	$p = 0.6835$
	Yeast*Initial cell mass	$F_{2,51} = 0.2707$	$p = 0.7640$



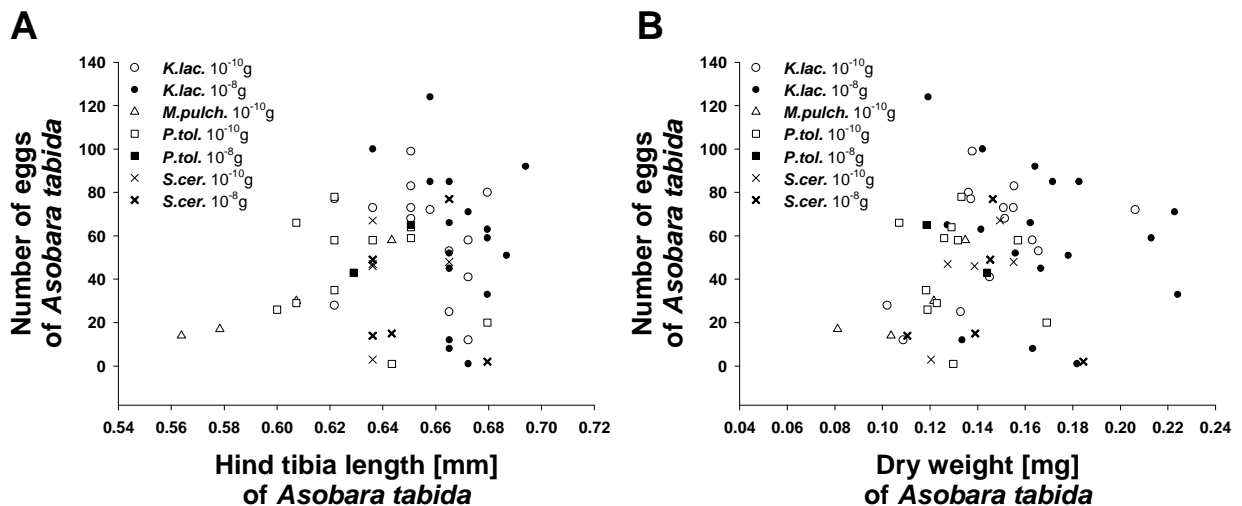
## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

Linear regressions between number of eggs of female *Asobara tabida* (dependent variable) and body size of *Asobara tabida*, measured as hind tibia length (Fig. 30A) as well as dry weight (independent variables) (Fig. 30B), carried out over all yeast species and both initial yeast cell masses, did not yield significant correlations between these traits (hind tibia versus number of eggs:  $R^2 = 0.01256$ ,  $F_{1,56} = 1.725$ ,  $t = 1.313$ ,  $p = 0.1944$ ; dry weight versus number of eggs:  $R^2 = 0.01465$ ,  $F_{1,56} = 1.847$ ,  $t = 1.359$ ,  $p = 0.1796$ ). There was, however, a significant positive correlation between the absolute fat content and hind tibia length ( $R^2 = 0.1909$ ,  $F_{1,56} = 14.45$ ,  $t = 3.801$ ,  $p = 0.0003572$ ) (Fig. 31A). A multiple regression between absolute fat content (dependent variable) and number of eggs as well as hind tibia length (independent variables) did not reveal a significant correlation between absolute fat content and number of eggs (Fig. 31B & Table 18).

**Table 18:** Multiple regression of number of eggs and hind tibia length on the absolute fat content of *Asobara tabida* over all yeast species and both initial yeast cell masses.  $F_{2,57} = 7.112$ ,  $R^2 = 0.177$ ,  $p = 0.002$ .

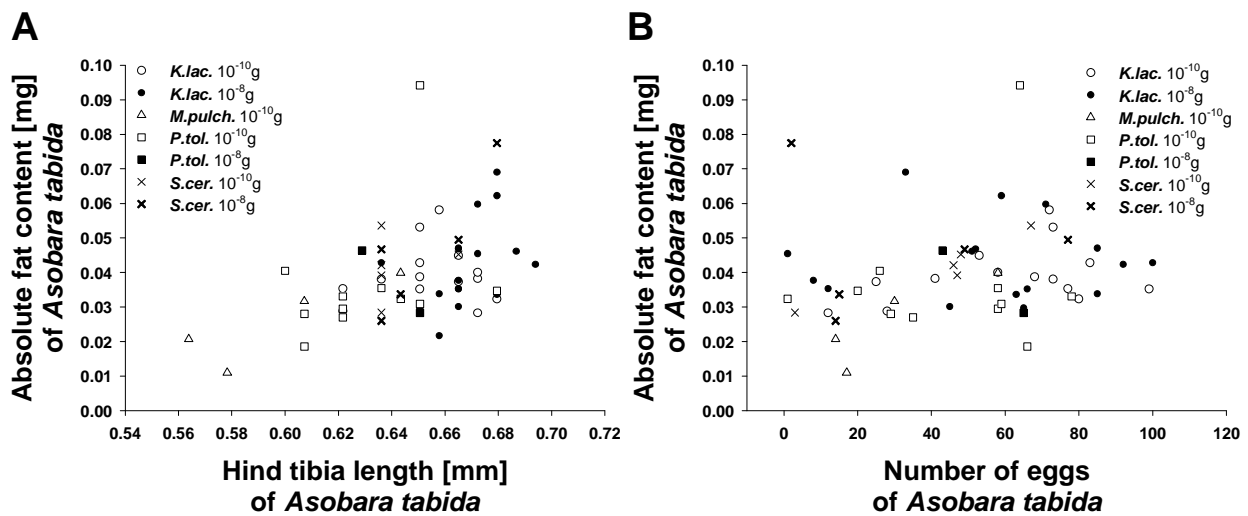
Character	Coefficient	Std. error	t-value
Constant	- 0.114	0.041	- 2.768
Number of eggs	$1.079 \times 10^{-5}$	0.000	0.180
Hind tibia length [mm]	0.236	0.064	3.679**

\*\* $p < 0.001$



**Fig. 30:** Scatter plots of individual values of **A)** Hind tibia length (in mm) versus number of eggs and **B)** Dry weight (in mg) versus number of eggs of *Asobara tabida*, over all dietary yeast species and both initial yeast cell masses ( $10^{-10}$ g and  $10^{-8}$ g). Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **P.tol.** = *Pichia toletana*, **S.cer.** = *Saccharomyces cerevisiae*.

## 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 31:** Scatter plots of individual values of **A)** Hind tibia length (in mm) versus absolute fat content (in mg) and **B)** Number of eggs versus absolute fat content (in mg) of *Asobara tabida*, over all dietary yeast species and both initial yeast cell masses ( $10^{-10}$ g and  $10^{-8}$ g). Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*.

### 5.4.4 Discussion

The results suggest that: (i) both the quality and the quantity of dietary yeast species may have a significant influence on fitness traits of *Drosophila melanogaster*. (ii) Fitness traits of *Asobara tabida* may also be significantly influenced, though mainly by the quality than the quantity of dietary yeast species. (iii) The degree of this influence as well as the kind of fitness traits that are influenced depends on the specific dietary yeast species. (iv) Not all measured fitness traits may experience a similar (favourable or unfavourable) influence by a specific yeast species. (v) There is a tendency of *Asobara tabida* to perform better in hosts fed on yeast species which favour the host's own larval and adult fitness traits. Even though yeast quantity may influence fitness traits of *Drosophila melanogaster*, it may only rarely affect the performance of *Asobara tabida*.

Compared to *ad libitum* conditions (see chapter 5.2), the differences in influence of the specific yeast species on fitness traits of unparasitised *Drosophila melanogaster* got more pronounced, when yeasts were offered at intermediate (initial cell mass  $10^{-8}$ g) and limited conditions (initial cell mass  $10^{-10}$ g). Here, in addition to development time and female dry weight, survival until eclosion and male dry weight were also significantly influenced. The significant differences can mostly be ascribed to *Metschnikowia pulcherrima*, which had an overall negative influence on most of the fitness traits measured in *Drosophila melanogaster*, whereby the influence got more pronounced at limited conditions ( $10^{-10}$ g) and, surprisingly, at an intermediate biomass ( $10^{-8}$ g). The bad performance at an initial cell mass of  $10^{-8}$ g compared to  $10^{-10}$ g may have been due to detrimental (probably toxic) ef-

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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fects of *Metschnikowia pulcherrima*, being more pronounced at the higher cell mass (though not at *ad libitum* conditions, see chapter 5.2). Thus, the influence of a specific yeast species may not be linearly correlated to the amount of biomass offered to *Drosophila melanogaster* larvae. Even though larvae may have consumed ‘enough’ biomass, some essential components may either have been lacking or some components may have negatively influenced the metabolism of *Drosophila melanogaster* larvae. On the other hand, larvae may not have devoured sufficient biomass (e.g. macronutrients), even though it was offered to them, due to insalubrity of *Metschnikowia pulcherrima*. Since I did not measure, how much biomass larvae actually consumed, I cannot necessarily attribute the bad performance to malnutrition (‘poorly-fed’ in the sense of not the adequate food). Larvae may just as well have refrained from eating a sufficient amount of biomass and may thus have been starved (undernutrition) concerning specific dietary components (‘poorly-fed’ in the sense of not enough food). Often, the response to limiting nutrients may be to increase consumption until the limiting requirements are met, irrespective of other nutrients that might be ingested in excess. Yet, this need not always be the case, since a range of situations in both insects and vertebrates has been described, where the ingestion of excess nutrients constrains overall intake, although overall intake would have had to be increased in order to meet requirements for limiting nutrients (SIMPSON & RAUBENHEIMER 1996; HAEGELE *et al.* 1999 and references therein).

The effect of initial cell mass on fitness traits of unparasitised larvae varied depending on the yeast species, and was more pronounced in some yeast species compared to others. This, however, also depended on the fitness trait in question, since not all measured fitness traits were similarly influenced by a specific yeast species, i.e. not all fitness traits were only favourably or only unfavourably influenced. *Saccharomyces cerevisiae*, for example, which had a highly beneficial influence on all fitness traits measured in unparasitised larvae at *ad libitum* conditions (chapter 5.2) as well as intermediate conditions ( $10^{-8}$ g), did not appear to yield sufficient food resources to support successful survival at limited food conditions ( $10^{-10}$ g). Dry weight of adult flies, on the other hand, was still comparably high, even at limited food conditions. *Kluyveromyces lactis* appeared to pose the most advantageous yeast species at limited food conditions, especially in terms of survival and development time, even though dry weight of adult flies was not as high as of those that had as larvae fed on *Saccharomyces cerevisiae*. This may, however, be due to the frequently observed trade-off between age and size at maturity.

Survival of parasitised larvae was generally lower than that of unparasitised ones, though not significantly so in all of the yeast species. This already occurred at *ad libitum* conditions as described in chapter 5.2. MOREAU *et al.* (2002) found that *Drosophila melanogaster* larvae parasitised by *Asobara tabida* exhibited longer periods of development and lower larval weights than controls, probably due to physiological costs. As para-

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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sitoid factors that may be responsible for these costs, they examined parasitoid venoms for their impact on host mortality. Artificial injections of venoms induced higher mortality rates and were also found responsible for the induction of a transient paralysis, naturally occurring after parasitisation. HOANG (2002), however, could hardly detect any costs in terms of decreased fitness traits (development time) in *Drosophila melanogaster* larvae that had survived parasitisation of *Asobara tabida*, and supposed that the major investment may rather be that of building and maintaining the [immune] machinery and not in actually running the machine (KRAAIJEVELD & GODFRAY 1997; HOANG 2002). Even if the main reason for enhanced mortality of parasitised compared to unparasitised larvae may have been due to the venom of *Asobara tabida*, the fact remains, that this did not hold true for all yeast species. That nutrition may also modulate virulence factors of a parasite could for example be observed in bumblebees infected with trypanosome intestinal parasites (BROWN *et al.* 2000). The condition-dependent virulence was, however, mainly caused by stress through starvation. Yet, differences in survival until pupariation between unparasitised and parasitised *Drosophila melanogaster* larvae in the study described here, occurred at *ad libitum* (*K.lac.*, *M.pulch.*, *S.cer.*), intermediate (*M.pulch.*, *S.cer.*) and limited conditions (*K.lac.*). This suggests that the quality rather than the quantity of yeast species is of importance. Even if the mechanisms are not known, dietary yeast species may both modulate resistance factors (encapsulation ability) of the host as well as virulence factors of the parasite.

At limited food conditions, the differences in the influence of the specific yeast species on the encapsulation ability of parasitised larvae (as shown in chapter 5.2), were evened out, and encapsulation ability was in general much lower at limited food conditions. Encapsulation response, or haemocyte concentration as a correlate, has been shown to vary considerably with - among other things - host nutritional status (SHAPIRO 1967; SCHMID-HEMPEL & SCHMID-HEMPEL 1998 and references therein). The low encapsulation ability of *Drosophila melanogaster* larvae in this experiment may thus have been due to the lack of macro- and/or micronutrients. Alternatively, however, the low encapsulation ability may have also been due to a decreased investment of larvae in their immune defence. According to an analytical model of HOUSTON *et al.* (2007), animals with low reserves choose to allocate less to defence than animals with higher reserves, because when reserves are low it may be more important to increase reserves to reduce the risk of starvation in the future. At the limited food conditions in this study, *Drosophila melanogaster* larvae may have invested less in their cellular immune defence, irregardless of the yeast species they had fed on. This would also imply that the yeast species do not necessarily always yield the same (favourable or less favourable) effect on encapsulation ability.

A generalisation concerning whether *Asobara tabida* performs better on poorly or well fed *Drosophila melanogaster* host larvae cannot be drawn, maybe also due to the difficulty in

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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defining ‘poorly-fed’ and ‘well-fed’ in this case. Neither *Drosophila melanogaster* nor *Asobara tabida* showed a consistent response in their fitness traits to either of the two initial yeast cell masses offered. The response rather varied depending on the specific yeast species offered at the two different initial cell masses, as already mentioned for *Metschnikowia pulcherrima*. *Asobara tabida* only very rarely responded to differences in initial cell mass at all. Yet, *Asobara tabida* responded to the quality of the microbial diet and tended to show a good performance on hosts feeding from yeast species that were also beneficial to the performance of the host itself, and sometimes (but rarely significantly so) when host larvae were offered higher amounts of biomass of this specific yeast species. So did *Kluyveromyces lactis*, having an overall beneficial influence on *Drosophila melanogaster* larvae, also positively affect fitness traits of *Asobara tabida*, such as survival, development time and size parameters. *Saccharomyces cerevisiae*, that did not pose a comparably advantageous food source for *Drosophila melanogaster* larvae at limited conditions, was also disadvantageous for the performance of *Asobara tabida*, e.g. for survival and development time. Surprisingly, even though dry weight of adult flies was enhanced when they had fed on *Saccharomyces cerevisiae*, irrespective of the initial cell mass, *Asobara tabida* females did not show a correspondingly high dry weight, but were heavier when host larvae had fed on *Kluyveromyces lactis*. *Pichia toletana*, influencing fitness traits in an intermediate way compared to *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, also influenced fitness traits of *Asobara tabida* in an intermediate way, and *Metschnikowia pulcherrima*, not posing a favourable food for *Drosophila melanogaster* larvae, led to a rather poor performance of *Asobara tabida*. That *Asobara tabida* rarely responded to the different initial cell masses offered to their host larvae may have been due to a not sufficiently large difference between the cell masses chosen in the experiment. It may, however, also have been due to the delayed development of *Asobara tabida* compared to that of their host. Parasitoid larvae mainly start to feed invasively and destructively when their host has pupariated. In the meantime, host larvae may have been able to compensate for food losses they had suffered from at the beginning of their development, when initial cell mass was still low. The fact that the food, the different yeast species, also grows and reproduces, led to different conditions in available biomass during larval development. A permanent or elongated starvation may have had a more striking influence on *Asobara tabida* than a low initial cell mass.

ELLERS (1996) presented a method to uncover potential trade-offs in *Asobara tabida* between survival and reproduction by measuring number of eggs and fat content of female parasitoids. She detected a positive correlation between the size of *Asobara tabida* (measured as hind tibia length) and fat content as well as number of eggs. Additionally, she found a negative correlation between the number of eggs and the amount of fat carried by female *Asobara tabida*. Similar correlations could not be detected in this study. Although

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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absolute fat content positively correlated with the size of the parasitoid, the number of eggs did not. Additionally, no negative correlation between the number of eggs and absolute fat content was found. *Asobara tabida* females, for example, whose larvae had fed on *Metschnikowia pulcherrima*, both carried the lowest mean number of eggs, but also had the lowest mean absolute fat content, compared to *Asobara tabida* females, whose larvae had fed on the other yeast species. The absence of a trade-off between reproduction and survival may be due to the lower number of replicates in this experiment compared to that carried out by ELLERS. Alternatively, the absence or undetectability of the trade-off may be due to a variation in the amount of fat stored in the eggs of *Asobara tabida* females. The method used by ELLERS (1996) is based on the presumption that eggs of *Asobara tabida* possess a similar amount of fat, irregardless of genetic or environmental conditions. This may, however, not be the case. The production of yolk-deficient (hydropic) and yolk-rich (anhydropic) eggs are reported in parasitoid species, and the yolk content is often associated with the ovigeny of a specific parasitoid and a parasitoid's developmental mode (idionbiont or koinobiont) (JERVIS *et al.* 2001). The ovigeny of *Asobara tabida* may itself vary between different strains and depending on food availability (ELLERS & VAN ALPHEN 1997). In order to include the absolute fat content of the eggs into the analysis, one would have to measure dry weight and fat content of the eggs separate to the rest of the *Asobara tabida* carcass. This may, however, pose problems concerning the dissection and may also exceed the measuring accuracy of many a balance. Yet, the apparent absence of the trade-off between reproduction and survival at the phenotypic level may not necessarily mean that there was no such trade-off in *Asobara tabida* in this experiment. According to GLAZIER (1999), life history trade-offs should be sought at the genotypic level, since phenotypic differences are directly influenced by the environment (here nutritional condition of the host), and may thus mask underlying genetic trade-offs predicted by life history models. These masking effects include, for example, genotype-environment interactions. Besides the fact that favourable conditions may allow organisms to increase their reproductive effort without survival costs, life history trade-offs may also be obscured by inter-individual variation in resource acquisition (GLAZIER 1999). A model of VAN NOORDWIJK & DE JONG (1986) for example, predicts that if individual variation in resource acquisition exceeds that of resource allocation, even positive correlations between reproduction and somatic growth/survival may occur (GLAZIER 1999). The nutritional conditions as different yeast species and different initial yeast cell masses in this experiment may have masked a potential trade-off between reproduction and survival in *Asobara tabida* females. The linear regressions between absolute fat content and number of eggs as well as hind tibia length were carried out across all yeast species and both initial yeast cell masses. Analyses should probably rather be carried out for single yeast species and merely one initial cell mass. Yet, the number of replicates in this experiment was not sufficient for a sound analy-

#### 5.4 Influence of microbial species (limited conditions) on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

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sis. Separate analyses I nevertheless carried out, also did not yield any negative correlation between fat content and number of eggs (probably now due to lack of sufficient replicates). Merely in *Saccharomyces cerevisiae* at the initial cell mass of  $10^{-10}$  g, a tendency was visible, but it was not significant.

Female *Asobara tabida* have been shown to be attracted to odours emitted by the food of their host (volatiles produced by microorganisms and those emitted by fermenting substrates) as well as the host itself (kairomones) (VET *et al.* 1984; VAN ALPHEN & VET 1986). These odours are used by *Asobara tabida* as major cues in host habitat and host location. JULIA STARK (2008) studied the host location behaviour of *Asobara tabida* (host habitat/patch location, on-patch host location) depending on the presence of different yeast species (*Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae*) on the food substrate of *Drosophila melanogaster* host larvae. In all of the experiments she had carried out, *Asobara tabida* had exhibited a more or less pronounced preference for *Kluyveromyces lactis*. Even though this yeast species could be shown to enhance the ability of *Drosophila melanogaster* to encapsulate offspring of *Asobara tabida* (chapters 5.2, 5.3 and 5.5), the developmental success as well as various adult fitness traits of *Asobara tabida* were favoured when host larvae had fed on *Kluyveromyces lactis* (this chapter). This suggests that, similar to its host *Drosophila melanogaster* that has been shown to exhibit a preference for those yeast species favouring their larval and adult fitness traits (DORSCH 2007, this study), also *Asobara tabida* seems to exhibit a preference for the yeast species, which favours its own performance. Thus, the preference-performance hypothesis, predicting a preference of organisms for that food which may favour their own performance and life history traits, may apply for *Drosophila melanogaster* and *Asobara tabida*, at least with regard to the yeast species investigated.

The eggs and young larvae of internal parasitoids obtain all of their nutrients from the haemolymph (VINSON & IWANTSCH 1980). Just as in ectoparasites such as fleas, where the physical and chemical properties of blood are known as important characteristics of a host, to which a host-specific flea is adapted (KRASNOV *et al.* 2003 and references therein), dietary compounds (as different microorganisms) may modulate the physical and chemical properties of a host's haemolymph, and influence the parasitoid's development.

Even though no consistent pattern may be visible with regard to the influence of specific yeast species and initial yeast cell masses on fitness traits of *Drosophila melanogaster* and *Asobara tabida*, the results strongly suggest, that the microbial organisms in the diet of *Drosophila melanogaster* host larvae carry over and modulate the developmental success of *Asobara tabida* and thus the outcome of the host-parasitoid interaction. Parasite development may, therefore, depend on a tritrophic interaction with all trophic levels influencing the final outcome (THOMPSON & REDAK 2008).

## 5.5 *Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida**

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### 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

#### 5.5.1 Introduction

There are two most frequently addressed hypotheses of why some animals mix their diet from a wide range of foods: (i) A nutritional benefit may arise from food mixing, due to the fact that eating a range of substitutable food types reduces an animal's reliance on any one, thus increasing overall resource availability. Alternatively, mixing foods may increase the quality of the overall diet by improving the nutrient balance, since by mixing complementary foods animals are able to regulate the balance of nutrients that they ingest and may even select foods that best fit potentially changing physiological needs (BERNAYS *et al.* 1994; BERNAYS & MINKENBERG 1997; RAUBENHEIMER & JONES 2006). (ii) Food mixing may also be beneficial by enabling foragers to ingest only subthreshold concentrations of a range of potential toxins (e.g. secondary plant compounds, allelochemicals), rather than pathological doses of any one (FREELAND & JANZEN 1974; BERNAYS *et al.* 1994; RAUBENHEIMER & JONES 2006).

Studies on food mixing in a wide range of organisms have shown improved performance of these organisms on mixtures of food relative to any single food item (BERNAYS *et al.* 1994; RAUBENHEIMER & JONES 2006 and references therein). The mixing of diets could hereby not only be shown in herbivorous organisms, but also in predatory animals, whose food spectrum is usually considered to predominantly consist of qualitatively equivalent prey (insect predators: EVANS *et al.* 1999; spiders: TOFT & WISE 1999; OELBERMANN & SCHEU 2002), and even in a parasitic plant, where simultaneous attack of a mixture of host species led to improved parasite performance (MARVIER 1998). The performance of herbivorous organisms may also be affected by intraspecific host plant variation, depending, however, on whether conspecific plant individuals sufficiently differ to promote diet-mixing behaviour (MODY *et al.* 2007). Heterogeneity as food source may hereby not only hold true in animal prey or higher plants. Marine algae, for example, are supposed to vary considerably in their nutritional, chemical and structural composition, thus providing a heterogeneous and variable food resource for marine invertebrates (CRUZ-RIVERA & HAY 2001).

However, the assumption that mixed diets will always be better than single diets is only adequate when no single diet comes close to the insect's nutrient requirements and when alternative foods are available which happen to complement each other in limiting nutrients or toxin content (RAUBENHEIMER & SIMPSON 1997 and references therein). In a lycosid spider, for example, it could be shown that the nutritional quality for generalist predators may differ significantly between prey species, that, however, mixed diets may not only be beneficial but also detrimental to spider performance (OELBERMANN & SCHEU 2002).

Regarding the control of nutrient intake in insects, the haemolymph plays a central role and provides a constantly updated summary of nutritional state, with haemolymph parameters



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being directly and indirectly linked to key aspects of feeding behaviour, namely food location, food selection and ingestion (SIMPSON & RAUBENHEIMER 1993).

The importance of yeasts in the nutrition of *Drosophila melanogaster* and other *Drosophila* species has long been recognised, e.g. as an important source for the supply of phosphorous (NORTHRUP 1917; BAUMBERGER 1917; KING 1954). Yeasts are a highly heterogeneous and complex metabolic group (RODRIGUES *et al.* 2006). Even of the most intensively studied yeast species *Saccharomyces cerevisiae*, many metabolic properties are yet to be fully understood due to the complexity of its metabolic network (WANG & HATZIMANIKATIS 2006).

Although the pathways of sugar utilisation follow the same theme in all yeasts, important biochemical and genetic variations on it exist (FLORES *et al.* 2000). Yeasts are capable of utilising a wide diversity of nitrogen compounds as nitrogen sources, of which more than 50 are known. Hereby, the nitrogen utilisation profiles markedly vary according to yeast species (MESSENGUY *et al.* 2006). Additionally, growth conditions, such as temperature and nutrition, may contribute to a substantial variation in yeast metabolism (BOUNDY-MILLS 2006). Ripe and deteriorating fruits are usually colonised by a plethora of different yeast (and other microbial) species, whereby the colonisation takes place in successional stages in which changes in host fruit chemistry, presence of yeast vectors and microbe-microbe interactions (e.g. yeast-yeast interactions and the production of (glyco) proteins/mycocins and glycolipids) are involved (MORAIS *et al.* 1995; ABRANCHES *et al.* 2001; GOLUBEV 2006). *Drosophila melanogaster* itself is an important vector supporting the dispersal of yeasts (with yeast spores being the primary dispersal stage via fly faeces), and also contributing to increased outbreeding among yeasts (REUTER *et al.* 2007).

Considering this, different yeast species may pose substantial differences in quality to *Drosophila melanogaster* and other *Drosophila* species that depend on the presence of yeast for a successful larval development and adult nutrition. These differences in yeast quality may promote a diet-mixing behaviour in *Drosophila melanogaster*, but also preferences for or rejection of specific yeast species. As could already be shown by DORSCH (2007) as well as in former chapters of this study, several fitness traits of *Drosophila melanogaster* may be affected by the quality and quantity of specific yeast species.

The experiments described in this chapter were carried out against the background of the following questions:

- (i) Does a mixed microbial diet (composed of a combination of two or more different dietary yeast species) influence preadult developmental success and adult fitness traits of unparasitised and parasitised *Drosophila melanogaster*?
- (ii) Does a mixed microbial diet (composed of a combination of two or more different dietary yeast species) influence developmental success and adult fitness traits of *Asobara tabida*?

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### 5.5.2 Material and Methods

#### (a) *Experimental setup*

**Experiment (I):** This experiment comprised three yeast species (*Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*), and three yeast species mixtures (*Kluyveromyces lactis* & *Metschnikowia pulcherrima*, *Kluyveromyces lactis* & *Saccharomyces cerevisiae*, *Kluyveromyces lactis* & *Metschnikowia pulcherrima* & *Saccharomyces cerevisiae*) as dietary treatments. Parasitised as well as unparasitised larvae were exposed to the different treatments.

**Experiment (II):** The bifactorial experiment comprised four yeast species (*Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae*) and one yeast species mixture (*Kluyveromyces lactis* & *Saccharomyces cerevisiae*) as first factor/treatment and two different ambient temperatures (20°C and 25°C) as second factor/treatment. Parasitised as well as unparasitised larvae were exposed to the different treatments. The two different ambient temperatures were chosen, since *Asobara tabida* had turned out to enter diapause at an ambient temperature of 25°C. In order to be able to follow parasitoid development until emergence of adult parasitoids, 20°C were chosen as a second ambient temperature. Yet, the ambient temperature of 25°C was not discarded, since all other experiments had already been carried out at 25°C, and I wanted to be able to compare some of the results of this chapter with those of the other chapters. The disadvantage was, that I had to leave out most of the yeast species mixtures, since the number of parasitised larvae needed for an even greater experiment would have been too large for one person to manage at a time.

#### (b) *Fly and parasitoid cultures*

See chapter 4.1.

#### (c) *Preparation of media:*

See chapter 4.3.

#### (d) *Preparation of larvae*

See chapter 4.4 and 4.5.

#### (e) *Gaining of parasitised larvae*

**Experiment (I):** For this experiment the observational method described in chapter 4.6.1 was used. One parasitoid each was allowed to parasitise approx. 12 larvae, of which two larvae each were transferred into each of the six yeast treatments, directly after the parasitoid had finished the 12 parasitisations.

**Experiment (II):** For this experiment the non-observational method described in chapter 4.6.2 was used, since the number of parasitised larvae needed was too high for one person to get the experiment started within a time frame that allowed the comparison of the different

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treatments. Otherwise, the time point of parasitisation would have been too far apart between a large number of larvae, and would not have allowed to treat them as replicates.

### **(f) *Preparation of microorganisms***

For this experiment *ad libitum* food conditions were prepared with 50% yeast suspensions (see chapter 4.2). The yeast species mixtures were prepared in mixing the same volume of the 50% yeast suspensions of the singular yeast species.

### **(g) *Experimental proceeding***

50µl each of the 50% yeast suspensions were pipetted into the prepared test tubes filled with Banana medium II. Hereupon one larva each was transferred into the tubes with a fine brush and the tubes covered with dental rolls (HARTMANN). Test tubes containing one parasitised larva each as well as tubes with one unparasitised larva each (control tubes) were prepared. The tubes were incubated in a climate chamber at 25°C and a 16:8 L/D cycle in Experiment (I). In Experiment (II) a climate chamber with an ambient temperature of 20°C and a 16:8 L/D cycle, as well as a climate chamber with an ambient temperature of 25°C and a 16:8 L/D cycle was used. The development of unparasitised larvae was followed until eclosion. Adult flies were dried and weighed. In Experiment (II), the mesothorax length of adult flies was additionally measured. Eclosed female *Asobara tabida* adults in Experiment II were dissected to determine egg load, were dried and weighed, and the fat content was measured.

### **(h) *Determination of encapsulation ability***

After eclosion of adult flies emerging from parasitised larvae, the adults were killed in the freezer at a temperature of -20°C and stored there until dissection. When adults were dissected, the absence or presence of capsules was noted. Only those individuals were included in the analysis that contained a capsule (parasitised, successful defence/encapsulation). Unparasitised flies (not containing a capsule) and superparasitised flies (inhabiting more than one capsule) were excluded from the analysis.

### **(i) *Determination of egg load and fat content in *Asobara tabida****

See chapter 4.8.4.

### **(j) *Statistical analysis***

See chapter 4.9. The analysis was carried out with yeast species, ambient temperature as well as parasitisation status (no, yes) as linear predictor.

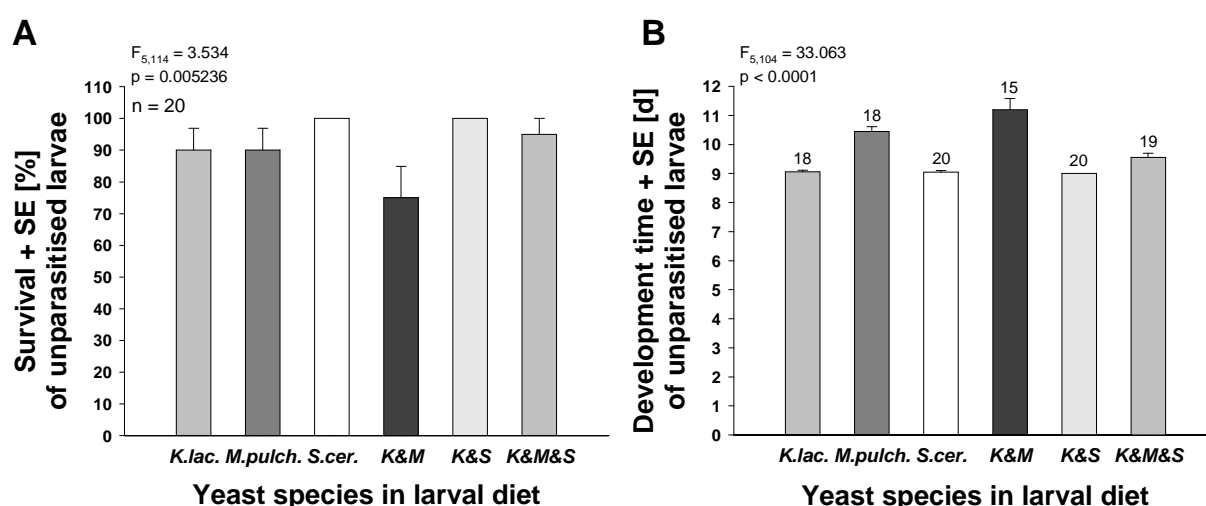
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### 5.5.3 Results

#### Experiment (I):

(a) *Unparasitised larvae*: The dietary yeast species and the combination of particular yeast species offered as a mixed diet, significantly affected the *survival* of unparasitised larvae until eclosion ( $F_{5,114} = 3.534$ ,  $p = 0.005236$ ). Survival was highest for larvae fed on *Saccharomyces cerevisiae* and a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (both  $100 \pm 0.00\%$ ), followed by a mixed diet composed of *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* ( $95.0 \pm 5.00\%$ ), *Kluyveromyces lactis* ( $90.0 \pm 6.88\%$ ), *Metschnikowia pulcherrima* ( $90.0 \pm 6.88\%$ ) and a mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima* ( $75.0 \pm 9.93\%$ ) (**Fig. 32A**).

A significant effect also resulted for the *development time* of unparasitised larvae until maturity ( $F_{5,104} = 33.063$ ,  $p < 0.0001$ ). When larvae either fed on *Kluyveromyces lactis*, *Saccharomyces cerevisiae*, or a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, development time was shortest and more or less similarly so (*K.lac.*:  $9.06 \pm 0.06$ d; *S.cer.*:  $9.05 \pm 0.05$ d; *K.lac.* & *S.cer.*:  $9.00 \pm 0.00$ d). They were followed by larvae fed on a mixed diet composed of *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* ( $9.56 \pm 0.14$ d), as well as larvae fed on *Metschnikowia pulcherrima* alone ( $10.44 \pm 0.17$ d). Larvae fed on a mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima*, took longest to fulfil their development ( $11.20 \pm 0.38$ d) (**Fig. 32B**).



**Fig 32:** **A)** *Survival* (in %) until eclosion **B)** *Development time* (in days) of unparasitised *Drosophila melanogaster* larvae, depending on the dietary yeast species and the yeast species mixture. Shown are mean values + standard error (SE).  $n$  = number of replicates. Numbers above columns in **B)** = number of replicates. Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **S.cer.** = *Saccharomyces cerevisiae*; as well as **K** = *Kluyveromyces lactis*, **M** = *Metschnikowia pulcherrima*, **S** = *Saccharomyces cerevisiae*.

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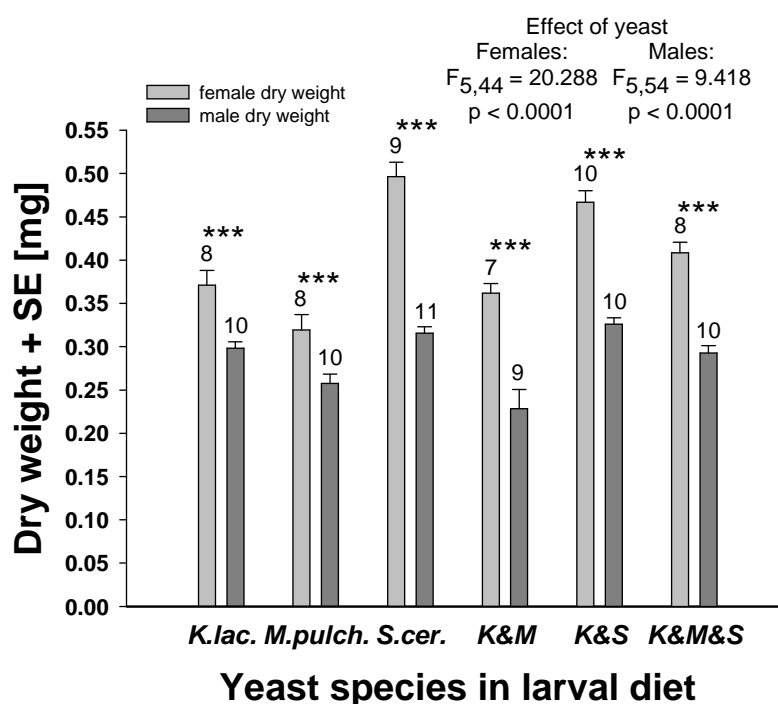
**Dry weight** of female as well as of male adult flies emerging from unparasitised larvae, was significantly affected by the dietary yeast species and the combination of particular yeast species offered as a mixed diet (female dry weight:  $F_{5,44} = 20.288$ ,  $p < 0.0001$ ; male dry weight:  $F_{5,54} = 9.418$ ,  $p < 0.0001$ ). The heaviest females as well as the heaviest males emerged from larvae that had fed on either *Saccharomyces cerevisiae* alone, or a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (female dry weight: *S.cer.*:  $0.4963 \pm 0.0167$ mg, *K.lac.* & *S.cer.*:  $0.4668 \pm 0.0135$ mg; male dry weight: *S.cer.*:  $0.3156 \pm 0.0073$ mg, *K.lac.* & *S.cer.*:  $0.3260 \pm 0.0074$ mg). In female flies these were followed by adults that as larvae had fed on a mixed diet composed of *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* ( $0.4083 \pm 0.0122$ mg), those fed on *Kluyveromyces lactis* alone ( $0.3711 \pm 0.0169$ mg), those fed on a mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima* ( $0.3619 \pm 0.0110$ mg), and finally those fed on *Metschnikowia pulcherrima* ( $0.3193 \pm 0.0178$ mg). In male flies, those fed on *Kluyveromyces lactis* alone, as well as those fed on a mixed diet composed of *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*, eclosed as similarly heavy adults (*K.lac.*:  $0.2981 \pm 0.0076$ mg; *K.lac.* & *M.pulch.* & *S.cer.*:  $0.2927 \pm 0.0084$ mg), followed by male flies fed on *Metschnikowia pulcherrima* ( $0.2578 \pm 0.0107$ mg) and a mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima* ( $0.2283 \pm 0.0222$ mg) (**Fig. 33**).

The influence of dietary yeast species and sex (factors), as well as the interaction between the two factors on dry weight of adult *Drosophila melanogaster* (response variable) can be seen in **Table 19**. The results of the ANCOVA show that the dietary yeast species as well as sex had a global significant influence on adult dry weight. Additionally, the interaction between yeast and sex significantly affected adult dry weight. The relative difference in adult dry weight between female and male flies was, for example, smaller when larvae had fed on *Kluyveromyces lactis* and *Metschnikowia pulcherrima* than when larvae had fed on the other yeast species and yeast species combinations (**Fig. 33**).

**Table 19:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence adult dry weight of *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Dry weight of adult <i>Drosophila melanogaster</i>	Yeast	$F_{5,104} = 29.5382$	$p < 0.0001$
	Sex	$F_{1,103} = 248.1801$	$p < 0.0001$
	Yeast*Sex	$F_{5,98} = 5.9645$	$p < 0.0001$

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**Fig. 33:** *Dry weight* (in mg) of adult flies emerging from unparasitised *Drosophila melanogaster* larvae, depending on the dietary yeast species and the yeast species mixture. Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *S.cer.* = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis*, **M** = *Metschnikowia pulcherrima*, **S** = *Saccharomyces cerevisiae*.

**(b) Parasitised *Drosophila melanogaster* larvae:** The dietary yeast species and the combination of particular yeast species offered as a mixed diet, did not significantly affect *survival* of parasitised larvae until pupariation, though only weakly so ( $F_{5,236} = 2.2195$ ,  $p = 0.05313$ ). Survival was highest when larvae had fed on *Kluyveromyces lactis* ( $90.2 \pm 4.70\%$ ), followed by those fed on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $87.5 \pm 5.30\%$ ), a mixed diet composed of *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* ( $87.2 \pm 5.42\%$ ), *Saccharomyces cerevisiae* alone ( $86.5 \pm 5.70\%$ ), *Metschnikowia pulcherrima* alone ( $73.8 \pm 6.87\%$ ), and finally by those fed on a mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima* ( $67.4 \pm 7.23\%$ ) (**Fig. 34A**).

When comparing *survival until pupariation* between unparasitised and parasitised larvae (**Fig. 34A**), there was a significant difference in survival when larvae had fed on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $F_{1,58} = 6.2041$ ,  $p = 0.01563$ ), on *Metschnikowia pulcherrima* alone ( $F_{1,60} = 4.5295$ ,  $p = 0.03743$ ), as well as on *Saccharomyces cerevisiae* alone ( $F_{1,55} = 6.8046$ ,  $p = 0.01169$ ). When larvae had fed on a mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima*, the difference

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in survival was not significant, though only weakly so ( $F_{1,61} = 3.9972$ ,  $p = 0.05004$ ). Larvae that had fed on *Kluyveromyces lactis* and a mixed diet composed of *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*, did not exhibit a significant difference in their survival until pupariation (*K.lac.*:  $F_{1,59} = 9.0 \times 10^{-4}$ ;  $p = 0.9766$ ; *K&M&S*:  $F_{1,57} = 0.9524$ ,  $p = 0.3332$ ). Survival until pupariation of unparasitised larvae slightly differed from survival until eclosion, due to mortality in the puparial stage when larvae had fed on *Metschnikowia pulcherrima* (survival until pupariation:  $95.0 \pm 5.00\%$ ) and a mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima* (survival until pupariation:  $90.0 \pm 6.88\%$ ) (survival until eclosion see **Fig. 32A**).

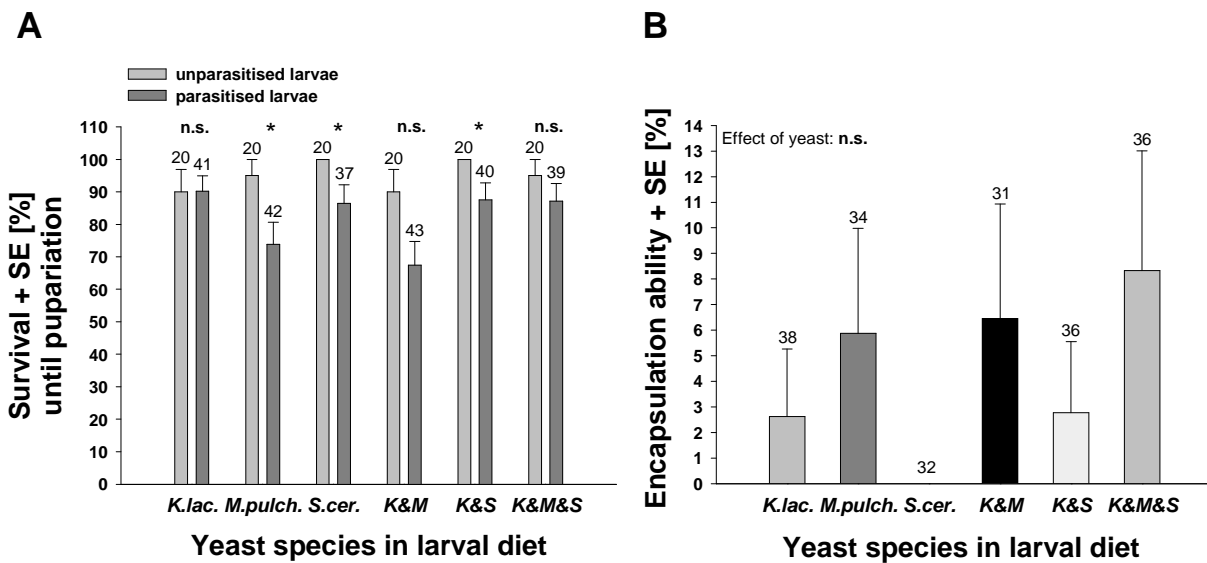
The influence of dietary yeast species and parasitisation status (no, yes) (factors), as well as the interaction between the two factors on survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 20**. The results of the ANCOVA show that the dietary yeast species as well as the parasitisation status had a global significant influence on survival until pupariation. Survival of parasitised larvae was usually lower than that of unparasitised larvae, except when larvae had fed on *Kluyveromyces lactis* where survival was nearly similarly high (**Fig. 34A**).

**Table 20:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until pupariation of unparasitised and parasitised <i>Drosophila melanogaster</i>	Yeast	$F_{5,356} = 2.6240$	$p = 0.0239934$
	Parasitisation	$F_{1,355} = 14.7463$	$p = 0.0001460$
	Yeast*Parasitisation	$F_{5,350} = 1.1056$	$p = 0.3570533$

The *encapsulation ability* of parasitised larvae was not significantly affected by the dietary yeast species and the combination of particular yeast species offered as a mixed diet ( $F_{5,201} = 1.1389$ ,  $p = 0.3411$ ). Surprisingly, encapsulation ability was in general extremely low, especially when compared to the results shown in chapter 5.2 and 5.3. Larvae fed on a mixed diet composed of *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*, were most successful in encapsulating *Asobara tabida* offspring ( $8.3 \pm 4.67\%$ ), followed by those fed on a mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima* ( $6.5 \pm 4.49\%$ ), those fed on *Metschnikowia pulcherrima* alone ( $5.9 \pm 4.10\%$ ), *Kluyveromyces lactis* alone ( $2.6 \pm 2.63\%$ ), and finally by those fed on *Saccharomyces cerevisiae*, who did not succeed in encapsulating a single parasitoid egg or larva ( $0.0 \pm 0.00\%$ ) (**Fig. 34B**).

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**Fig. 34:** **A)** *Survival* (in %) until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae **B)** *Encapsulation ability* (in %) of parasitised larvae, depending on the dietary yeast species and the yeast species mixture. Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **S.cer.** = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis*, **M** = *Metschnikowia pulcherrima*, **S** = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ .

**(c) *Asobara tabida*:** Surprisingly, no adult parasitoid emerged from parasitised larvae in this experiment. Unfortunately, I did not note the number of parasitoids presumably still alive, since at that time I did not know that *Asobara tabida* may enter diapause at an ambient temperature of 25°C, even when the temperature is kept constant.

### Experiment (II):

**(a) Unparasitised *Drosophila melanogaster* larvae:** The dietary yeast species fed to unparasitised larvae had a significant effect on their *survival*, though only at an ambient temperature of 25°C (20°C:  $F_{4,95} = 1.0407$ ,  $p = 0.3904$ ; 25°C:  $F_{4,95} = 2.7366$ ,  $p = 0.03324$ ). Irregardless of the yeast species or combination of yeast species larvae had fed on, their survival was comparably high. Merely when larvae had fed on *Metschnikowia pulcherrima* at an ambient temperature of 25°C, survival was decreased (20°C: *K.lac.*:  $95.0 \pm 5.00\%$ , *M.pulch.*:  $90.0 \pm 6.88\%$ , *P.tol.*:  $95.0 \pm 5.00$ , *S.cer.*:  $100.0 \pm 0.00\%$ , *K.lac.&S.cer.*:  $90.0 \pm 6.88\%$ ; 25°C: *K.lac.*:  $95.0 \pm 5.00\%$ , *M.pulch.*:  $85.0 \pm 8.19\%$ , *P.tol.*:  $95.0 \pm 5.00$ , *S.cer.*:  $100.0 \pm 0.00\%$ , *K.lac.&S.cer.*:  $100.0 \pm 0.00$ ) (**Fig. 35A**). A significant effect of ambient temperature on survival was only found for larvae that had fed on a mixed diet composed of *Kluyveromyces lac-*



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*tis* and *Saccharomyces cerevisiae* ( $F_{1,38} = 5.468$ ,  $p = 0.02473$ ), but not in any of the other dietary treatments (*K.lac.*:  $F_{1,38} = 0$ ,  $p = 1$ , *M.pulch.*:  $F_{1,38} = 0.2184$ ,  $p = 0.6429$ , *P.tol.*:  $F_{1,38} = 0$ ,  $p = 1$ , *S.cer.*:  $F_{1,38} = 0$ ,  $p = 1$ ).

The influence of dietary yeast species and ambient temperature (factors), as well as the interaction between the two factors on survival until eclosion of unparasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 21**. The results of the ANCOVA show that only the dietary yeast species had a weak significant influence on survival until eclosion of unparasitised *Drosophila melanogaster* larvae.

**Table 21:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until eclosion of unparasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until eclosion of unparasitised <i>Drosophila melanogaster</i>	Yeast	$F_{4,195} = 2.5133$	$p = 0.04308$
	Temperature	$F_{1,194} = 0.1349$	$p = 0.71384$
	Yeast*Temperature	$F_{4,190} = 1.0207$	$p = 0.39784$

The **development time** of unparasitised larvae until maturity was at both ambient temperatures significantly affected by the dietary yeast species (**20°C**:  $F_{4,89} = 59.368$ ,  $p < 0.0001$ ; **25°C**:  $F_{4,90} = 37.124$ ,  $p < 0.0001$ ). The development of larvae took in general longer at an ambient temperature of 20°C compared to 25°C. The shortest development time at both temperatures was found in larvae fed on *Pichia toletana* (**20°C**:  $12.8 \pm 0.09$ d; **25°C**:  $8.0 \pm 0.00$ d). They were followed by those fed on *Kluyveromyces lactis* (**20°C**:  $14.2 \pm 0.1754$ d; **25°C**:  $8.7 \pm 0.11$ d) and *Saccharomyces cerevisiae* (**20°C**:  $13.7 \pm 0.15$ d; **25°C**:  $8.8 \pm 0.12$ d), as well as those fed on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (**20°C**:  $13.7 \pm 0.11$ d; **25°C**:  $8.6 \pm 0.11$ d). Larvae fed on *Metschnikowia pulcherrima* took longest to fulfil their development, irregardless of the ambient temperature (**20°C**:  $17.9 \pm 0.56$ d; **25°C**:  $11.6 \pm 0.56$ d) (**Fig. 35B**). In all of the dietary treatments, development time was significantly affected by the ambient temperature (*K.lac.*:  $F_{1,36} = 744.9$ ,  $p < 0.0001$ , *M.pulch.*:  $F_{1,33} = 60.868$ ,  $p < 0.0001$ , *P.tol.*:  $F_{1,36} = 3947.4$ ,  $p < 0.0001$ , *S.cer.*:  $F_{1,38} = 666.04$ ,  $p < 0.0001$ ; *K.lac.* & *S.cer.*:  $F_{1,36} = 988.95$ ,  $p < 0.0001$ ).

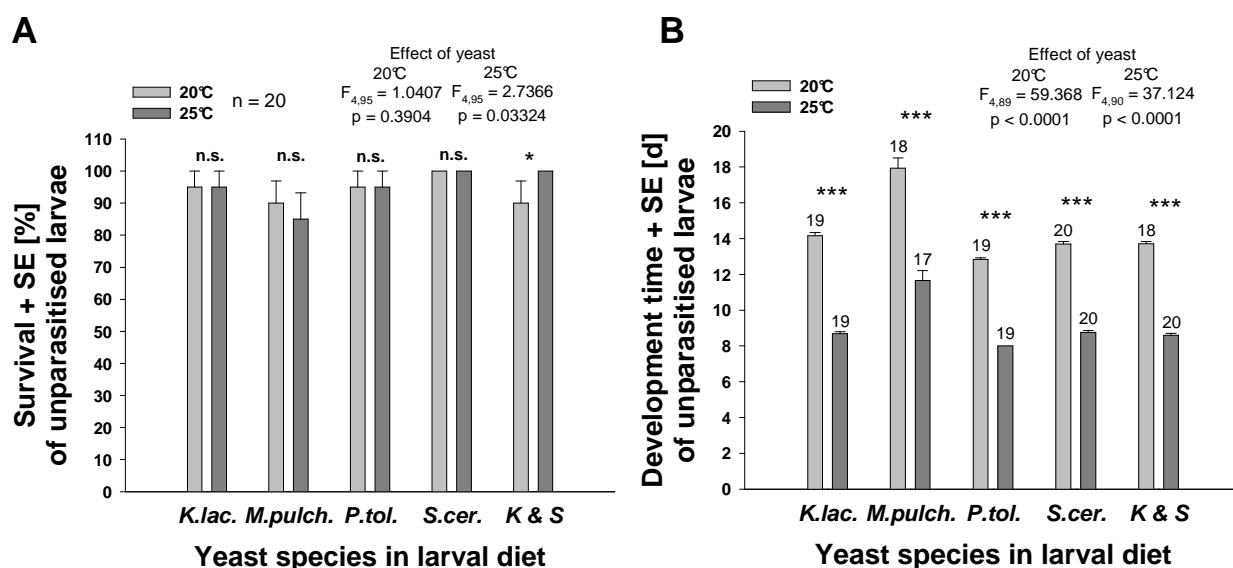
The influence of dietary yeast species and ambient temperature (factors), as well as the interaction between the two factors on development time of unparasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 22**. The results of the ANCOVA show that the dietary yeast species as well as ambient temperature had a global significant influence on development time of unparasitised *Drosophila melanogaster* larvae. At

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an ambient temperature of 20°C, development time was generally longer than at 25°C. The significant influence of the interaction between yeast and temperature on development time indicates that the relative differences in development time between 20°C and 25°C differed among the dietary yeast species. The relative difference in development time of larvae that had fed on *Metschnikowia pulcherrima* was, for example, greater than of larvae that had fed on the other yeast species and yeast species combinations (Fig. 35B).

**Table 22:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence development time of unparasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Development time of unparasitised <i>Drosophila melanogaster</i>	Yeast	$F_{4,184} = 98.924$	$p = 0.0239934$
	Temperature	$F_{1,183} = 1253.768$	$p = 0.0239934$
	Yeast*Temperature	$F_{4,179} = 2.585$	$p = 0.03864$



**Fig. 35:** **A)** Survival (in %) until eclosion **B)** Development time (in days) of unparasitised *Drosophila melanogaster* larvae, depending on the dietary yeast species, the yeast species mixture, and the ambient temperature during larval development (20°C and 25°C). Shown are mean values + standard error (SE). n = number of replicates. Numbers above columns in **B)** = number of replicates. Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **P.tol.** = *Pichia toletana*, **S.cer.** = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis* and **S** = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ , \*\*\*  $p < 0.001$

Neither the dietary yeast species or combination of yeast species, nor the ambient temperature during larval development, had a significant effect on the *mesothorax length* of female adult flies emerging from unparasitised larvae (Effect of yeast: 20°C:  $F_{4,47} = 1.8796$ ,  $p = 0.1297$ ; 25°C:  $F_{4,44} = 1.3216$ ,  $p = 0.2768$ ) (Effect of temperature: *K.lac.*:  $F_{1,17} = 0.4713$ ,  $p = 0.5016$ ,

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*M.pulch.*:  $F_{1,13} = 0.1737$ ,  $p = 0.6836$ , *P.tol.*:  $F_{1,25} = 1.4841$ ,  $p = 0.2345$ , *S.cer.*:  $F_{1,21} = 0.3408$ ,  $p = 0.5656$ ; *K.lac.* & *S.cer.*:  $F_{1,14} = 0.9469$ ,  $p = 0.347$ ). Female adults largely had a similar mesothorax length, irregardless of dietary yeast species and ambient temperature (**20°C**: *K.lac.*:  $1.0558 \pm 0.0292\text{mm}$ , *M.pulch.*:  $0.9756 \pm 0.0578\text{mm}$ , *P.tol.*:  $1.0411 \pm 0.0213\text{mm}$ , *S.cer.*:  $1.0947 \pm 0.0223\text{mm}$ , *K.lac.&S.cer.*:  $1.0851 \pm 0.0396\text{mm}$ ; **25°C**: *K.lac.*:  $1.0837 \pm 0.0282\text{mm}$ , *M.pulch.*:  $1.0031 \pm 0.0227\text{mm}$ , *P.tol.*:  $1.0793 \pm 0.0181\text{mm}$ , *S.cer.*:  $1.0551 \pm 0.0304\text{mm}$ , *K.lac.* & *S.cer.*:  $1.0392 \pm 0.0261\text{mm}$ ) (**Fig. 36A**).

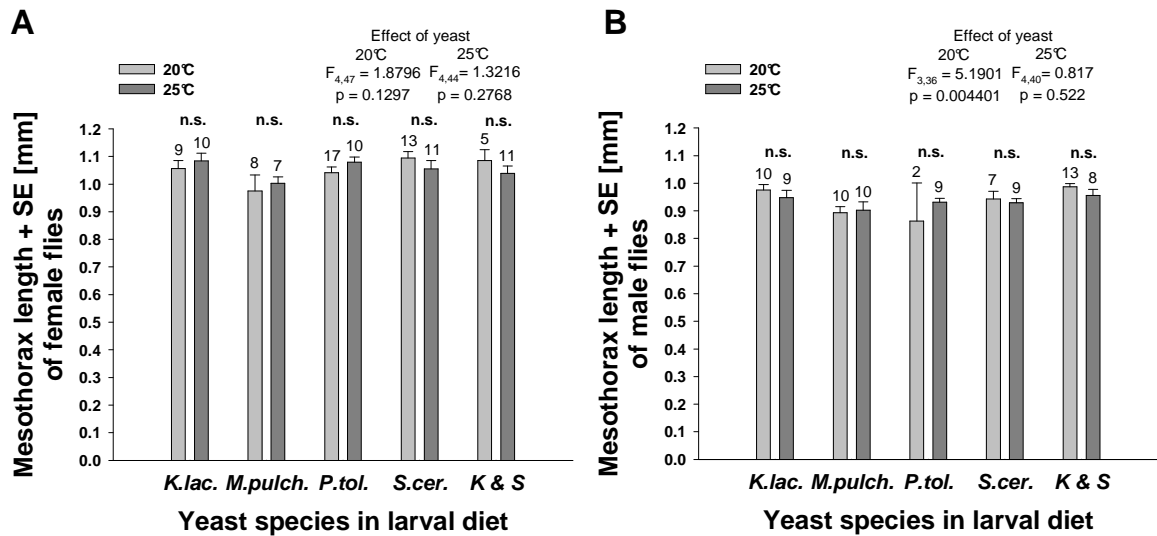
In contrast to females, the *mesothorax length* of *male flies* was significantly affected by the dietary yeast species, though only at an ambient temperature of 20°C (**20°C**:  $F_{4,37} = 3.8834$ ,  $p = 0.009876$ ; *P.tol.* excluded:  $F_{3,36} = 5.1901$ ,  $p = 0.004401$ ; **25°C**:  $F_{4,40} = 0.817$ ,  $p = 0.522$ ), but was not significantly affected by the temperature itself (*K.lac.*:  $F_{1,17} = 0.6668$ ,  $p = 0.4255$ , *M.pulch.*:  $F_{1,18} = 0.0561$ ,  $p = 0.8154$ , *S.cer.*:  $F_{1,14} = 0.2284$ ,  $p = 0.6401$ ; *K.lac.* & *S.cer.*:  $F_{1,19} = 2.1054$ ,  $p = 0.1631$ ) (data for *P.tol.* were not analysed, since there were only two values at 20°C). Males with the shortest mesothorax length emerged from larvae fed on *Metschnikowia pulcherrima* (**20°C**:  $0.8936 \pm 0.0212\text{mm}$ ; **25°C**:  $0.9023 \pm 0.0300\text{mm}$ ) and *Pichia toletana* (**20°C**:  $0.8632 \pm 0.1378\text{mm}$ ; **25°C**:  $0.9317 \pm 0.0141\text{mm}$ ). Males of all the other treatments largely had a similar mesothorax length (**20°C**: *K.lac.*:  $0.9749 \pm 0.0201\text{mm}$ ; *S.cer.*:  $0.9430 \pm 0.0283\text{mm}$ , *K.lac.&S.cer.*:  $0.9876 \pm 0.0111\text{mm}$ ; **25°C**: *K.lac.*:  $0.9478 \pm 0.0268\text{mm}$ , *S.cer.*:  $0.9284 \pm 0.0155\text{mm}$ , *K.lac.* & *S.cer.*:  $0.9557 \pm 0.0217\text{mm}$ ) (**Fig. 36B**).

The influence of dietary yeast species, ambient temperature and sex (factors), as well as the interaction between the three factors on mesothorax length of adult *Drosophila melanogaster* (response variable) can be seen in **Table 23**. The results of the ANCOVA show that the dietary yeast as well as sex had a global significant influence on mesothorax length. Female flies generally exhibited a greater mesothorax length than male flies (**Fig. 36**).

**Table 23:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence mesothorax length of adult *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Mesothorax length of adult <i>Drosophila melanogaster</i>	Yeast	$F_{4,183} = 6.6793$	$p < 0.0001$
	Temperature	$F_{1,182} = 0.6641$	$p = 0.4163$
	Sex	$F_{1,181} = 80.3667$	$p < 0.0001$
	Yeast*Temperature	$F_{4,177} = 1.3280$	$p = 0.2616$
	Yeast*Sex	$F_{4,173} = 1.0201$	$p = 0.3985$
	Temperature*Sex	$F_{1,172} = 0.0230$	$p = 0.8796$
	Yeast*Temperature*Sex	$F_{4,168} = 0.4003$	$p = 0.8082$

## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 36:** *Mesothorax length* (in mm) of adult *Drosophila melanogaster* flies emerging from unparasitised larvae **A**) *Mesothorax length* of female flies and **B**) *Mesothorax length* of male flies, depending on the dietary yeast species, the yeast species mixture, and the ambient temperature during larval development (20°C and 25°C). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: : **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **P.tol.** = *Pichia toletana*, **S.cer.** = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis* and **S** = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant.

The *dry weight of female flies* turned out to be more prone to an influence by the dietary yeast species fed to unparasitised larvae. There was a significant effect of the dietary yeast species at both ambient temperatures (20°C:  $F_{4,47} = 11.838$ ,  $p < 0.0001$ ; 25°C:  $F_{4,44} = 6.0613$ ,  $p < 0.001$ ). For larvae feeding on *Pichia toletana*, also an effect of ambient temperature was found ( $F_{1,25} = 8.822$ ,  $p = 0.006486$ ). Female dry weight in the other dietary yeast treatments was not significantly affected (*K.lac.*:  $F_{1,17} = 0.3712$ ,  $p = 0.5504$ , *M.pulch.*:  $F_{1,13} = 0.4264$ ,  $p = 0.5251$ , *S.cer.*:  $F_{1,22} = 1.3829$ ,  $p = 0.2522$ ; *K.lac.* & *S.cer.*:  $F_{1,14} = 1.8577$ ,  $p = 0.1944$ ). The dry weight of female flies was generally higher at an ambient temperature of 20°C compared to 25°C, irregardless of the dietary yeast treatment. The heaviest females emerged from larvae that had fed on *Saccharomyces cerevisiae* (20°C:  $0.4800 \pm 0.0142$ mg; 25°C:  $0.4478 \pm 0.0245$ mg), a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (20°C:  $0.4804 \pm 0.0145$ mg; 25°C:  $0.4344 \pm 0.0214$ mg) and *Kluyveromyces lactis* (20°C:  $0.4523 \pm 0.0204$ mg; 25°C:  $0.4343 \pm 0.0213$ mg), followed by those fed on *Pichia toletana* (20°C:  $0.4255 \pm 0.0078$ mg; 25°C:  $0.3901 \pm 0.00804$ mg), and *Metschnikowia pulcherrima* (20°C:  $0.3339 \pm 0.0264$ mg; 25°C:  $0.3115 \pm 0.0208$ mg) (**Fig. 37A**).

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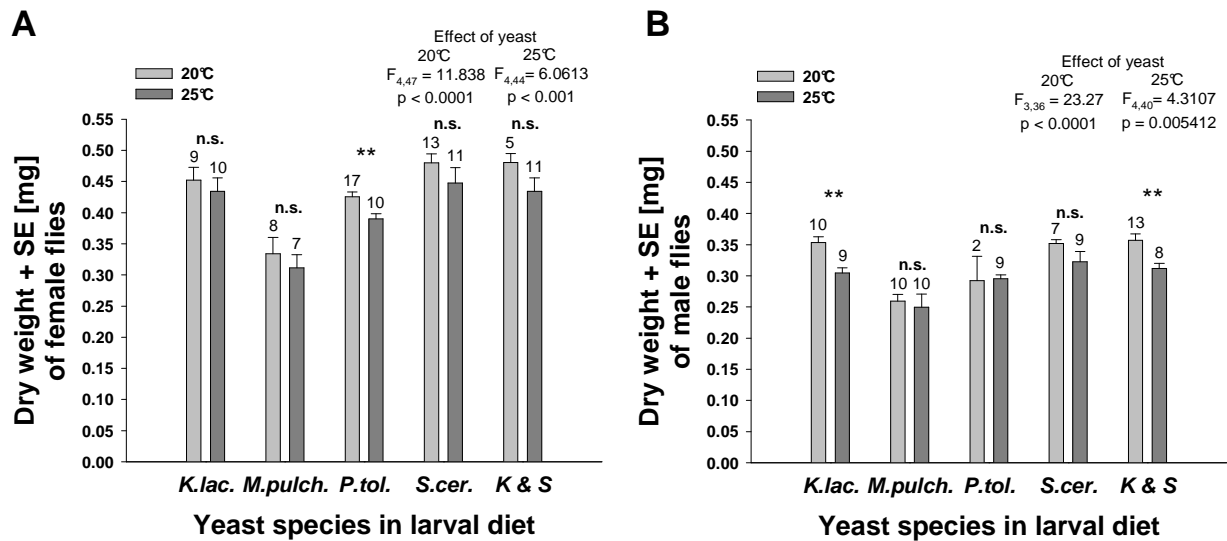
Similar to female flies, *dry weight* of *male flies* was also significantly affected by the dietary yeast species at both ambient temperatures (20°C:  $F_{4,37} = 17.092$ ,  $p < 0.0001$ ; *P.tol.* excluded, since there were only two values:  $F_{3,36} = 23.27$ ,  $p < 0.0001$ ; 25°C:  $F_{4,40} = 4.3107$ ,  $p = 0.005412$ ). In male flies, however, ambient temperature had a significant effect on the dry weight of those adults that as larvae had fed on *Kluyveromyces lactis* ( $F_{1,17} = 15.477$ ,  $p = 0.001070$ ) and on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $F_{1,19} = 10.108$ ,  $p = 0.004937$ ). Male flies of the other treatments were not significantly affected in their dry weight (*M.pulch.*:  $F_{1,18} = 0.1601$ ,  $p = 0.6938$ ; *P.tol.*:  $F_{1,9} = 0.0215$ ,  $p = 0.8867$ ; *S.cer.*:  $F_{1,14} = 2.2447$ ,  $p = 0.1563$ ). Except for larvae fed on *Pichia toletana*, there was a general tendency of dry weight being increased at an ambient temperature of 20°C compared to 25°C. Just as in females, the heaviest males emerged from larvae fed on *Kluyveromyces lactis* (20°C:  $0.3535 \pm 0.00916\text{mg}$ ; 25°C:  $0.3044 \pm 0.008394\text{mg}$ ), *Saccharomyces cerevisiae* (20°C:  $0.3520 \pm 0.00590\text{mg}$ ; 25°C:  $0.3224 \pm 0.0163\text{mg}$ ), and a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (20°C:  $0.3569 \pm 0.0101\text{mg}$ ; 25°C:  $0.3116 \pm 0.00837\text{mg}$ ), followed by those fed on *Pichia toletana* (20°C:  $0.2923 \pm 0.0389\text{mg}$ ; 25°C:  $0.2952 \pm 0.00647\text{mg}$ ), and *Metschnikowia pulcherrima* (20°C:  $0.2593 \pm 0.0111\text{mg}$ ; 25°C:  $0.2498 \pm 0.0208\text{mg}$ ) (Fig. 37B).

The influence of dietary yeast species, ambient temperature and sex (factors), as well as the interaction between the three factors on adult dry weight of *Drosophila melanogaster* (response variable) can be seen in Table 24. The results of the ANCOVA show that the dietary yeast species, ambient temperature as well as sex had a global and highly significant influence on dry weight. At an ambient temperature of 20°C dry weight was generally higher than at 25°C. Additionally, female flies were generally heavier than male flies (Fig. 37).

**Table 24:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence adult dry weight of *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Dry weight of adult <i>Drosophila melanogaster</i>	Yeast	$F_{4,183} = 38.5923$	$p < 0.0001$
	Temperature	$F_{1,182} = 20.2289$	$p < 0.0001$
	Sex	$F_{1,181} = 205.0518$	$p < 0.0001$
	Yeast*Temperature	$F_{4,177} = 0.5103$	$p = 0.72820$
	Yeast*Sex	$F_{4,173} = 2.2462$	$p = 0.06617$
	Temperature*Sex	$F_{1,172} = 0.0298$	$p = 0.86315$
	Yeast*Temperature*Sex	$F_{4,168} = 0.4870$	$p = 0.74526$

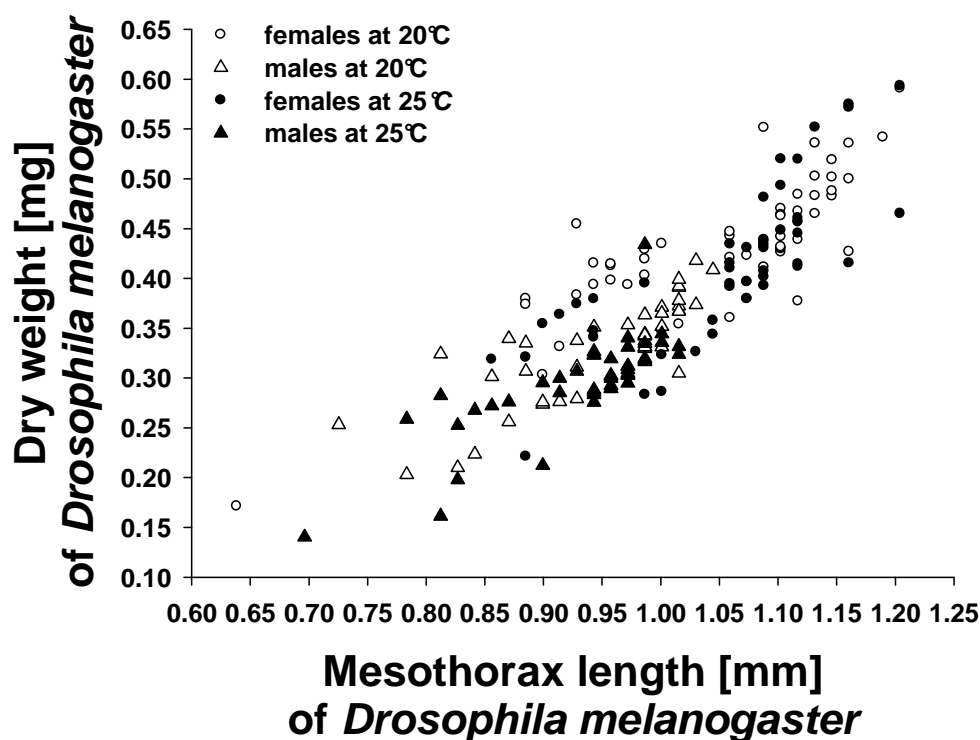
## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 37:** Dry weight (in mg) of adult *Drosophila melanogaster* flies emerging from unparasitised larvae **A)** Dry weight of female flies **B)** Dry weight of male flies, depending on the dietary yeast species, the yeast species mixture, and the ambient temperature during larval development (20°C and 25°C). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **P.tol.** = *Pichia toletana*, **S.cer.** = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis* and **S** = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant. Significance levels: n.s. = not significant, \*\*  $p < 0.01$ .

A correlation between adult dry weight and mesothorax length of female as well as male *Drosophila melanogaster* at both environmental temperatures and over all dietary yeast species, yielded a significant positive correlation between the two traits (Pearson's correlation coefficient  $R^2 = 0.854$ ,  $p < 0.0001$ ) (**Fig. 38**). Thus, although it could be assumed that small flies may possess a comparably high weight, the same as large flies may be thinner and exhibit a comparably low weight, a significant positive correlation between body size (measured as mesothorax length) and body weight (measured as dry weight) of adult flies may hold true for more than 85% of all cases, even across a range of different environmental conditions (nutrition & temperature).

## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 38:** Scatter plot of individual values of mesothorax length (in mm) versus dry weight (in mg) of female as well as male *Drosophila melanogaster* at both environmental temperatures (20°C and 25°C) and over all dietary yeast species.

**(b) Parasitised *Drosophila melanogaster* larvae:** The dietary yeast species significantly affected the *survival* of parasitised larvae until pupariation at both ambient temperatures (20°C:  $F_{4,143} = 9.1721$ ,  $p < 0.0001$ ; 25°C:  $F_{4,129} = 11.024$ ,  $p < 0.0001$ ). The ambient temperature itself had no significant effect on survival (*K.lac.*:  $F_{1,56} = 0.8869$ ,  $p = 0.3504$ ; *M.pulch.*:  $F_{1,64} = 1.1928$ ,  $p = 0.2789$ ; *P.tol.*:  $F_{1,51} = 1.1147$ ,  $p = 0.2960$ ; *S.cer.*:  $F_{1,53} = 0.8909$ ,  $p = 0.3495$ ; *K.lac.* & *S.cer.*:  $F_{1,48} = 0.3891$ ,  $p = 0.5357$ ). In treatments with a difference in survival between the two ambient temperatures, survival was higher at 20°C compared to 25°C. Survival was mostly rather high in all of the dietary yeast treatments (20°C: *K.lac.*:  $93.3 \pm 4.63\%$ , *P.tol.*:  $92.0 \pm 5.54\%$ , *S.cer.*:  $81.8 \pm 6.82\%$ , *K.lac.* & *S.cer.*:  $70.4 \pm 8.96\%$ ; 25°C: *K.lac.*:  $85.7 \pm 6.73\%$ , *P.tol.*:  $82.1 \pm 7.37\%$ , *S.cer.*:  $90.9 \pm 6.27\%$ , *K.lac.* & *S.cer.*:  $78.3 \pm 8.79\%$ ), with the exception of larvae fed on *Metschnikowia pulcherrima* that surprisingly exhibited a decreased survival even at 25°C (20°C:  $33.3 \pm 8.33\%$ ; 25°C:  $21.2 \pm 7.23\%$ ) (**Fig. 39A & B**).

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When comparing *survival until pupariation* between unparasitised and parasitised larvae at an ambient temperature of 20°C, a significant difference was found in survival of larvae fed on *Metschnikowia pulcherrima* ( $F_{1,51} = 17.308$ ,  $p = 0.0001221$ ) and those fed on *Saccharomyces cerevisiae* ( $F_{1,51} = 9.4934$ ,  $p = 0.003321$ ), but not in larvae fed on *Kluyveromyces lactis* ( $F_{1,48} = 0.0579$ ,  $p = 0.8108$ ), *Pichia toletana* ( $F_{1,43} = 0.1573$ ,  $p = 0.6936$ ) and a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $F_{1,45} = 2.7146$ ,  $p = 0.1064$ ). At an ambient temperature of 25°C survival between unparasitised and parasitised larvae fed on *Metschnikowia pulcherrima* ( $F_{1,51} = 21.157$ ,  $p < 0.0001$ ), *Saccharomyces cerevisiae* ( $F_{1,40} = 4.8678$ ,  $p = 0.03317$ ), as well as a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $F_{1,41} = 12.170$ ,  $p = 0.001174$ ), differed significantly. This was again not the case for larvae fed on *Kluyveromyces lactis* ( $F_{1,46} = 1.1218$ ,  $p = 0.2951$ ) and *Pichia toletana* ( $F_{1,46} = 1.8715$ ,  $p = 0.1779$ ). Survival of unparasitised larvae until pupariation was hereby similar to survival until eclosion (see **Fig. 35A**), since mortality only occurred prior to pupariation.

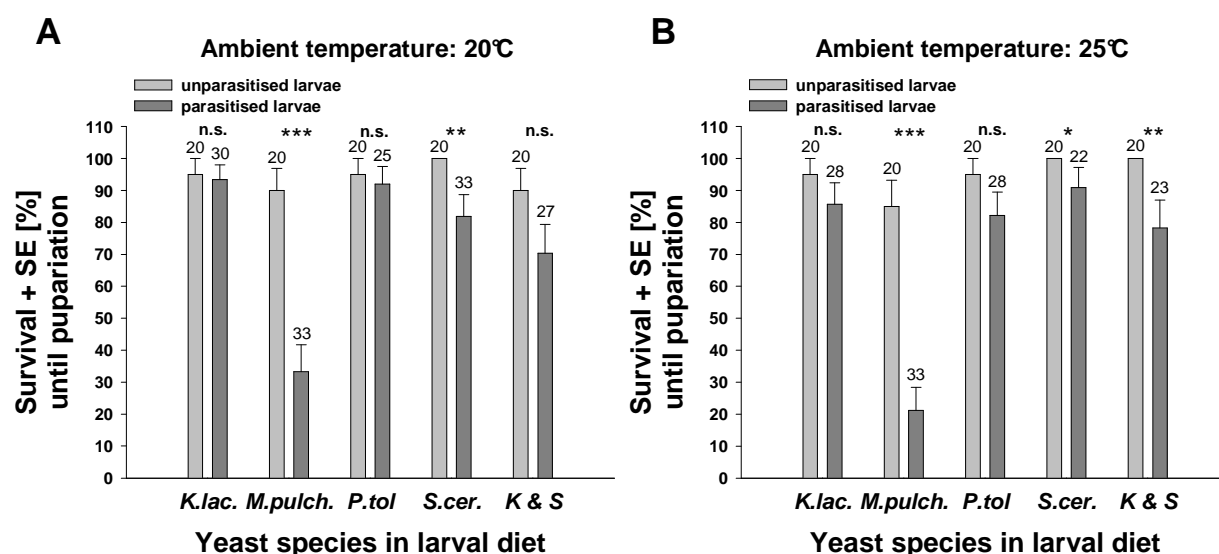
The influence of dietary yeast species, ambient temperature and parasitisation status (no, yes) (factors), as well as the interaction between the three factors on survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 25**. The results of the ANCOVA show that merely the dietary yeast species as well as the parasitisation status had a highly significant influence on survival. Survival of unparasitised larvae was generally higher than that of parasitised larvae (**Fig. 39**).

**Table 25:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until pupariation of unparasitised and parasitised <i>Drosophila melanogaster</i> larvae	Yeast	$F_{4,477} = 20.6124$	$p < 0.0001$
	Temperature	$F_{1,476} = 0.1079$	0.74267
	Parasitisation	$F_{1,475} = 57.3633$	$p < 0.0001$
	Yeast*Temperature	$F_{4,471} = 1.3668$	0.24444
	Yeast* Parasitisation	$F_{4,467} = 1.9980$	0.09378
	Temperature* Parasitisation	$F_{1,466} = 1.1812$	0.27768
	Yeast*Temperature* Parasitisation	$F_{4,462} = 0.3730$	0.82790



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**Fig. 39:** *Survival* (in %) until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae **A**) at an ambient temperature of 20°C and **B**) at an ambient temperature of 25°C, depending on the dietary yeast species and the yeast species mixture. Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **P.tol.** = *Pichia toletana*, **S.cer.** = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis* and **S** = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

At an ambient temperature of 25°C, the dietary yeast species had a significant effect on the *encapsulation ability* of parasitised larvae ( $F_{4,93} = 2.7328$ ,  $p = 0.03357$ ), though not at 20°C ( $F_{4,100} = 1.317$ ,  $p = 0.2688$ ). There was also a significant effect on encapsulation ability in larvae fed on *Saccharomyces cerevisiae* regarding ambient temperature ( $F_{1,42} = 6.6835$ ,  $p = 0.01329$ ). Temperature did not significantly affect encapsulation of larvae fed on the other dietary yeast species and combination of yeasts (*K.lac.*:  $F_{1,47} = 1.2595$ ,  $p = 0.2674$ ; *M.pulch.*:  $F_{1,25} = 0.0145$ ,  $p = 0.9053$ ; *P.tol.*:  $F_{1,43} = 2.9808$ ,  $p = 0.09144$ ; *K.lac.* & *S.cer.*:  $F_{1,36} = 0.1664$ ,  $p = 0.6858$ ). Encapsulation ability was highest in larvae fed on *Metschnikowia pulcherrima*, with only a slight difference between the two ambient temperatures (20°C:  $30.8 \pm 13.32\%$ ; 25°C:  $28.6 \pm 12.53\%$ ). Larvae fed on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, were also quite successful in encapsulating *Asobara tabida* offspring, and here they were more successful at an ambient temperature of 25°C compared to 20°C (20°C:  $15.8 \pm 8.59\%$ ; 25°C:  $21.1 \pm 9.61\%$ ). Larvae fed on *Kluyveromyces lactis* and *Pichia toletana* exhibited a similar encapsulation ability that was generally higher at the ambient temperature of 25°C (*K.lac.*: 20°C:  $7.4 \pm 5.14\%$ ; 25°C:  $18.2 \pm 8.42\%$ ; *P.tol.*: 20°C:  $4.5 \pm 4.55\%$ ; 25°C:  $21.7 \pm 8.79\%$ ). Surprisingly, the encapsulation ability of larvae fed on *Saccharomyces cerevisiae* was comparably high at an ambient temperature of 20°C ( $12.5 \pm$

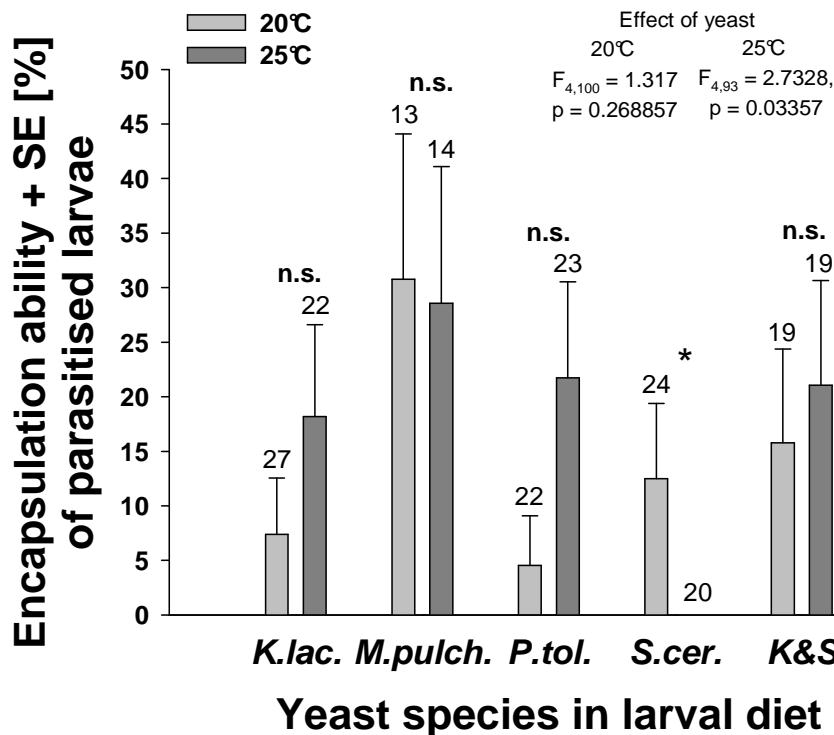
## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

6.90%). At 25°C, however, larvae were not able to encapsulate a single parasitoid ( $0.0 \pm 0.00\%$ ) (Fig. 40).

The influence of dietary yeast species and ambient temperature (factors), as well as the interaction between the two factors on the encapsulation ability of parasitised *Drosophila melanogaster* larvae (response variable) can be seen in Table 26. The results of the ANCOVA did not yield any significant effects of the above factors on encapsulation ability.

**Table 26:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the encapsulation ability of parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Encapsulation ability of parasitised <i>Drosophila melanogaster</i>	Yeast	$F_{4,198} = 1.9115$	$p = 0.11008$
	Temperature	$F_{1,197} = 0.8549$	$p = 0.35633$
	Yeast*Temperature	$F_{4,193} = 2.0124$	$p = 0.09429$



**Fig. 40:** Encapsulation ability (in %) of parasitised *Drosophila melanogaster* larvae, depending on the dietary yeast species, the yeast species mixture, and the ambient temperature during larval development (20°C and 25°C). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*, as well as *K* = *Kluyveromyces lactis* and *S* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ .

## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

(c) *Asobara tabida*: Just as in **Experiment (I)**, no adult *Asobara tabida* emerged at an ambient temperature of 25°C. This time, however, puparia were checked and the number of presumably diapausing parasitoids noted. In comparison to 25°C, where 100% of the parasitoids entered diapause, irregardless of the dietary yeast treatment, the proportion of parasitoids that also entered diapause at 20°C, differed significantly among the different dietary yeast treatments ( $F_{4,23} = 4.3122$ ,  $p = 0.00951$ ) (*K.lac.*:  $19.0 \pm 0.00\%$ , *M.pulch.*:  $20.2 \pm 0.45\%$ , *P.tol.*:  $0.0 \pm 0.00\%$ , *S.cer.*:  $22.2 \pm 0.40\%$ , *K.lac.* & *S.cer.*:  $6.25 \pm 0.58\%$ ).

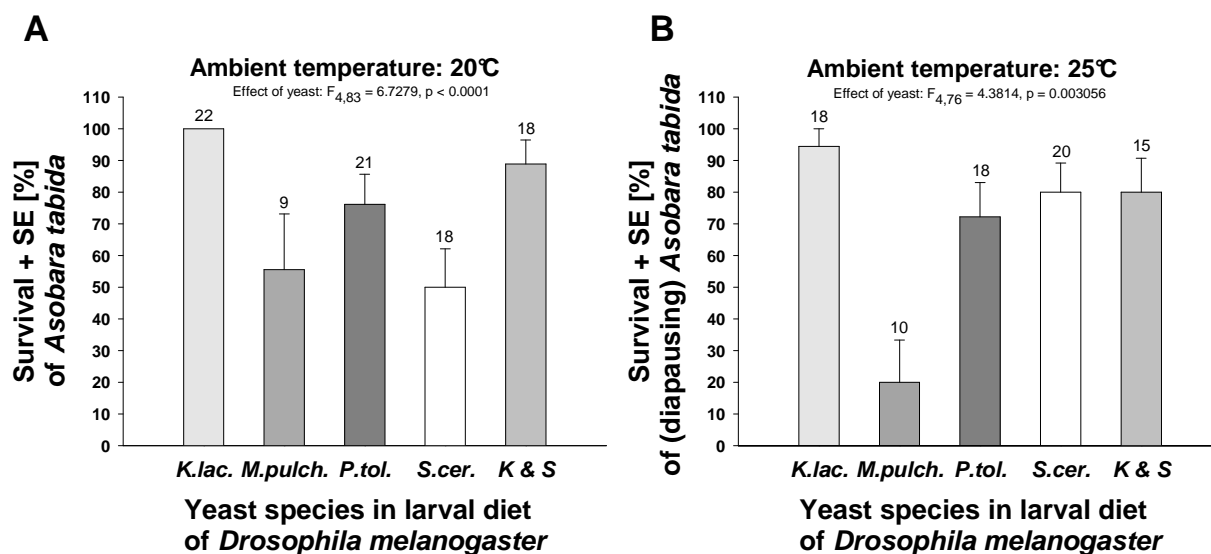
The influence of dietary yeast species and ambient temperature (factors), as well as the interaction between the two factors on survival of diapausing *Asobara tabida* (response variable) can be seen in **Table 27**. The results of the ANCOVA show that the dietary yeast species of *Drosophila melanogaster* host larvae as well as ambient temperature had a global significant influence on survival of diapausing *Asobara tabida*.

**Table 27:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival of diapausing *Asobara tabida*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival of diapausing <i>Asobara tabida</i>	Yeast	$F_{4,104} = 6.5023$	$p = 0.0001092$
	Temperature	$F_{1,103} = 17.6306$	$p < 0.0001$
	Yeast*Temperature	$F_{4,99} = 1.7281$	$p = 0.1497843$

The dietary yeast species had a significant effect on the *survival* of parasitoids that entered diapause at an ambient temperature of 25°C ( $F_{4,76} = 4.3814$ ,  $p = 0.003056$ ), as well as on the survival of parasitoids that emerged as adults at an ambient temperature of 20°C ( $F_{4,83} = 6.7279$ ,  $p < 0.0001$ ). At an ambient temperature of 20°C, parasitoids whose host larvae had fed on *Kluyveromyces lactis* alone, as well as a on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, exhibited the highest survival until eclosion as adult parasitoids (*K.lac.*:  $100.0 \pm 0.00\%$ ; *K.lac.* & *S.cer.*:  $88.9 \pm 7.62\%$ ), followed by those whose host larvae had fed on *Pichia toletana* ( $76.2 \pm 9.52\%$ ), *Metschnikowia pulcherrima* ( $55.6 \pm 17.57\%$ ), and *Saccharomyces cerevisiae* ( $50.0 \pm 12.13\%$ ). At an ambient temperature of 25°C, parasitoids whose host larvae had fed on *Kluyveromyces lactis* and *Saccharomyces cerevisiae* alone, as well as on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, exhibited the highest survival until prepupation (*K.lac.*:  $94.4 \pm 5.56\%$ ; *S.cer.*:  $80. \pm 9.18\%$ ; *K.lac.* & *S.cer.*:  $80.0 \pm 10.69\%$ ), followed by those, whose host larvae had fed on *Pichia toletana* ( $72.2 \pm 10.86\%$ ) and *Metschnikowia pulcherrima* ( $20.0 \pm 13.33\%$ ). I did not test for an effect of ambient temperature on survival, since it did not seem correct to me, to compare the data of diapausing parasitoids with that of eclosed ones (**Fig. 41A & B**).

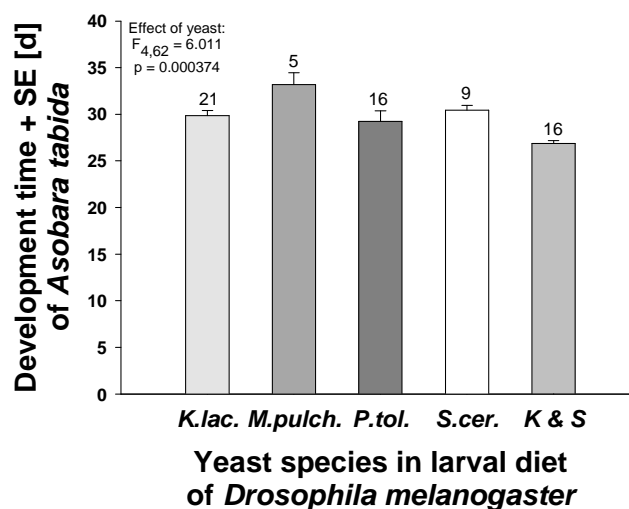
## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 41:** *Survival* (in %) of *Asobara tabida* **A**) Survival until eclosion at an ambient temperature of 20°C **B**) Survival until prepupation (late 3<sup>rd</sup> instar) at an ambient temperature of 25°C, depending on the dietary yeast species and the dietary yeast species mixture of their host larvae (*Drosophila melanogaster*). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*, as well as *K* = *Kluyveromyces lactis* and *S* = *Saccharomyces cerevisiae*.

The *development time* of parasitoids until eclosion as adults at an ambient temperature of 20°C was also significantly affected by the dietary yeast species ( $F_{4,62} = 6.011$ ,  $p = 0.000374$ ). Development time was shortest for parasitoids whose larvae had fed on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $26.9 \pm 0.30$ d), followed by those whose host larvae had fed on *Pichia toletana* ( $29.3 \pm 1.11$ d), *Kluyveromyces lactis* ( $29.9 \pm 0.54$ d), *Saccharomyces cerevisiae* ( $30.4 \pm 0.50$ d), and *Metschnikowia pulcherrima* ( $33.2 \pm 1.24$ d) (**Fig. 42**).

## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

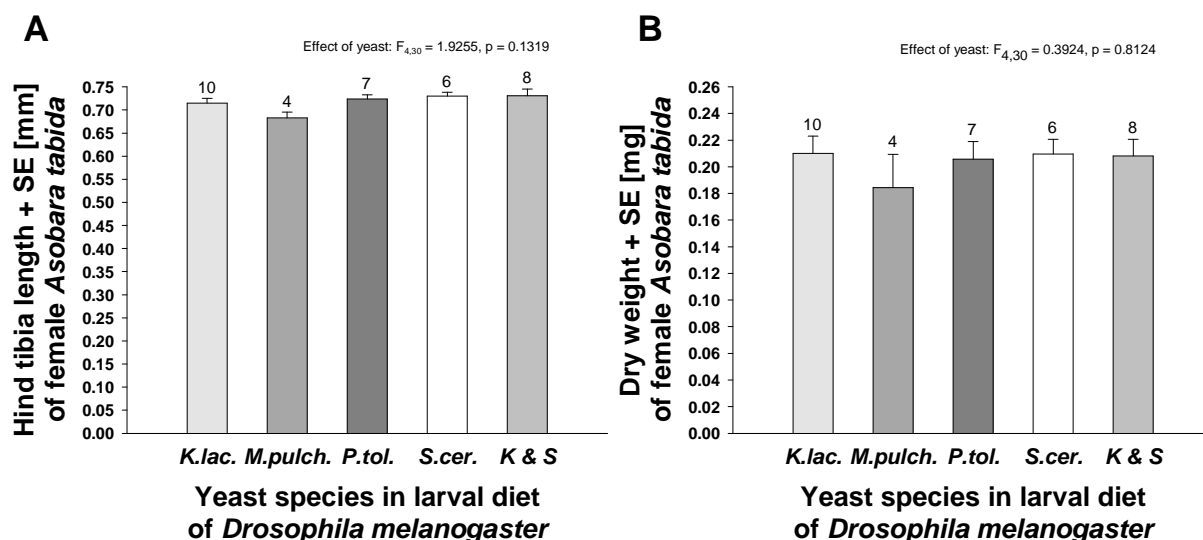


**Fig. 42:** *Development time* (in days) of *Asobara tabida* at an ambient temperature of 20°C, depending on the dietary yeast species and the dietary yeast species mixture of their host larvae (*Drosophila melanogaster*). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **P.tol.** = *Pichia toletana*, **S.cer.** = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis* and **S** = *Saccharomyces cerevisiae*.

The body size of female *Asobara tabida*, measured as *hind tibia length*, was not significantly influenced by the dietary yeast species of their host larvae ( $F_{4,30} = 1.9255$ ,  $p = 0.1319$ ). *Asobara tabida* females whose host larvae had fed on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, had the longest hind tibiae ( $0.7310 \pm 0.0144$ mm), followed by those whose host larvae had fed on *Saccharomyces cerevisiae* alone ( $0.7301 \pm 0.00814$ mm), *Pichia toletana* ( $0.7239 \pm 0.0090$ mm), *Kluyveromyces lactis* ( $0.7149 \pm 0.0105$ mm) and *Metschnikowia pulcherrima* ( $0.6831 \pm 0.0123$ mm) (**Fig. 43A**).

Similarly, the body size of female *Asobara tabida*, measured as *dry weight*, was also not significantly influenced by the dietary yeast species of host larvae ( $F_{4,30} = 0.3924$ ,  $p = 0.8124$ ). Here, female *Asobara tabida* whose host larvae had fed on *Kluyveromyces lactis* ( $0.2101 \pm 0.0130$ mg) exhibited the highest dry weight, followed by those whose larvae had fed on *Saccharomyces cerevisiae* ( $0.2096 \pm 0.0111$ mg), a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $0.2083 \pm 0.0124$ mg), *Pichia toletana* ( $0.2058 \pm 0.0131$ mg), and *Metschnikowia pulcherrima* ( $0.1843 \pm 0.0250$ mg) (**Fig. 43B**).

## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



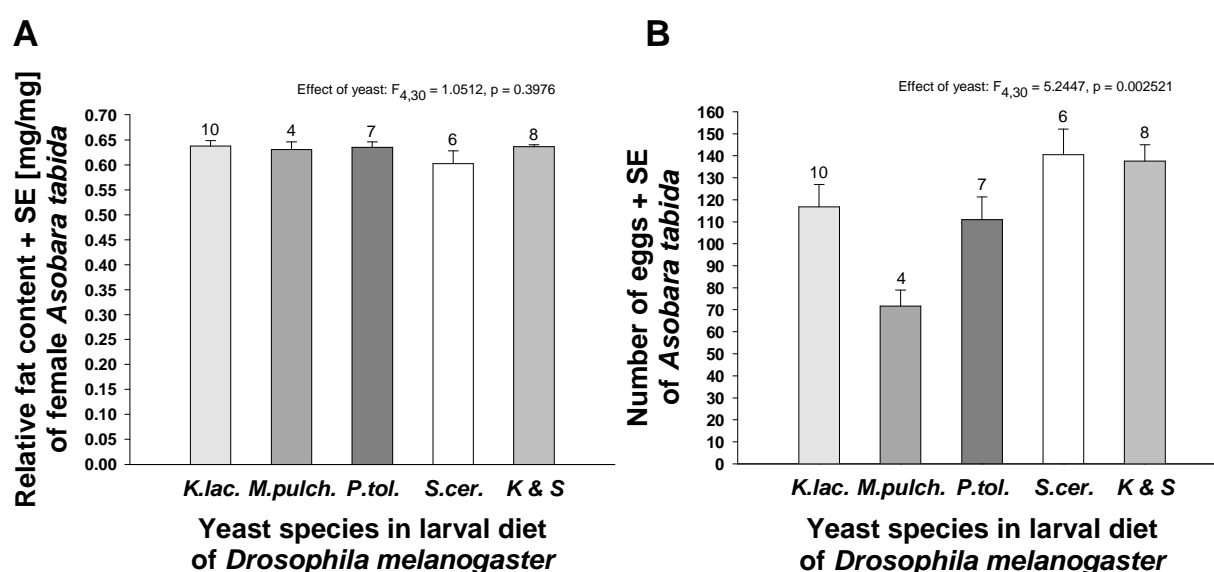
**Fig. 43:** **A)** *Hind tibia length* (in mm) and **B)** *Dry weight* (in mg) of female *Asobara tabida* at an ambient temperature of 20°C, depending on the dietary yeast species and the dietary yeast species mixture of their host larvae (*Drosophila melanogaster*). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*, **M.pulch.** = *Metschnikowia pulcherrima*, **P.tol.** = *Pichia toletana*, **S.cer.** = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis* and **S** = *Saccharomyces cerevisiae*.

The dietary yeast species of *Drosophila melanogaster* host larvae did not significantly affect the **relative fat content** of female *Asobara tabida* ( $F_{4,30} = 1.0512$ ,  $p = 0.3976$ ). Those *Asobara tabida* females had the highest relative fat content, whose host larvae had fed on *Kluyveromyces lactis* ( $0.6372 \pm 0.0113$ mg), followed by those whose host larvae had fed on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $0.6364 \pm 0.0034$ mg), *Pichia toletana* ( $0.6348 \pm 0.0111$ mg), *Metschnikowia pulcherrima* ( $0.6306 \pm 0.0156$ mg), and *Saccharomyces cerevisiae* ( $0.6023 \pm 0.0255$ mg) (**Fig. 44A**).

The absolute **number of eggs** of freshly hatched virgin *Asobara tabida*, however, was significantly influenced by the dietary yeast species of the *Drosophila melanogaster* host larvae ( $F_{4,30} = 5.2447$ ,  $p = 0.002521$ ). Females whose host larvae had fed on *Saccharomyces cerevisiae* carried the highest number of eggs ( $140.5 \pm 11.61$ ), followed by those fed on a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* ( $137.6 \pm 7.38$ ), *Kluyveromyces lactis* ( $116.8 \pm 10.16$ ), *Pichia toletana* ( $111.0 \pm 10.24$ ), and *Metschnikowia pulcherrima* ( $71.8 \pm 7.25$ ) (**Fig. 44B**).

## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*

The absolute number of eggs was corrected for a potential influence of the size parameters hind tibia length and dry weight. For this, the absolute number of eggs of each *Asobara tabida* female was divided by the hind tibia length of the same female on the one hand and by the dry weight of the individual female on the other hand. The graphs very much resembled that of the absolute number of eggs and are therefore not shown here. The results of a statistical analysis with number of eggs per hind tibia length as well as number of eggs per dry weight both also yielded a significant influence of dietary yeast species (number of eggs per hind tibia length:  $F_{4,30} = 4.5985$   $p = 0.005142$ ; number of eggs per dry weight:  $F_{4,30} = 3.1292$ ,  $p = 0.02898$ ).



**Fig. 44:** **A)** *Relative fat content* (in mg per mg dry weight) and **B)** *Number of eggs* of female *Asobara tabida* at an ambient temperature of 20°C, depending on the dietary yeast species and the dietary yeast species mixture of their host larvae (*Drosophila melanogaster*). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*, as well as *K* = *Kluyveromyces lactis* and *S* = *Saccharomyces cerevisiae*.

A linear regression between the number of eggs (dependent variable) and hind tibia length (independent variable) of female *Asobara tabida* revealed a significant positive correlation between the two traits ( $R^2 = 0.1664$ ,  $F_{1,33} = 7.787$ ,  $t = 2.790$ ,  $p = 0.0086$ ) (**Fig. 45A**). The number of eggs did, however, not positively correlate with dry weight ( $R^2 = 0.06679$ ,  $F_{1,33} = 3.433$ ,  $t = 1.853$ ,  $p = 0.07286$ ) (**Fig. 45B**). Absolute fat content also positively correlated with hind tibia length ( $R^2 = 0.3351$ ,  $F_{1,33} = 18.14$ ,  $t = 4.259$ ,  $p = 0.0001606$ ) (**Fig. 46A**).

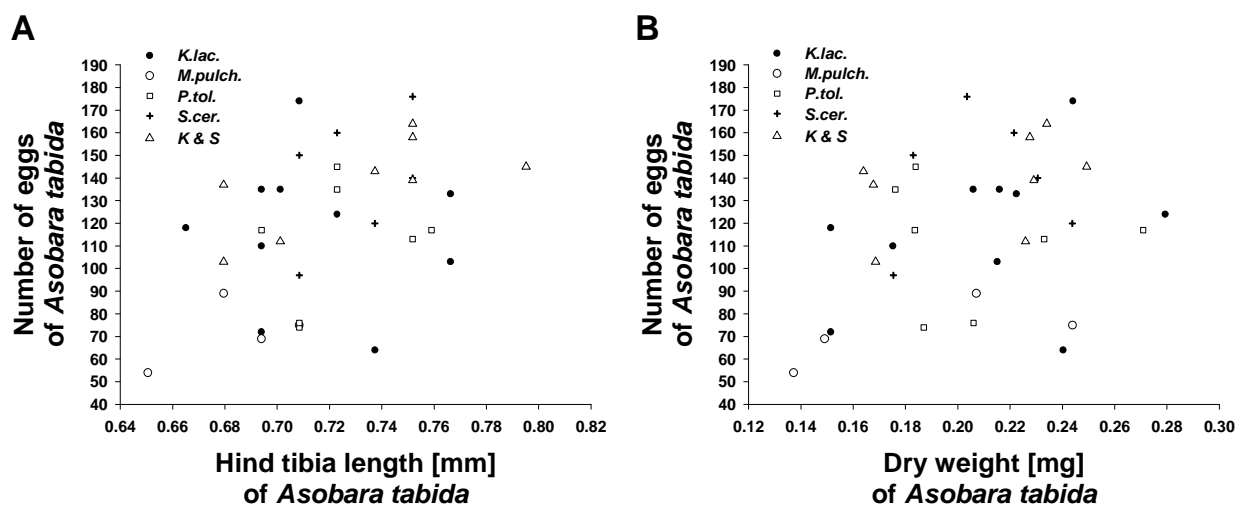
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Due to the positive correlation between absolute fat content and hind tibia length, as well as the number of eggs and hind tibia length, a multiple regression was carried out, with absolute fat content as dependent variable and hind tibia length as well as number of eggs as independent variables. It did not show a negative correlation between the number of eggs and the absolute fat content (Table 28).

**Table 28:** Multiple regression of number of eggs and hind tibia length on absolute fat content of *Asobara tabida* females, whose host larvae had fed on different dietary yeast species.  $F_{2,32} = 8.840$ ,  $R^2 = 0.316$ ,  $p = 0.001$ .

Character	Coefficient	Std. error	t-value
Constant	- 0.164	0.74	- 2.204
Number of eggs	0.00002677	0.000	0.245
Hind tibia [mm]	0.403	0.110	3.669***

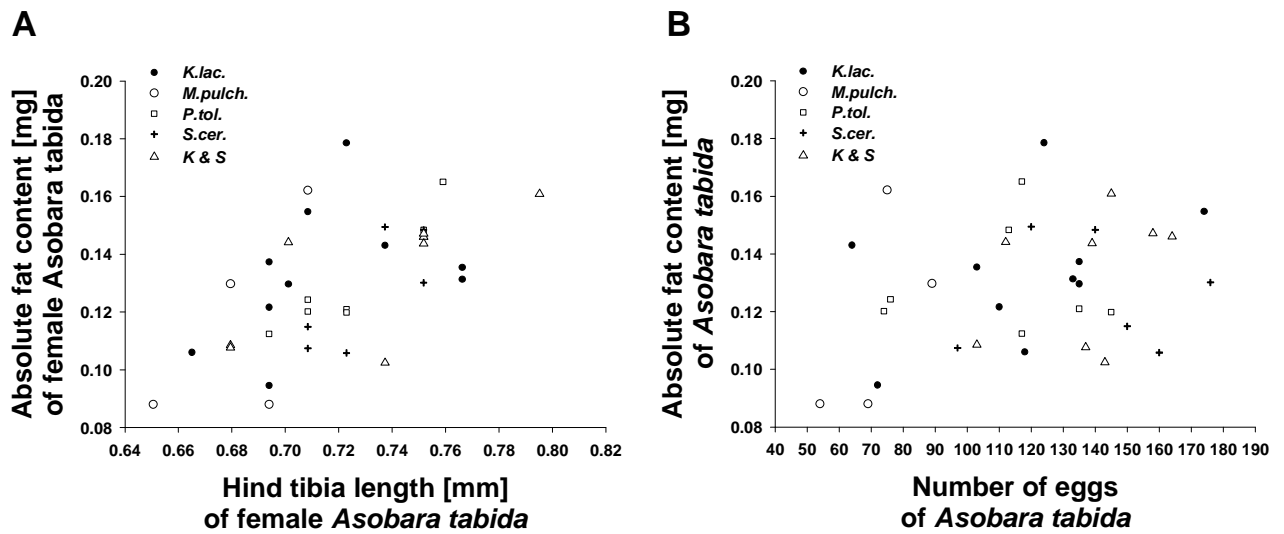
\*\*\*  $p < 0.001$



**Fig. 45:** Scatter plots of individual values of **A**) Hind tibia length (in mm) versus number of eggs and **B**) Dry weight (in mg) versus number of eggs of female *Asobara tabida* over all dietary yeast species. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*, as well as *K* = *Kluyveromyces lactis* and *S* = *Saccharomyces cerevisiae*.



## 5.5 Influence of a mixed microbial diet on fitness traits of *Drosophila melanogaster* and *Asobara tabida*



**Fig. 46:** Scatter plots of individual values of **A**) Hind tibia length (in mm) versus absolute fat content (in mg) and **B**) Number of eggs versus absolute fat content (in mg) of female *Asobara tabida* over all dietary yeast species. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*, as well as **K** = *Kluyveromyces lactis* and **S** = *Saccharomyces cerevisiae*.

### 5.5.4 Discussion

The results of the two experiments allow drawing the following conclusions: (i) A mixture of yeast species in the larval diet of *Drosophila melanogaster* may not necessarily pose a beneficial influence on fitness traits of *Drosophila melanogaster* and *Asobara tabida* compared to singular yeast species. Whether a mixture poses a favourable or unfavourable influence, strongly depends on which yeast species are offered in combination. (ii) The quality of a diet composed of a single yeast species which does not pose a good food source for *Drosophila melanogaster*, may not be enhanced by mixing with a qualitatively higher yeast species; the quality may even be impaired. (iii) There is a tendency that the quality of specific yeast species (and yeast species mixtures) for *Drosophila melanogaster* and *Asobara tabida* varies depending on the ambient temperature. (iv) As has already been shown in the former chapter, not all measured fitness traits may experience a similar (favourable or unfavourable) influence by a specific yeast species or yeast species mixture. (v) The already observed tendency of *Asobara tabida* to perform better on yeast species beneficial to fitness traits of *Drosophila melanogaster* host larvae may be modulated by ambient temperature and may not hold true for all measured fitness traits.

The significant differences in fitness traits (survival, development time, male size measured as mesothorax length, body weight measured as dry weight of adult flies) observed among unparasitised *Drosophila melanogaster* larvae were mainly due to the dietary yeast

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species *Metschnikowia pulcherrima*, that had already in former chapters been shown to be rather unfavourable for the performance of *Drosophila melanogaster* larvae. Significant differences in some fitness traits (development time and male dry weight) were also due to *Pichia toletana*, which at an ambient temperature of 20°C appeared to favour a significantly shorter development time, being, however, accompanied by significantly reduced dry weight in male *Drosophila melanogaster*. This had not occurred to a similar degree at an ambient temperature of 25°C.

The combination of *Metschnikowia pulcherrima* with the qualitatively more favourable yeast species *Kluyveromyces lactis* did, surprisingly, rather lead to an impairment of larval performance (e.g. in survival, development time and male dry weight). The performance was not significantly worse than when larvae had fed on *Metschnikowia pulcherrima* alone. Yet, compared to larvae that had fed on *Kluyveromyces lactis* alone, the mixed diet composed of *Kluyveromyces lactis* and *Metschnikowia pulcherrima* led to a significant impairment in larval survival, larval development and male adult dry weight. This unfavourable influence disappeared, when *Metschnikowia pulcherrima* was offered in a combination with *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, probably due to the lower proportion that each yeast species (and here especially *Metschnikowia pulcherrima*) was offered the more species were combined in one mixture.

The combination of the two qualitatively comparably high yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae* in the larval diet did not lead to a significantly better (or worse) performance of *Drosophila melanogaster* larvae. *Kluyveromyces lactis* as well as *Saccharomyces cerevisiae* alone appeared to already pose a sufficiently advantageous diet. Only with regard to encapsulation ability of parasitised larvae, the combination of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* appeared to be more favourable compared to *Saccharomyces cerevisiae* alone, though not to *Kluyveromyces lactis* alone. This, however, depended on ambient temperature, since only at 25°C the yeast combination of *Kluyveromyces lactis* and *Saccharomyces cerevisiae* favourably influenced encapsulation ability compared to *Saccharomyces cerevisiae* alone. Yet, as already shown and discussed in former chapters, encapsulation ability appears to be in general prone to a pronounced variability due to sex-related (not shown in this study) and inter-individual (see chapter 7) differences.

As evidence in the former chapters has already suggested, some yeast species may pose a more beneficial food source for parasitised compared to unparasitised larvae. Besides its beneficial influence on encapsulation ability, *Kluyveromyces lactis* appeared to promote a better survival for parasitised larvae than for example *Metschnikowia pulcherrima*, *Saccharomyces cerevisiae* and a mixed diet composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, where in both experiments a significant difference was found between survival of unparasitised and parasitised larvae, though not when larvae had fed on *Kluy-*

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*veromyces lactis* alone. With regard to survival of parasitised larvae, also *Pichia toletana* appears to be a beneficial yeast species, since there was also no significant difference, in unparasitised as well as parasitised larvae, and survival was comparably high as in *Kluyveromyces lactis*.

It has been shown in predatory insects, that ingestion of “alternative foods”, even though inadequate for specific fitness traits (for example reproduction) when consumed alone, may enhance fitness traits of a predator when the predator also has access to its “essential food” (EVANS *et al.* 1999). The presence of *Metschnikowia pulcherrima*, usually inadequate for supporting fitness traits of *Drosophila melanogaster*, may at least pose the option for parasitised larvae to feed from this yeast species in order to potentially benefit from its positive influence on encapsulation ability. As will be shown in chapter 7, *Drosophila melanogaster* larvae do, however, not choose to feed from this yeast species even when parasitised.

With regard to fitness traits of *Asobara tabida*, the yeast species mixture composed of *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, posed a significantly more favourable influence on survival and development time than *Saccharomyces cerevisiae* alone, and - regarding development time - also *Kluyveromyces lactis* alone. As already shown in the former chapter, *Metschnikowia pulcherrima*, posing a rather inadequate diet to *Drosophila melanogaster* larvae, also turned out to unfavourably influence the development of *Asobara tabida*. At an ambient temperature of 20°C, also the number of eggs in female *Asobara tabida* was significantly reduced, besides a significantly decreased survival and enhanced development time, as had already occurred at an ambient temperature of 25°C and intermediate and limited food conditions, respectively (see chapter 5.4). This may affirm the already discussed tendency of *Asobara tabida* to perform better in hosts whose dietary yeast species favour fitness traits of the host itself. An either overall favourable or unfavourable influence on the performance of *Asobara tabida* may, however, not hold true for all measured fitness traits and may depend on ambient temperature. In this regard, the yeast *Saccharomyces cerevisiae* poses a puzzling phenomenon. Even though most fitness traits of *Drosophila melanogaster* larvae experienced a beneficial influence by *Saccharomyces cerevisiae*, the survival of *Asobara tabida* at an ambient temperature of 20°C was significantly lower compared to for example *Kluyveromyces lactis*, and even as low as when host larvae had fed on *Metschnikowia pulcherrima*. Yet, *Asobara tabida* females carried the highest number of eggs when fed on *Saccharomyces cerevisiae*, significantly higher than in females whose host larvae had fed on *Metschnikowia pulcherrima*. The significant difference in number of eggs carried by *Asobara tabida* was in fact only due to *Metschnikowia pulcherrima*. Parasitoids whose host larvae had fed on this yeast species had significantly fewer eggs than parasitoids whose larvae had eaten from all the other yeast species and combination of yeast species. When yeasts were offered at intermediate and limited

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conditions at an ambient temperature of 25°C (see chapter 5.4) the number of eggs had been less strongly influenced by host diet compared to experiment II. This may have either been due to an effect of temperature or food availability. Unfortunately, the effect of temperature on number of eggs at *ad libitum* conditions cannot be tested, since no parasitoids emerged at 25°C in the experiments I and II of this chapter. The richer food conditions in experiment II compared to the experiment in chapter 5.4 may have caused a decreased variability in number of eggs among females of the same yeast treatment. It has been commonly observed for several fitness traits, for example for development time and dry weight in *Drosophila mercatorum*, that variability increases when the environment is deteriorating, e.g. when the medium is poor (GEBHARDT & STEARNS 1988). Periods of food deprivation and extended quantitative or qualitative food-limitation were also shown to cause intraspecific variations in reproduction among clones of *Daphnia pulicaria* (EPP 1996).

Similar to the results in chapter 5.4, again no trade-off between reproduction and survival could be detected. There was no significant negative correlation between the absolute fat content and the number of eggs in *Asobara tabida*, which may have supported the presence of a trade-off. Possible reasons for the absence or the apparent absence of a trade-off has already been discussed in the former chapter.

At the ambient temperature of 25°C, at which Experiment I and one treatment of Experiment II were carried out, all surviving parasitoids entered diapause. Besides showing a large geographic variation in diapause, effects of temperature and photoperiod have been reported in *Asobara tabida* (KRAAIJEVELD & VAN DER WEL 1994; KRAAIJEVELD & VAN ALPHEN 1995c and references therein). KRAAIJEVELD AND VAN ALPHEN (1995c) also found a host-specific effect on diapause, with significantly more parasitoids entering diapause in *Drosophila melanogaster* than in *Drosophila subobscura*. According to KRAAIJEVELD AND VAN ALPHEN (1995c) diapause in *Asobara tabida* is most clearly influenced by temperature, whereby western European parasitoids are supposed to show an increasing percentage in diapause at lower temperatures. Only few data are available on diapause in *Asobara tabida* at 25°C, but KRAAIJEVELD (unpublished) has reported an experiment with different parasitoid strains and *Drosophila melanogaster* and *Drosophila subobscura* as hosts, where 100% of parasitoids entered diapause at 25°C (KRAAIJEVELD & VAN ALPHEN 1995c; data itself unpublished). KRAAIJEVELD & VAN ALPHEN (1995c) reported that although all experiments and rearings had been carried out under constant circumstances, the time of the year in which the experiments were done seemed to influence diapause induction, with fewer parasitoids entering diapause when the experiments were done in a period with longer days. Longer day lengths can, however, not explain the induction of diapause in the experiments described here. In chapter 5.4, carried out in mid November 2007 (day length approx. 8hours and 30minutes), almost all surviving parasitoids emerged (with

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maybe a few diapausing ones, which at that time I assumed to be dying). In the experiments described in this chapter, carried out in mid April (day length approx. 14hours and 30minutes) and end of May (day length approx. 16hours and 45minutes), day length was much longer, but all parasitoids entered diapause. KRAAIJEVELD & VAN ALPHEN (1995c), however, reported another, even stronger effect on the induction of diapause. One of the parasitoid strains used by them showed a cyclical emergence pattern where one (or rarely two) generation with many adult offspring alternated with a few generations in which hardly any adult parasitoids emerged. Maybe such a pattern also exists in the *Asobara tabida* strain used in the experiments described in this study. This, however, remains speculative and would have to be affirmed by a series of experiments at 25°C. In Experiment II, which was partly carried out at an ambient temperature of 20°C, also some parasitoids appeared to have entered diapause. The fact that the proportion of diapausing parasitoids differed significantly between the yeast treatments may suggest the influence of host microbial diet on diapause induction in *Asobara tabida*. With regard to the only few replicates available, this shall so far only be a suggestion which might be worth looking at more closely in the future.

The results in Experiment II revealed potential influences of ambient temperature on fitness traits of *Drosophila melanogaster* and *Asobara tabida*. Dry weight in adult *Drosophila melanogaster* was generally higher at an ambient temperature of 20°C than 25°C, irregardless of the dietary yeast species fed to them as larvae. Development time of larvae usually took longer at an ambient temperature of 20°C than 25°C, also irrespective of the dietary yeast species fed to them. The tendency of *Drosophila melanogaster*, having a shorter development time and a lower dry weight at higher temperatures, was also found by GEBHARDT & STEARNS (1993). It has been shown that mean critical weight of *Drosophila melanogaster* is lower at higher temperatures. The probability in *Drosophila melanogaster* of reaching the pupal stage depends upon larval weight and larval physiology. Larval physiology, independent of larval weight, is affected by feeding conditions. Both mean critical weight and mean final larval weight (which largely determines the final weight at eclosion), result from the interaction between growth rate and development rate. A stronger increase in development rate with temperature as compared to growth rate results in a reduction of weight with temperature (GEBHARDT & STEARNS 1993; DE MOED *et al.* 1999). According to PRASAD & JOSHI (2003), there is some evidence suggesting that cold-reared larvae have an increased critical weight and reduced efficiency of conversion of food to biomass, but there are also some observations that contradict these findings.

In contrast to adult dry weight, mesothorax length was hardly affected by ambient temperature even though a significant positive correlation between mesothorax length and adult dry weight existed. Metric parameters such as thorax and wing length have been shown to be influenced by changes in both cell size and cell number, which themselves may re-

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sponse to ambient temperature and food conditions. Hereby, temperature and food interact in determining the reaction norms of body size in *Drosophila melanogaster* and interact in determining the genetic variation in body size and its components (DE MOED *et al.* 1997, see also for more details on the responses of cell size and cell number on temperature- and food-conditions).

With regard to encapsulation ability, no consistent response to ambient temperature could be found in Experiment II. WALKER (1959), who kept *Drosophila melanogaster* at temperatures ranging from 16 to 28°C, reported that temperatures between 18-20°C appeared to be optimal for effective host melanotic encapsulation to occur. In the experiment described here, potential effects of ambient temperature may have been masked or superimposed by the influence of the dietary yeast species. According to VASS & NAPPI (2000), the importance of diet in the manifestation of cellular melanotic encapsulation responses in insects is an important factor that should not be ignored.

Despite the tendencies in response of various fitness traits of *Drosophila melanogaster* as well as *Asobara tabida* to dietary yeast species and species mixtures, results never were highly consistent. The reasons for the great variability may be due to large inter-individual differences. Yeast species or species mixtures favourable for one individual, may not necessarily be as favourable, but even detrimental for another individual. Results on these potential inter-individual differences will be presented in chapter 7. The variability may also be due to sex-related differences. There may, for example, be a difference in diet-mixing behaviour between the sexes, as could be shown in a ladybeetle where females did not exhibit a switching response to different prey aphids compared to males (SOARES *et al.* 2004). A source of variability may, however, be the 'food' itself, which is not composed of dead organic or anorganic matter, but which comprises live organisms, that themselves may exhibit substantial inter-species or inter-strain differences in their physiology and thus their value as food source for higher trophic levels.

## 6. Influence of dietary microorganisms on the haemocyte count of *Drosophila melanogaster* larvae

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### 6 Influence of dietary microorganisms on the haemocyte count of *Drosophila melanogaster* larvae

#### 6.1 Introduction

The immune response of *Drosophila melanogaster* against parasitoid eggs present in their haemocoel (*encapsulation*) is mainly accomplished by haemocytes, especially by lamellocytes and crystal cells, as well as plasmatocytes (see chapter 2.2). The success of this host defence is supposed to be, at least partially, related to the number of haemocytes circulating in the haemolymph within a few hours after parasitisation, as has been shown in *Drosophila melanogaster* and *Drosophila simulans* parasitised by *Asobara tabida* (ESLIN & PRÉVOST 1995). The observation that the success of *Drosophila melanogaster* to encapsulate eggs or larvae of *Asobara tabida* may depend on the dietary yeast species fed to host larvae after parasitisation (see results in chapter 5.2), raised the question, whether these differences in encapsulation ability may be related to a different number of circulating haemocytes in the haemolymph of *Drosophila melanogaster* larvae, depending on the yeast species they had fed on. In several animals farmed in aquaculture, such as *Haliotis midae* (abalone) or *Panaeus vannamei* (White shrimp) and *Panaeus monodon* (Tiger prawn), dietary probiotic organisms could be shown to have an immunostimulatory effect, partially, by enhancing the number and phagocytic activity of circulating haemocytes (RENGPIPAT *et al.* 2000; GULLIAN *et al.* 2004; MACEY & COINE 2005; RODRIGUEZ *et al.* 2007).

Based on these observations, the hypothesis was, that those larvae more successful in encapsulating *Asobara tabida* offspring (as seen in chapter 5.2), had been so, due to an immunostimulatory effect of the dietary yeast species they had fed on, and which had enhanced the number of circulating haemocytes in the larval haemolymph. Hereby, it was of interest, whether such a potentially immunostimulatory effect was only active in parasitised or also in unparasitised larvae. Thus, parasitised as well as unparasitised larvae were investigated with regard to the number of circulating haemocytes and depending on the dietary yeast species consumed. Since it was not known, when the potential effect of the dietary yeast species would occur, I decided to choose the time spans of 6hrs, 12hrs and 24hrs after parasitisation, based on the observation, that the encapsulation process starts approximately 4-6hrs after parasitisation and is supposed to be completed at around 48hrs after parasitisation (WERTHEIM *et al.* 2005). As exemplary yeast species, I decided to choose *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, which had both yielded a more or less similarly successful larval development. Larvae fed on *Kluyveromyces lactis*, however, had tended to exhibit enhanced encapsulation ability in comparison to those fed on *Saccharomyces cerevisiae*.

## ***6. Influence of dietary microorganisms on the haemocyte count of Drosophila melanogaster larvae***

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### **6.2 Material and Methods**

#### **(a) *Experimental setup***

The experiment comprised six treatments in accordance to the two different yeast species in the larval diet (*Kluyveromyces lactis* and *Saccharomyces cerevisiae*), and the three different time spans after parasitisation (6hrs, 12hrs and 24hrs) that larvae were investigated with regard to the number of circulating haemocytes in the haemolymph. Parasitised as well as unparasitised larvae were exposed to the six different treatments. Since *Asobara tabida* females favour to attack 1<sup>st</sup> and early 2<sup>nd</sup> instar larvae for oviposition, freshly hatched larvae (one day after dechoriation of eggs, two days after oviposition of female flies) were used for the experiment. This also had the advantage of larvae having the same nutritional background (banana medium) without a prior contact to any yeast species. The disadvantage, however, was, that young larvae do not yield high amounts of haemolymph and have fewer haemocytes than older larvae.

#### **(b) *Fly and parasitoid cultures***

See chapter 4.1.

#### **(c) *Preparation of media:***

See chapter 4.3.

#### **(d) *Preparation of larvae***

See chapter 4.4 and 4.5.

#### **(e) *Gaining of parasitised larvae***

For this experiment the observational method described in chapter 4.6.1 was used to gain parasitised larvae. Parasitised larvae were removed immediately after oviposition and were transferred onto a fresh Petri dish containing Banana medium III. One parasitoid each was allowed to parasitise 12 larvae. After that the parasitoid was exchanged. Two of the thus gained parasitised larvae were transferred into each treatment (two different yeast species and three different time spans after parasitisation) directly after one parasitoid had finished the 12 parasitisations. The time span of 6hrs, 12hrs or 24hrs, respectively, was calculated from that moment on when half of the time had elapsed that the parasitoid needed for parasitising the 12 larvae.

#### **(f) *Preparation of microorganisms***

For preparation of microorganisms and 50% yeast suspensions see chapter 4.2.



## 6. Influence of dietary microorganisms on the haemocyte count of *Drosophila melanogaster* larvae

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### (g) *Experimental proceeding*

50µl each of the yeast suspensions were pipetted into the prepared test tubes filled with Banana medium II. Hereupon one larva each was transferred into the tube with a fine brush and the tube covered with dental rolls (HARTMANN). The tubes were incubated in a climate chamber at 25°C and a 16:8 L/D cycle.

### (h) *Preparation of specimens*

Unfortunately, the number of treatments and replicates that had to be investigated by one person at the same time, did not allow the usage of a haemocytometer, but made it necessary to prepare specimens that could be stored and investigated for haemocyte count at some later point in time. Thus, the method used for obtaining circulating haemocytes was a combination of the methods carried out by LANOT *et al.* (2001) and HUANG *et al.* (2005). After the time span (6, 12 and 24hrs) had elapsed, each larva was washed out of the test tube using tap water and a nylon gaze sieve. The larva was thoroughly washed in PBS buffer and gently put on filter paper to dry. The larva was then bled on a microscopic slide. For this, the posterior end of the larva was gently held with Dumont forceps and the anterior end cut off with ophthalmic scissors (approximately on the level of the mouthparts' basis). The larva was held over the slide and haemolymph was allowed to drip onto the slide just before the larval tissues started sticking to the glass. The carcass of parasitised larvae was transferred onto a fresh microscopic slide, dipped into a droplet of tap water and dissected to look for a parasitoid egg (or capsule) in order to verify parasitisation. Sometimes during bleeding, the egg already plopped out of the larva together with the haemolymph and thus made further dissection of the larval carcass unnecessary.

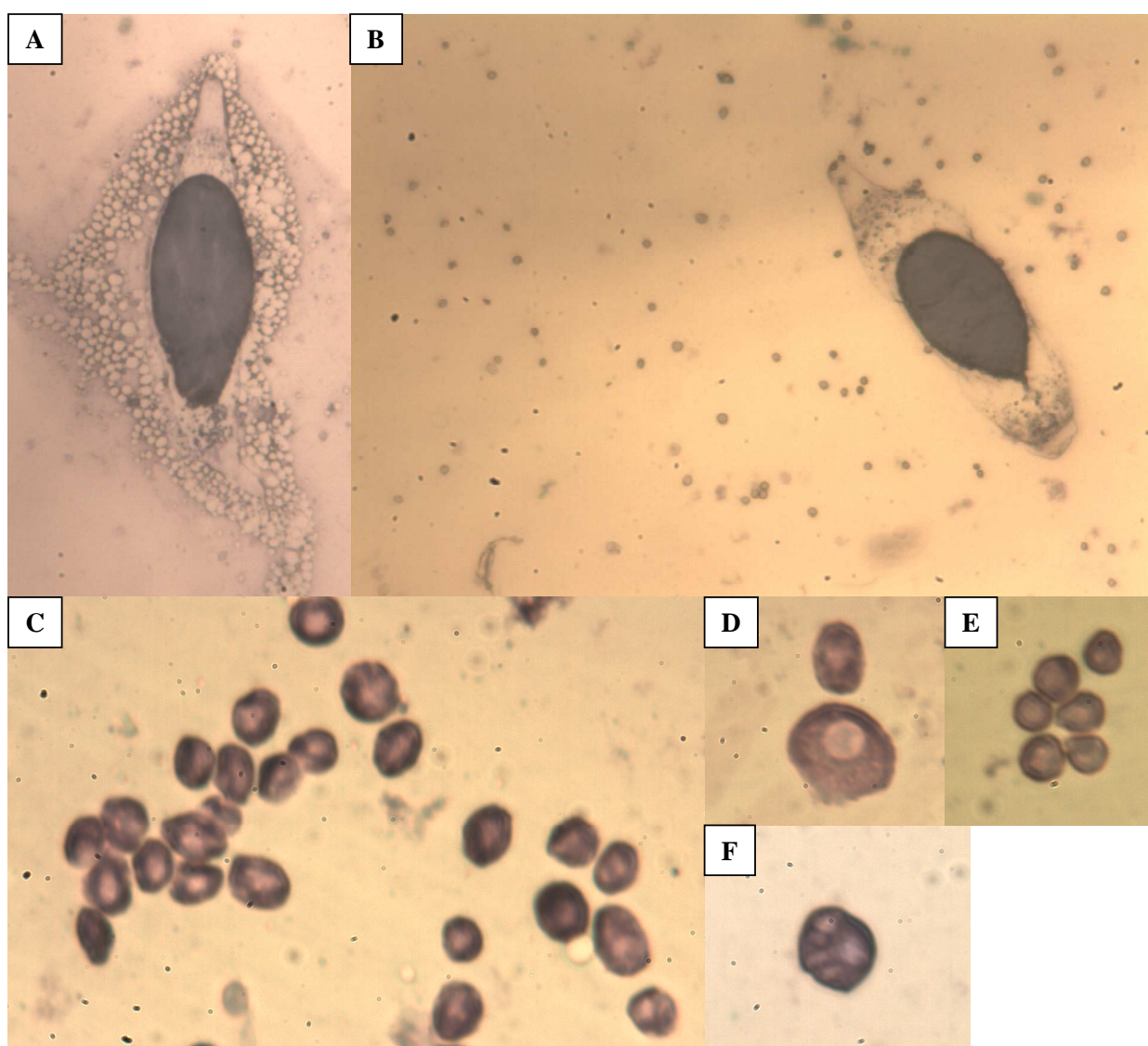
The haemolymph specimens were allowed to dry for approx. half an hour and were then fixed in 100% methanol for 10 minutes. After drying under a flue, the specimens were dyed with Giemsa stain (SIGMA ALDRICH) (10%, 20ml stem solution plus 180ml PBS buffer for one dyeing vessel) for one hour. The specimens were gently dipped into fresh PBS buffer for brief destaining, then washed in distilled water and dried. Specimens could then directly (or at a later point in time) be put under a microscope for counting haemocytes.

### (i) *Haemocyte count*

All types of haemocytes (plasmatocytes, crystal cells and lamellocytes) (**Fig. 47**) were counted (*total haemocyte count*, also *THC*) at a total magnification of 480 (object lense: 40x, eyepiece: 12x). The whole specimen was scanned and all haemocytes counted by meandering through the specimen. This was repeated four times. The arithmetic mean of all four counts was finally used for further calculation.

## 6. Influence of dietary microorganisms on the haemocyte count of *Drosophila melanogaster* larvae

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**Fig. 47:** Photos of specimens of *Drosophila melanogaster* larvae as they were prepared for the total haemocyte count. **A)** Egg of *Asobara tabida* embedded in parts of the fat body. **B)** Egg of *Asobara tabida* and several haemocytes. **C)** Plasmatocytes. **D)** Plasmatocyte (above) and lamellocyte (below). **E)** Several plasmatocytes. **F)** Crystal cell.

### (j) Statistical analysis

See chapter 4.9. The analysis was carried out with yeast species, time span after parasitisation as well as parasitisation status (no, yes) as linear predictor and total haemocyte count (THC) of unparasitised and parasitised *Drosophila melanogaster* larvae as response variable.

## 6. Influence of dietary microorganisms on the haemocyte count of *Drosophila melanogaster* larvae

### 6.3 Results

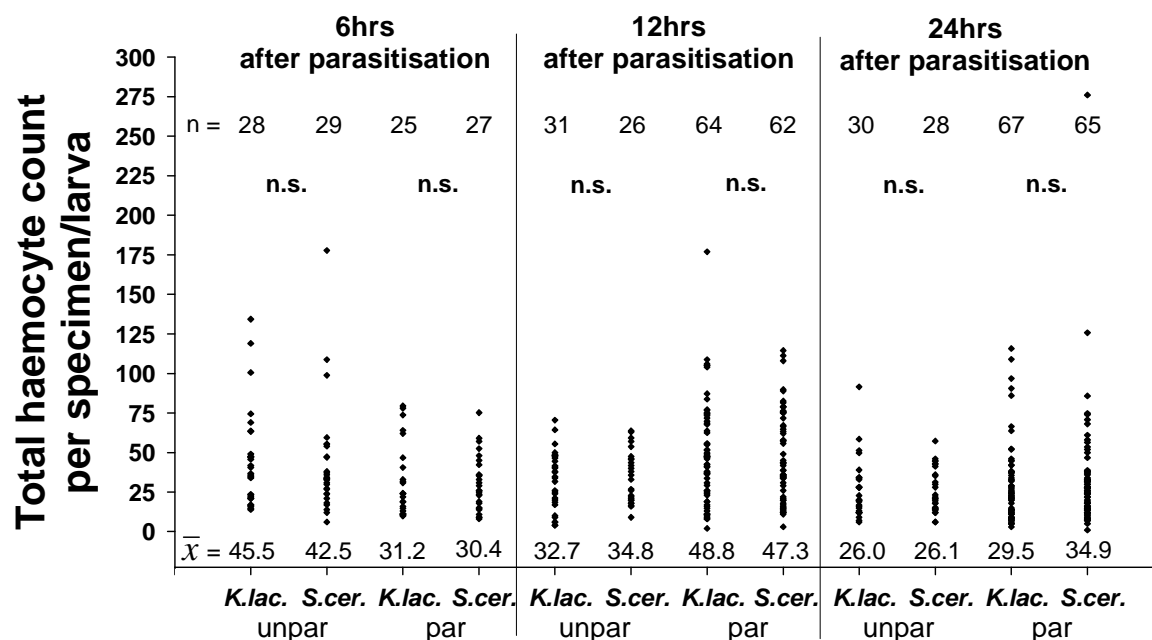
At none of the three time spans after parasitisation did the dietary yeast species have a significant influence on the number of circulating haemocytes in the haemolymph of *Drosophila melanogaster* (THC), neither in unparasitised nor in parasitised larvae (6hrs after parasitisation: unparasitised:  $F_{1,55} = 0.1161$ ,  $p = 0.7346$ ; parasitised:  $F_{1,50} = 0.022$ ,  $p = 0.8827$ ; 12hrs after parasitisation: unparasitised:  $F_{1,55} = 0.2059$ ;  $p = 0.6518$ ; parasitised:  $F_{1,124} = 0.0718$ ,  $p = 0.7891$ ; 24hrs after parasitisation: unparasitised:  $F_{1,56} = 0.0001$ ,  $p = 0.9928$ ; parasitised:  $F_{1,130} = 0.9674$ ,  $p = 0.3272$ ). Accordingly, the total haemocyte count of larvae was similar within each time span (6hrs after parasitisation: unparasitised: *K.lac.*:  $45.5 \pm 5.86$ , *S.cer.*:  $42.5 \pm 6.41$ ; parasitised: *K.lac.*:  $31.2 \pm 4.58$ , *S.cer.*:  $30.4 \pm 3.33$ ; 12hrs after parasitisation: unparasitised: *K.lac.*:  $32.7 \pm 3.25$ , *S.cer.*:  $34.8 \pm 3.20$ ; parasitised: *K.lac.*:  $48.8 \pm 4.04$ , *S.cer.*:  $47.3 \pm 3.55$ ; 24hrs after parasitisation: unparasitised: *K.lac.*:  $26.0 \pm 3.33$ , *S.cer.*:  $26.1 \pm 2.63$ ; parasitised: *K.lac.*:  $29.5 \pm 3.05$ , *S.cer.*:  $34.9 \pm 4.68$ ) (**Fig. 48**). In the haemocoel of parasitised *Drosophila melanogaster* larvae, only eggs of *Asobara tabida* and no parasitoid larvae or capsules were found.

The influence of dietary yeast species, time span and parasitisation status (no, yes) (factors), as well as the interaction between the three factors on the total haemocyte count of parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 29**. The results of the ANCOVA merely show a significant influence of time span as well as parasitisation on total haemocyte count.

**Table 29:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the total haemocyte count of parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Total haemocyte count of <i>Drosophila melanogaster</i>	Yeast	$F_{1,480} = 0.1539$	$p = 0.6950150$
	Time span	$F_{1,479} = 13.4218$	$p = 0.0002768$
	Parasitisation	$F_{1,478} = 4.6622$	$p = 0.0313350$
	Yeast*Time span	$F_{1,477} = 0.9813$	$p = 0.3223884$
	Yeast*Parasitisation	$F_{1,476} = 0.0365$	$p = 0.8484981$
	Parasitisation*Time span	$F_{1,475} = 1.0969$	$p = 0.2954872$
	Yeast*Time span* Parasitisation	$F_{1,474} = 0.4458$	$p = 0.5046695$

## 6 Influence of dietary microorganisms on the haemocyte count of *Drosophila melanogaster* larvae



**Fig. 48:** Individual values of the total haemocyte count in the haemolymph of unparasitised (**unpar**) and parasitised (**par**) 1<sup>st</sup> instar *Drosophila melanogaster* larvae (parasitised by *Asobara tabida*), depending on the dietary yeast species (*Kluyveromyces lactis* and *Saccharomyces cerevisiae*), and the time span after parasitisation (6hrs, 12hrs and 24hrs) larvae were bled. Numbers below scatter plots = mean values. Numbers above scatter plots = number of data points (replicates). Abbreviations of yeast species: **K.lac.** = *Kluyveromyces lactis*; **S.cer.** = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant.

### 6.4 Discussion

The results suggest that the enhanced encapsulation ability observed in *Drosophila melanogaster* larvae fed on *Kluyveromyces lactis* compared to those fed on *Saccharomyces cerevisiae* (chapters 5.2 and 5.3), was not based on a higher number of circulating haemocytes in the larval haemolymph.

Several aspects may, however, have blurred potential differences in haemocyte count. One possible aspect may be the method used for obtaining haemocyte numbers. Even though larvae of approximately the same size were chosen for bleeding, small differences in haemolymph volume, not detectable by this comparably rough method of estimating larval size, may have led to differences in haemocyte count that were greater than the potential differences mediated by the dietary yeast species. Differences in haemocyte number within each treatment were indeed rather high (see **Fig. 48**). This high variance may also be ascribed to the young age and the smallness of the larvae chosen for this experiment. The amount of haemolymph and the haemocyte number of freshly hatched 1<sup>st</sup> instar larvae are comparably low, so that deviations lead to a relatively greater error than in high haemocyte numbers. The total number of blood cells in 1<sup>st</sup> instar *Drosophila melanogaster* larvae is lower than 200 per ani-

## 6 Influence of dietary microorganisms on the haemocyte count of *Drosophila melanogaster* larvae

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mal, with numbers increasing progressively during the larval stages and reaching up to more than 5000 per animal at the time of pupariation (LANOT *et al.* 2001). Additionally, the method used here for collecting haemolymph underestimates haemocyte numbers, since usually all of the haemolymph cannot be recovered on the coverslip, as also described by LANOT *et al.* (2001). Even though this underestimation holds true for all specimens and may yield reproducible and comparable values, the problem of low haemocyte numbers remains.

How great need differences in haemocyte count actually be to lead to differences in encapsulation ability? ESLIN & PRÉVOST (1995), who studied haemograms in *Drosophila simulans* and *Drosophila melanogaster*, stated, that *Drosophila simulans* larvae, being more successful in encapsulating *Asobara tabida* offspring, had 4-5 times more haemocytes in their haemolymph than *Drosophila melanogaster* larvae. They counted approximately 10-150 cells per larva in 3 day old larvae (haemolymph was diluted with buffer) and 100-300 cells per larva in 6 to 7 day old larvae. The youngest larvae used for parasitisation were 2 days old (after hatching at 20°C). Thus, larvae used in the experiment described here, were younger and probably smaller than those used by ESLIN & PRÉVOST, even when considering the temperature difference (here 25°C). The number of cells approximately counted was mostly below 100, with the most frequently occurring value being 12 (haemolymph was undiluted), the minimum value being 1, the maximum value being 276. If an increase in haemocyte number by four or five is already sufficient for enhancing encapsulation ability, the great variability in the data may have blurred a potential difference in haemocyte count. High variability in the data due to methodical drawbacks might be avoided, if the experiment was carried out using 2<sup>nd</sup> or even 3<sup>rd</sup> instar larvae as well as haemocytometers for investigating total haemocyte count.

Beside the already mentioned drawbacks of the method itself, with which haemocyte numbers were investigated, another source for the high variability in haemocyte count may be due to great inter-individual differences. Even though there is a strong tendency of *Kluyveromyces lactis*, compared to *Saccharomyces cerevisiae*, to enhance encapsulation ability in a large proportion of *Drosophila melanogaster* larvae, this may not hold true for all larvae. For a (smaller) proportion of larvae, *Saccharomyces cerevisiae* may even be the yeast species more beneficial to them than *Kluyveromyces lactis*. Indications for this possibility will be given by the results described in the following chapter 7. Thus, the enhanced encapsulation ability observed may still have been due to a higher number of haemocytes circulating in the haemolymph, whereby in some larvae *Kluyveromyces lactis* may have been the one enhancing THC, yet in another proportion of larvae *Saccharomyces cerevisiae*.

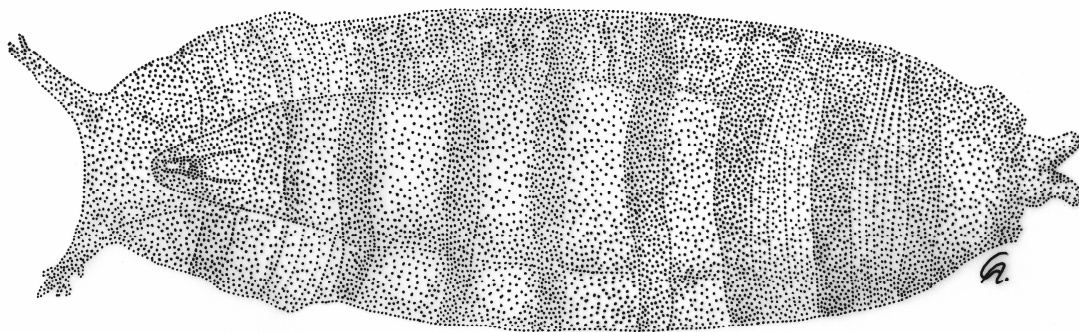
As already discussed in chapter 5, a potential increase in the number of circulating haemocytes may not be the only mechanistic explanation for the differing influence of different yeast species on the encapsulation ability of *Drosophila melanogaster* larvae. A potential modulation of the Phenoloxidase cascade has already been suggested, as well as a potential modulation of virulence factors posed by *Asobara tabida*, such as egg adhesiveness to host tissue as well as venoms. Specific *Saccharomyces cerevisiae* strains in the diet of *Artemia*

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larvae, for example, were shown to support *Artemia* growth and induce a higher Phenoloxidase activity (ROJAS-GARCÍA *et al.* 2008). An enhancement of Phenoloxidase activity and other immune parameters could also be found in *Litopenaeus vannamei* (White shrimp) having fed on *Lactobacillus plantarum* (CHIU *et al.* 2007), as well as the use of a marine *Candida* in the diet of Indian white shrimp *Fenneropenaeus indicus* (SAJEEVAN *et al.* 2006). Regarding a potential modulation of virulence factors, various probiotic bacterial strains could, for example, be shown to have an inhibitory effect on the expression of genes coding for several Shiga-toxins in a Shiga-toxin producing *Escherichia coli* (CAREY *et al.* 2008).

Alternatively, specific yeast species (or other microbial species) in the diet of *Drosophila melanogaster* may stimulate the immune system, preparing it for a ‘serious’ attack. Dietary microorganism (especially probiotic microorganisms), have been shown to be capable of triggering an immune response cascade in the form of an immune system priming, thereby decreasing the risk of a ‘serious’ infection and outweighing potential trade-offs or unfavourable influences on other fitness traits (FREITAK *et al.* 2007). Since there are still more bridges to be gapped concerning the knowledge on cellular immune responses against parasitoids than on humoral immune responses (especially regarding immune recognition), potential points of action of dietary compounds on cellular immune responses are yet to be discovered.



## 7 Food choice of *Drosophila melanogaster* larvae

### 7.1 Introduction

*I think it could be plausibly argued that changes in diet are more important than changes in dynasty or even of religion [George Orwell].*

*Medicine and food are of the same origin [Japanese saying].*

*Not all pharmacists are human [Clayton & Wolfe 1993].*

Animals over all taxa have been shown to continuously regulate their food intake, involving frequent shifts between different foods, whereby their choice of foods or nutrients are non-random and beneficiary to their fitness (WALDBAUER & FRIEDMAN 1991; see ‘*dietary self-selection*’). The information an animal gains from its food to enable it to self-select its diet, may stem from chemosensory stimuli and a metabolic feedback (WALDBAUER & FRIEDMAN 1991).

The choice of food in both humans and (other) animals is shaped by a complex interplay of different influential factors. In humans, diet preferences are assumed to be more than a matter of nutritional value, and are reported to be mainly guided by the food’s taste, including chemical senses of taste and olfaction, perception of texture, as well as a hedonic component. The taste responses themselves may be influenced by genetic, physiological and metabolic variables (including sex and age), as well as psychological variables (KEANE & WILLETTS 1994; KÖSTER 2003; DREWNOWSKI 1997). Abundance or scarcity of particular nutrients and the presence of toxic secondary metabolic compounds may have acted as selective pressures, shaping hedonic responses to flavours and textures (GALEF 1996). Food preferences may, however, also be linked to behavioural, attitudinal, socio-cultural and economic aspects (DREWNOWSKI 1997). The interaction between the individual and the ecological/biological and social environment, as well as economic constraints, are major determinants, shaping food availability and food selection (KEANE & WILLETTS 1994; GALEF 1996). Ambience is also supposed to play a role in human food choice (STROEBELE & DE CASTRO 2004), as well as the impression made on conspecifics (VARTANIAN *et al.* 2007). In humans and (other) animals, also emotions, serving distinct motivational functions and controlling behavioural mechanisms, have been found to affect eating responses, from food choice to metabolism and ingestion, varying both across individuals and different kinds of emotions (GIBSON 2006; MACHT 2008). Food choice may among other things also be influenced by food quality (CRUZ-RIVERA & HAY 2000), nutritional status (MOORE & HOWARTH 1996; WU *et al.* 2005), seasonality (NORSCIA *et al.* 2006), number of items to

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choose from (HUTCHINSON 2005), predation pressure (STACHOWICZ & HAY 1999), parasite avoidance (HUTCHINGS *et al.* 2006), immune status (TEIXEIRA *et al.* 2007), maternal diet (ALTBACKER *et al.*) and preference-performance relationships (POORE & STEINBERG 1999; SCHEIRS & DE BRUYN 2002; VIDELA *et al.* 2006; see also 'preference-performance hypothesis', THOMPSON 1988).

Since organisms require resources for growth, reproduction, etc., the quantity of nutritional intake of both macro- and micronutrients, as well as the quality of the food chosen and finally consumed, are important features for an organism's fitness. Not to obtain too many nutrients of any kind may hereby be just as important as obtaining enough (RAUBENHEIMER *et al.* 2005). The importance of maximum intake of energy per unit time as predicted by the 'optimal foraging theory', is hereby often outweighed, among other things, by the importance of achieving a nutritional balance (RAUBENHEIMER & SIMPSON 1997), or whether 'prey' is mobile or immobile (SIH & CHRISTENSEN; see also 'optimal diet theory'). Food items of predatory carnivorous animals are often nutritionally similar and more or less balanced, so that these animals may focus on maximising caloric intake. Animals that eat foods with differing nutritional value may have to focus on mixing and selecting food items in order to achieve a nutritionally balanced diet (GALEF 1996; BEGON *et al.* 2005). In herbivorous animals, the presence of plant secondary metabolites (PSMs) (including alkaloids, terpenes, phenolics, glucosinolates, cyanogenic glycosides), which often have developed in plants as evolutionary response to herbivory, is reported to influence diet preferences and the amount eaten of each plant species or specific tissue (SINGER *et al.* 2004; PÖYKKÖ *et al.* 2005; MARSH *et al.* 2006; MCLEAN & DUNCAN 2006) (see also 'detoxification limitation hypothesis' or 'toxin dilution/avoidance hypothesis', coined by FREELAND & JANZEN 1974). PSMs may, hereby, not have a fixed effect on feeding behaviour in herbivores, but their effect may rather depend on detoxification mechanisms and the detoxification state, as well as the costs of detoxification of each individual (MARSH *et al.* 2006). Additionally, the predictions made by the detoxification limitation hypothesis, e.g. in reference to generalist and specialist herbivores, diet mixing and dilution of toxins; etc., may be too simple, since especially wild herbivores have to face other constraints and factors, such as social interactions, nutritional status, home range, predation pressure, etc., which may also influence feeding behaviour. The actual amount eaten of a specific plant is probably more dependent on PSMs than the initial food plant selection, e.g. due to mechanisms that help avoiding the ingestion of toxins in the first place and thus spare the animal the detoxification process (MARSH *et al.* 2006). Although reflexive responses of the olfactory and gustatory system may act as a rough guide to discriminate between non-toxic and toxic foods in an animal's natural habitat, this system can be modified. Food selection is characterised by a high plasticity, i.e. responses to tastes can be altered by a range of experiences and associative learning (GALEF 1996; EGAS & SABELIS 2001).



## 7. Food choice of *Drosophila melanogaster* larvae

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It is argued that proteins and amino acids influence food choice and intake more than other macronutrients, and protein often has a highly satiating effect, so that e.g. high protein diet is often associated with decreased food intake (TOME 2004). Since amino acids are neither synthesised nor stored in metazoans, amino acid sensory mechanisms (located in the brain), conserved across eukaryotic species, enable animals to detect the depletion of specific indispensable amino acids and replenish them in order to maintain homeostasis (GIETZEN & ROGERS 2006). Sterols are essential compounds in an insect's diet, needed for structural and physiological purposes, such as components of cell membranes and the production of the moulting hormone ecdysteroid, but insects are not able to synthesise sterols *de novo* (CLAYTON 1964; NAGATA *et al.* 2006; BEHMER & NES 2003). Thus, their presence or absence in a specific food may influence diet choice. It has, for example, been shown, that yeast species may greatly vary in their sterol content, e.g. ergosterol, with sterol content being extraordinarily sensitive to the conditions of culture (BILLS *et al.* 1930).

Various species of *Drosophila* depend on the presence of yeasts on the various decaying plant substrates (BEGON 1982). It has been shown, that different species of yeast may differ in their attractiveness to different species of *Drosophila*, and that different yeast species are not equally adequate to support the development of *Drosophila* larvae (LINDSAY 1958; COOPER 1960; DORSCH 2007). DORSCH (2007) could already show, that the yeast species *Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae*, may have a differing impact on various fitness traits of *Drosophila melanogaster*, such as survival, development time and dry weight of eclosed adults. Additionally, larval and adult *Drosophila melanogaster* exhibited different preferences for these different yeast species. CARSON *et al.* (1956) had observed that the yeasts of *Drosophila* breeding sites were strikingly different from those found in the crops of adult flies of the same species from the same region, suggesting that adult yeast foods of *Drosophila* in nature are different from larval yeast foods and that adult flies do not regularly feed from their breeding sites.

Parasitoids are known to pose a major threat to *Drosophila* larvae in the wild. The infection with parasites and pathogens may have a drastic impact on an infected organism, including its metabolism and its behaviour (BARBER *et al.* 2000; JOG & WATVE 2005). Physiological changes produced by parasitism are often manifested by altered host behaviour (THOMPSON & KAVALIERS 1004). Effects on behaviour may include changes in diet choice that may be due to manipulation of host behaviour by the parasite, or modified nutritional requirements, either caused by the demands of the parasite or pathogen, or by the activation of defence mechanisms, including immune defences. The energetic constraints imposed on an infected organism and potential constraints in physical fitness, may also force an organism to change feeding behaviour (BARBER *et al.* 2000). A number of animals have been shown to exhibit behaviours of self-medication (also called zoopharmacognosy),

## 7. Food choice of *Drosophila melanogaster* larvae

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i.e. they use substances (topical application and proximity, absorption, ingestion) produced by other organisms or substances of abiotic origin, as defence against parasites and pathogens, and thus increase their fitness (CLAYTON & WOLFE 1993; LOZANO 1998). Animals may, hereby, show prophylactic behaviour, e.g. by collecting plant compounds that defend themselves against parasites (CHRISTE *et al.* 2003; CHAPUISAT *et al.* 2007; CASTELLA *et al.* 2008), but they may also show therapeutic behaviour. Evidence has e.g. been found, of not an incidental but direct use of plants for their medicinal value, especially among primates (HUFFMAN 1997), but also in other animals (VILLALBA *et al.* 2006). To compensate for increased costs associated with infection and immune defence, animals have been shown to exhibit compensatory shifts in diet choice and consumption (THOMPSON *et al.* 2001, 2005a; SINGER *et al.* 2004; THOMPSON & REDAK 2005; LEE *et al.* 2006). The diet choice, however, may also influence the developmental success of the parasite (THOMPSON *et al.* 2005b; THOMPSON & REDAK 2008).

The results in chapter 5.2 suggest that different yeast species may have a substantially differing impact on fitness traits of parasitised *Drosophila melanogaster* larvae, having been attacked by *Asobara tabida*, especially in reference to the immune defence against the invader (encapsulation ability). Thus, it was hypothesised, that parasitised *Drosophila melanogaster* larvae are able to detect the qualitative differences of specific yeast species in reference to their impact on immune defence, and self-select the particular yeast species accordingly, if given the choice. An additional hypothesis was, that larvae (parasitised or unparasitised) experience a beneficial impact on various fitness traits (survival, development time, dry weight of adults, encapsulation ability of parasitised larvae), when they are allowed to fulfil their development on the preferred yeast species instead of the one they did not prefer.

The food choice experiments were carried out in the form of classical so called ‘cafeteria experiments’ (WALDBAUER & FRIEDMAN 1991), where *Drosophila melanogaster* larvae were simultaneously offered different yeast species on the same plant substrate. The experiment was designed on the basis of experiments carried out by COOPER (1960).

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### 7.2 Material and Methods

#### (a) Experimental setup

**Experiment I:** Parasitised and unparasitised larvae were offered the three yeasts species *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* (two at a time) in the three possible pairs of combinations, being *Kluyveromyces lactis* & *Metschnikowia pulcherrima*, *Kluyveromyces lactis* & *Saccharomyces cerevisiae*, and *Metschnikowia pulcherrima* & *Saccharomyces cerevisiae*. Since it was not known, when parasitisation would take effect, three time spans after parasitisation were chosen (3hrs, 6hrs & 24hrs) at which the food choice experiments were carried out. During the hours prior to the food choice experiments, larvae were kept on a substrate which should be as neutral as possible concerning the nitrogen source usually provided by yeasts. Thus larvae were kept on yeast free banana medium (Banana medium III). This, however, posed the problem of larvae being (N-) starved during those hours, since banana alone is insufficient for a successful development of larvae. Hence, unparasitised larvae had to undergo the same treatment and their food choice compared to that of parasitised larvae in order to consider a possible impact of (N-) starvation. Additionally, the influence of N-starvation on encapsulation ability was checked in another experiment which is presented in chapter 5.3. Due to the fact of larvae frequently not being parasitised despite having been exposed to parasitoids, potentially parasitised larvae had to be dissected after each food choice experiment in order to verify actual parasitisation. Only those larvae that had actually been parasitised (i.e. containing a parasitoid egg, larva or a capsule) were included in the statistical analysis.

**Experiment II:** The food choice experiment was repeated with the two yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, since they appeared to be of equal quality for the larvae in reference to their development time and survival, but had turned out to have a significantly different influence on encapsulation ability (see chapter 5.2). Additionally, the time span of 24 hours after parasitisation was chosen as only time span, since no capsules were found in the dissected larvae of the former experiment (thus food choice could still be relevant for the larvae for immune defence) and in order to make certain, that most larvae had started with their immune defence reaction against the parasitoid egg.

**Experiment III:** In a third food choice experiment, again only with *Kluyveromyces lactis* and *Saccharomyces cerevisiae* as offered yeast species, parasitised as well as unparasitised larvae were allowed to choose. To pursue their development, they were subsequently either transferred on the yeast species they had preferred or on the one they had not preferred to consume. Simultaneously, parasitised and unparasitised larvae that had not been allowed to choose any of the two yeast species were just as well transferred either on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* to pursue their development.

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### **(b) Food choice arenas**

As food choice arenas, Petri dishes with a diameter of 55mm were chosen (see **Fig. 50A**). They were each filled with 8ml Nipagin agar. After cooling down, two holes (diameter 8mm) were bored with a metal borer in a distance of 30mm from each other (calculated from the margin of each hole). The holes were filled with Banana medium IV (containing a high amount of agar in order to avoid larvae digging themselves too far into the medium and thus impeding counting them). 24hrs prior to the food choice experiments, 1 $\mu$ l of a 50% yeast suspension was pipetted onto each of the banana patches, and the arenas were then incubated over night in a climate chamber at 25°C and a 16:8 L/D cycle.

### **(c) Preparation of microorganisms**

50% yeast suspensions were used for preparing the yeast patches in the food choice arenas, as well as for the experimental tubes containing Banana medium II. The yeast suspensions were gained following the proceedings described in chapter 4.2.

### **(d) Preparation of larvae**

See chapter 4.4 and 4.5.

### **(e) Gaining of parasitised larvae**

Since having to stick to a rather tight time schedule with a large number of larvae being parasitised approximately at the same time, the non observational method described in chapter 4.6.2 was chosen to gain parasitised larvae. For **Experiment III** two sets of parasitised and unparasitised larvae were prepared, 24hrs prior to the food choice of larvae. One set was prepared, so that larvae could be used within the first approx. three hours, a second set for the next approx. three hours the observation of the larval food choice lasted.

### **(f) Temporary storage of larvae**

During the time spans of 3hrs, 6hrs and 24hrs after parasitisation, parasitised as well as unparasitised larvae were transferred onto Petri dishes (diameter 55mm) containing Banana medium III. The Petri dishes were stored in a moist chamber and incubated at 25°C and a 16:8 L/D cycle. Keeping the larvae on these Petri dishes also had the advantage of parasitised larvae being pooled on one Petri dish so that larvae entering the same food choice arena did not stem from the same Petri dish where the parasitisation had taken place. Unwanted possible effects of parasitisation dish and/or parasitoids might thus have been avoided.

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### (g) Experimental proceedings

**Experiments I & II:** The food choice arenas were arranged in the way that each yeast species in a combination of two was alternately positioned on the left and on the right side in order to avoid effects of position or light conditions on the larval food choice. Once the time spans after parasitisation had elapsed, 20 larvae each were transferred into the food choice arenas and placed near the edge of the Petri dish roughly equidistant to each banana-yeast patch. Larvae were then left for one hour. Immediately after the hour had elapsed, arenas containing unparasitised larvae were put under a stereo microscope and the position and number of larvae on each yeast patch was counted. Arenas containing parasitised larvae were put into the freezer (-20°C) in order to “freeze” their position on the patches since they had to undergo the rather time consuming procedure of being dissected in order to verify that they had actually been parasitised. Whether larvae were still able to change their position during the first minutes in the freezer and thus altering the results, had been checked beforehand in a preliminary experiment.

**Experiment III:** The food choice arenas were arranged in the way as described above, but approx. only six or eight at a time at most. *One* parasitised or unparasitised larva, respectively, was transferred into the food choice arena and placed near the edge of the Petri dish roughly equidistant to each banana-yeast patch. The larvae were observed during their roaming around, and just before they had made the decision to enter a particular banana-yeast patch and plunge their mouth hooks into the yeast colonies, they were seized with a brush and transferred into a prepared experimental tube with Banana medium II and either a 50% yeast suspension of *Kluyveromyces lactis* or *Saccharomyces cerevisiae*. A larva that had chosen *Kluyveromyces lactis*, for example, was transferred into a tube with *Kluyveromyces lactis*. The next larva choosing to devour *Kluyveromyces lactis*, however, was transferred into a tube with *Saccharomyces cerevisiae*. The same was done for larvae that chose to devour *Saccharomyces cerevisiae*. For each larva, the yeast preferred in the food choice arena was noted as well as into which experimental tube (either with *Kluyveromyces lactis* or *Saccharomyces cerevisiae*) it was transferred.

### (h) Editing of data

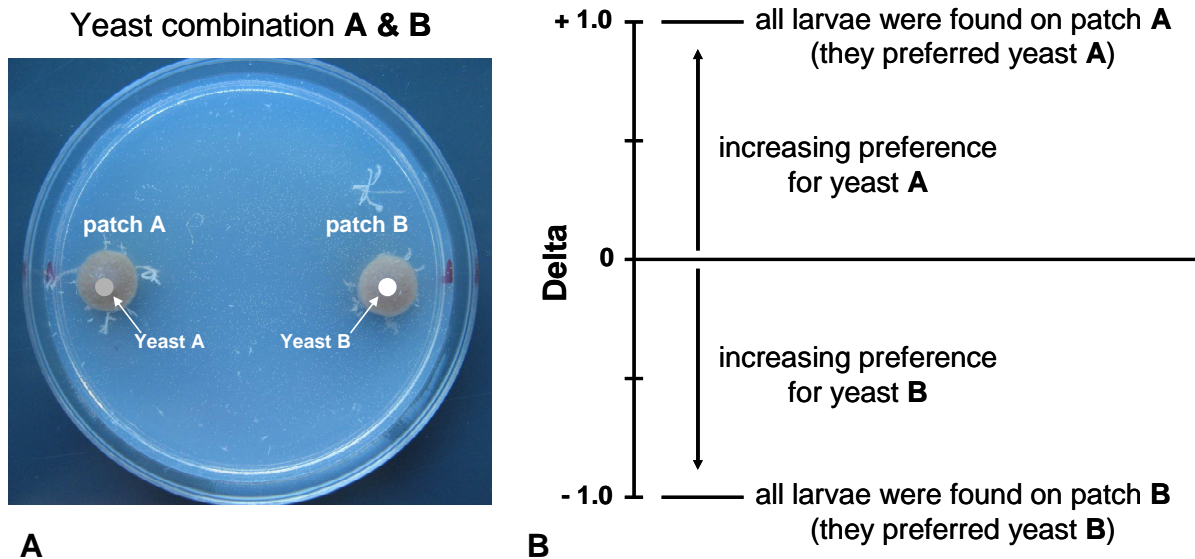
The food choice experiments I and II yielded as result the number of larvae found after one hour on each of the two yeast-banana patches per arena, whereby, in parasitised larvae, only those larvae found on the yeast-banana patches were included into the following calculation, which actually turned out to have carried a parasitoid egg in the haemocoel.

Subsequently, the following delta value was calculated (here for an assumed yeast-combination of **A & B** on patches **A** and **B**) (**Fig. 49A**):

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$$\text{Delta} = \frac{(\text{number of larvae on patch A} - \text{number of larvae on patch B})}{(\text{number of larvae on patch A} + \text{number of larvae on patch B})}$$

**Delta** values can only comprise numbers between **-1.0** and **+1.0**. If Delta is **-1.0**, all larvae were found on patch **B** and none on patch **A**, i.e. larvae exhibited a strong preference for yeast **B**. If Delta is **+1.0**, all larvae were found on patch **A** and none on patch **B**, i.e. larvae exhibited a strong preference for yeast **A**. If Delta is **0**, an equal number of larvae were found on patch **A** as well as on patch **B**, i.e. the larvae did not exhibit any preference, neither for yeast **A** nor for yeast **B**. Values between **0** and **+1.0** suggest that larvae exhibit a preference for yeast **A**, whereas values between **-1.0** and **0** suggest that larvae exhibit a preference for yeast **B** (**Fig. 49B**).



**Fig. 49:** Illustration and interpretation of the **Delta** value. **A)** Food choice arena with the assumed yeast combination **A & B** and with the patches **A** and **B**. **B)** Delta values ranging between **-1.0** and **+1.0** and the interpretation of the values. For details see chapter 7.2 (h).

### i) Statistical analysis

In **Experiment I & II**, all Delta values were transformed by adding **+1.0** to each value in order to extinguish all negative values. The transformed delta values were tested for normal distribution with the Shapiro-Wilk test and for homogeneity of variances with Levene's test. In order to test for potential effects of yeast species and time span after parasitisation on food choice of unparasitised compared to parasitised larvae, GLMs (family: Gaussian; link: identity) were applied in case of normally distributed and variance-

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homogenous data. For not normally distributed and variance-inhomogenous data, GLMs (family: quasipoisson; link: identity) were applied. In order to test, whether there was a significant difference in the number of larvae found on each of the two yeast patches (i.e. whether there was a preference for one of the yeast species) within one time span and for unparasitised and parasitised larvae respectively (test for significant difference of the Delta value to the intercept, either 0, for non-transformed, or +1 for transformed Delta values), single-sample t-tests (for normally distributed and variance-homogenous data) and GLMs (family: quasipoisson; link: identity) were applied.

In **Experiment III**, differences in fitness traits were analysed using GLMs. For differences in survival and encapsulation ability GLMs (family: quasibinomial; link: logit) were used, for differences in development time GLMs (family: quasipoisson; link: identity), and for differences in dry weight GLMs (family: Gaussian; link: identity, or family: quasipoisson; link: identity, respectively).

### 7.3 Results

(I) Food choice experiment with the three yeast species *Kluyveromyces lactis*, *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* at the three time spans of 3hrs, 6hrs and 24hrs after parasitisation of parasitised larvae.

**Yeast combination *Kluyveromyces lactis* & *Saccharomyces cerevisiae*:** When larvae could choose between the two yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (**Fig. 50A**), there was no significant difference between the number of **unparasitised larvae** found on the patch with *Kluyveromyces lactis* and those found on the patch with *Saccharomyces cerevisiae* in each of the three time spans after parasitisation (no significant deviation of the Delta value from the intercept) (3hrs:  $t = 0.4274$ ,  $df = 9$ ,  $p\text{-value} = 0.6791$ ; 6hrs:  $t = 0.1369$ ,  $df = 9$ ,  $p\text{-value} = 0.894$ ; 24hrs:  $t = -0.3389$ ,  $df = 9$ ,  $p\text{-value} = 0.7424$ ). Thus, unparasitised larvae did neither exhibit a preference for *Kluyveromyces lactis* nor for *Saccharomyces cerevisiae*, irregardless of the time span after parasitisation that larvae were allowed to choose. The Delta value hardly differed between the time spans and did not show a significant deviation from the intercept (3hrs:  $0.0475 \pm 0.1112$ , 6hrs:  $0.0155 \pm 0.1133$ , 24hrs:  $-0.0356 \pm 0.1049$ ).

For **parasitised larvae**, only at the time span of 24hrs after parasitisation, a significant difference was found between the number of larvae found on *Kluyveromyces lactis* and those found on *Saccharomyces cerevisiae* (3hrs:  $t = 0.3483$ ,  $df = 9$ ,  $p\text{-value} = 0.7356$ ; 6hrs:  $t = 2.0436$ ,  $df = 9$ ,  $p\text{-value} = 0.07134$ , 24hrs:  $t = 3.2313$ ,  $df = 9$ ,  $p\text{-value} = 0.0103$ ). The Delta value increased from 3hrs until 24hrs after parasitisation (3hrs:  $0.0382 \pm 0.1097$ , 6hrs:  $0.1363 \pm 0.0667$ , 24hrs:  $-0.2178 \pm 0.0674$ ). Whereas at the first two time spans after para-

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sitisation, also parasitised larvae did not exhibit a preference for any of the two yeast species offered, at 24hrs after parasitisation parasitised larvae preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae* in contrast to unparasitised larvae, suggested by the fact that there was a significant deviance of the Delta value from the intercept.

In none of the time spans a significant difference between the food choice of unparasitised compared to parasitised larvae occurred (3hrs:  $F_{1,18} = 0.0036$ ,  $p = 0.953$ ; 6hrs:  $F_{1,18} = 0.8439$ ,  $p = 0.3704$ ; 24hrs:  $F_{1,18} = 4.1289$ ,  $p = 0.05718$ ), i.e. unparasitised as well as parasitised larvae both exhibited the same yeast preferences.

**Yeast combination *Kluyveromyces lactis* & *Metschnikowia pulcherrima*:** When larvae could choose between the two yeast species *Kluyveromyces lactis* and *Metschnikowia pulcherrima* (**Fig. 50B**), a significant difference could be found between the number of **unparasitised larvae** found on the patch with *Kluyveromyces lactis* and the patch with *Metschnikowia pulcherrima*, in each of the three time spans after parasitisation (significant deviation of the Delta value from the intercept) (3hrs:  $t = 4.986$ ,  $df = 9$ ,  $p\text{-value} = 0.0007533$ ; 6hrs:  $t = 4.5442$ ,  $df = 9$ ,  $p\text{-value} = 0.001397$ , 24hrs:  $t = 7.0316$ ,  $df = 9$ ,  $p\text{-value} < 0.0001$ ). Thus, unparasitised larvae exhibited a preference for *Kluyveromyces lactis* over *Metschnikowia pulcherrima* at all time spans after parasitisation that larvae were allowed to choose. The Delta value showed merely a slight decrease from 3hrs until 24hrs after parasitisation, suggesting that the preference got less strong over time (3hrs:  $0.4679 \pm 0.0938$ , 6hrs:  $0.4067 \pm 0.0895$ , 24hrs:  $0.3734 \pm 0.0531$ ).

In **parasitised larvae**, only at the time spans of 6hrs and 24hrs after parasitisation, the number of larvae found on *Kluyveromyces lactis* was significantly different from that found on *Metschnikowia pulcherrima* (significant deviation of the Delta value from the intercept only at 6hrs and 24hrs) (3hrs:  $t = 1.1961$ ,  $df = 9$ ,  $p\text{-value} = 0.2622$ ; 6hrs:  $t = 3.6943$ ,  $df = 9$ ,  $p\text{-value} = 0.004964$ ; 24hrs:  $t = 7.8117$ ,  $df = 9$ ,  $p\text{-value} < 0.0001$ ). Thus, at 3hrs after parasitisation, parasitised larvae did not exhibit a preference for any of the two yeast species offered. This changed, however, at the time spans of 6hrs and 24hrs after parasitisation, where parasitised larvae exhibited a preference for *Kluyveromyces lactis* over *Metschnikowia pulcherrima* that was slightly stronger than in unparasitised larvae, suggested by the higher Delta values (3hrs:  $0.1155 \pm 0.0965$ , 6hrs:  $0.4327 \pm 0.1171$ , 24hrs:  $0.5648 \pm 0.0723$ ). Thus, in contrast to unparasitised larvae, the Delta value increased from 3hrs until 24hrs after parasitisation.

At the time span of 3hrs and 24 hrs after parasitisation, a significant difference between the food choice of unparasitised compared to parasitised larvae occurred (3hrs:  $F_{1,18} = 6.8515$ ,  $p = 0.01744$ ; 24hrs:  $F_{1,18} = 4.552$ ,  $p = 0.0469$ ). No significant effect was found at the time span of 6hrs ( $F_{1,18} = 0.0291$ ,  $p = 0.8666$ ).



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**Yeast combination *Metschnikowia pulcherrima* & *Saccharomyces cerevisiae*:** When larvae could choose between the two yeast species *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae* (**Fig. 50C**), the number of *unparasitised larvae* found on the patch with *Metschnikowia pulcherrima* was significantly different from that on the patch with *Saccharomyces cerevisiae* in all time spans after parasitisation (significant deviation of the Delta value from the intercept) (3hrs:  $t = -4.8368$ ,  $df = 9$ ,  $p\text{-value} = 0.0009251$ ; 6hrs:  $t = -6.2558$ ,  $df = 9$ ,  $p\text{-value} = 0.0001486$ ; 24hrs:  $t = -2.4992$ ,  $df = 9$ ,  $p\text{-value} = 0.03391$ ). Unparasitised larvae, therefore, preferred *Saccharomyces cerevisiae* over *Metschnikowia pulcherrima* at all three time spans after parasitisation, suggested by the negative Delta values (3hrs:  $-0.4790 \pm 0.0990$ , 6hrs:  $-0.3990 \pm 0.0638$ , 24hrs:  $-0.2476 \pm 0.0991$ ). This preference got slightly less strong over time, indicated by the fact that the Delta values got less negative from 3hrs until 24hrs after parasitisation.

In *parasitised larvae*, merely at the time spans of 3hrs and 6hrs after parasitisation did the number of larvae found on the patch with *Metschnikowia pulcherrima* significantly differ from that on the patch with *Saccharomyces cerevisiae* (3hrs:  $t = -2.8922$ ,  $df = 9$ ,  $p\text{-value} = 0.01782$ ; 6hrs:  $t = -4.9086$ ,  $df = 9$ ,  $p\text{-value} = 0.0008376$ ). No significant difference was found at the time span of 24hrs ( $t = -1.4385$ ,  $df = 9$ ,  $p\text{-value} = 0.1841$ ). Thus, parasitised larvae also preferred *Saccharomyces cerevisiae* over *Metschnikowia pulcherrima*, though only at the first two time spans after parasitisation. The Delta values also got less negative over time, but increasingly so compared to unparasitised larvae (3hrs:  $-0.3344 \pm 0.1156$ , 6hrs:  $-0.3517 \pm 0.1097$ , 24hrs:  $-0.1000 \pm 0.0695$ ).

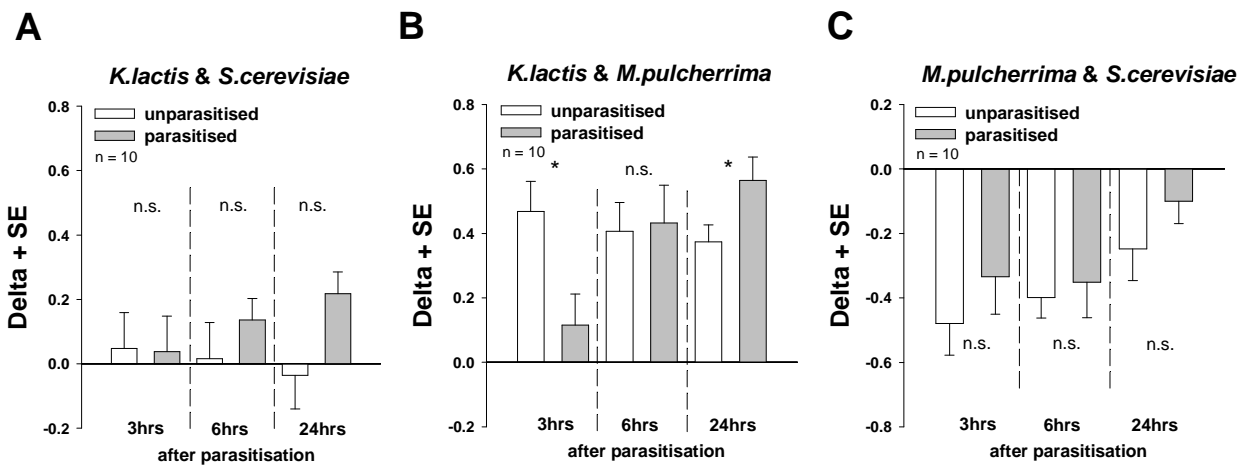
In none of the time spans a significant difference in food choice of unparasitised compared to parasitised larvae occurred (3hrs:  $F_{1,18} = 0.9019$ ,  $p = 0.3549$ ; 6hrs:  $F_{1,18} = 0.2436$ ,  $p = 0.6276$ ; 24hrs:  $F_{1,18} = 1.4888$ ,  $p = 0.2382$ ), i.e. unparasitised as well as parasitised larvae both exhibited the same yeast preferences.

The influence of yeast combination, time span after parasitisation and parasitisation status (no, yes) (factors) as well as the interaction between the three factors on the delta value of unparasitised as well as parasitised *Drosophila melanogaster* larvae (response variable) in the three yeast combinations *Kluyveromyces lactis* & *Saccharomyces cerevisiae*, *Kluyveromyces lactis* & *Metschnikowia pulcherrima*, and *Metschnikowia pulcherrima* & *Saccharomyces cerevisiae* can be seen in **Table 30**. The results of the ANCOVA merely show a highly significant influence of the yeast species combination, as well as the time span after parasitisation that the food choice experiments were carried out.

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**Table 30:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the delta value of unparasitised as well as parasitised *Drosophila melanogaster* larvae in the three yeast combinations *Kluyveromyces lactis* & *Saccharomyces cerevisiae*, *Kluyveromyces lactis* & *Metschnikowia pulcherrima*, and *Metschnikowia pulcherrima* & *Saccharomyces cerevisiae*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Delta value	Yeast combination	$F_{2,177} = 84.3294$	$p < 0.0001$
	Time span	$F_{1,176} = 9.3866$	$p = 0.002547$
	Parasitisation	$F_{1,175} = 2.9863$	$p = 0.085807$
	Yeast combination*Time span	$F_{2,173} = 1.4428$	$p = 0.239179$
	Yeast combination*Parasitisation	$F_{2,171} = 1.3141$	$p = 0.271461$
	Time span*Parasitisation	$F_{1,170} = 3.4563$	$p = 0.064761$
	Yeast combination*Time span*Parasitisation	$F_{2,168} = 1.1760$	$p = 0.311046$



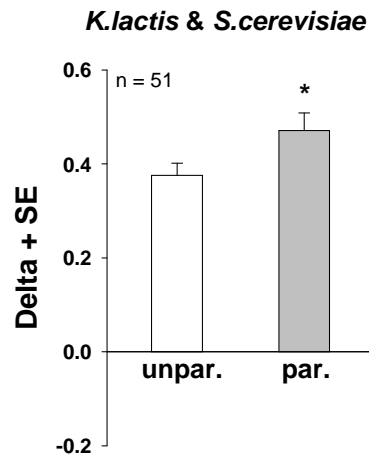
**Fig. 50:** Results of the larval food choice experiment for unparasitised as well as parasitised larvae for the time spans of 3, 6 and 24hrs after parasitisation of parasitised larvae and for the yeast combinations **A)** *Kluyveromyces lactis* & *Saccharomyces cerevisiae*, **B)** *Kluyveromyces lactis* & *Metschnikowia pulcherrima* and **C)** *Metschnikowia pulcherrima* & *Saccharomyces cerevisiae*. Shown are mean values + standard error (SE). n = number of replicates. Significance levels: n.s. = not significant, \*  $p < 0.05$ .

**(II)** Food choice experiment with the two yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae* at the time span of 24hrs after parasitisation of parasitised larvae.

In *unparasitised larvae*, the number of larvae found on the patch with *Kluyveromyces lactis* differed significantly from that found on the patch with *Saccharomyces cerevisiae* (significant deviation of the Delta value from the intercept) ( $t = 14.7073$ ,  $df = 101$ ,  $p < 0.0001$ ). This was also the case in *parasitised larvae* ( $t = 39.35$ ,  $df = 101$ ,  $p < 0.0001$ ). Un-

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parasitised as well as parasitised larvae exhibited a preference for *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, suggested by the distinctly positive Delta value (unparasitised larvae:  $0.3758 \pm 0.0256$ ; parasitised larvae:  $0.4714 \pm 0.0374$ ). The preference in parasitised larvae, however, was more pronounced (**Fig. 51**). The difference in the Delta value (and thus the preference for *Kluyveromyces lactis*) between unparasitised and parasitised larvae was significant ( $F_{1,202} = 4.503$ ,  $p = 0.03505$ ).



**Fig. 51:** Results of the larval food choice experiment for unparasitised (**unpar.**) as well as parasitised (**par.**) larvae for the time span of 24hrs after parasitisation of parasitised larvae and for the yeast combination *Kluyveromyces lactis* (**K.lactis**) & *Saccharomyces cerevisiae* (**S.cerevisiae**). Shown are mean values + standard error (SE). n = number of replicates. Significance levels: \*  $p < 0.05$

**(III) Food choice** experiment with *Kluyveromyces lactis* and *Saccharomyces cerevisiae* at the time span of 24hrs after parasitisation of parasitised larvae and a **crosswise transfer** of larvae either on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* for larval development.

**Unparasitised larvae**, that were not allowed to choose (no choice/control larvae), but were randomly transferred either on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* to pursue their development, showed no significant difference in their **survival** until eclosion as adult flies, between those feeding on *Kluyveromyces lactis* and those feeding on *Saccharomyces cerevisiae* ( $F_{1,86} = 0.123$ ;  $p = 0.7267$ ). Their survival was similarly high, irrespective of the yeast species they had fed on (development on *K.lac.*:  $96.0 \pm 2.90\%$ ; development on *S.cer.*:  $97.4 \pm 2.63\%$ ) (**Fig. 52A**).

Unparasitised larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, also did not exhibit a significant difference in their survival, irrespective of whether having fed on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* ( $F_{1,165} = 0.5788$ ,  $p = 0.4479$ ). Their survival was similarly high (development on *K.lac.*:  $95.3 \pm 2.28\%$ ; development on *S.cer.*:  $97.5 \pm 1.74\%$ ). Additionally, there was no significant difference in sur-

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vival between larvae that chose to consume *Kluyveromyces lactis* over *Saccharomyces cerevisiae* and those that were not allowed to choose any of the two yeast species (no choice larvae), irrespective of whether they had pursued their development on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* (development on *K.lac.*:  $F_{1,134} = 0.0317$ ,  $p = 0.8589$ ; development on *S.cer.*:  $F_{1,117} = 0.0027$ ,  $p = 0.9586$ ). Their survival was almost identically high (development on *K.lac.*: choice larvae:  $95.3 \pm 2.28\%$ ; no choice larvae:  $96.0 \pm 2.80\%$ ; development on *S.cer.*: choice larvae:  $97.5 \pm 1.74\%$ ; no choice larvae:  $97.3684 \pm 2.63\%$ ).

Unparasitised larvae that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis* did not exhibit a significant difference in their survival, irregardless of whether having fed on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* ( $F_{1,109} = 3.0832$ ,  $p = 0.08191$ ). The survival of larvae developing on *Kluyveromyces lactis* was lower than that of larvae developing on *Saccharomyces cerevisiae*, though not significantly so (development on *K.lac.*:  $90.9 \pm 3.91\%$ ; development on *S.cer.*:  $98.2 \pm 1.79\%$ ). There was also no significant difference between larvae that chose to consume *Saccharomyces cerevisiae* over *Kluyveromyces lactis* and no choice larvae, again, irrespective of whether they had fed on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* during their larval development (development on *K.lac.*:  $F_{1,103} = 1.1094$ ,  $p = 0.2947$ ; development on *S.cer.*:  $F_{1,92} = 0.0747$ ,  $p = 0.7852$ ). Their survival was also very similar (development on *K.lac.*: choice larvae:  $90.9 \pm 3.91\%$ ; no choice larvae:  $96.0 \pm 2.80\%$ ; development on *S.cer.*: choice larvae:  $98.2 \pm 1.79\%$ ; no choice larvae:  $97.4 \pm 2.63\%$ ).

When comparing choice larvae with one another (i.e. those that had either preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, or vice versa), no significant difference in survival was found, irregardless of the yeast species (*Kluyveromyces lactis* or *Saccharomyces cerevisiae*) larvae had fed on during their development (development on *K.lac.*:  $F_{1,139} = 1.0598$ ,  $p = 0.3051$ ; development on *S.cer.*:  $F_{1,135} = 0.0728$ ,  $p = 0.7877$ ). They exhibited a similarly successful survival (development on *K.lac.*: choice of *K.lac.*:  $95.3 \pm 2.28\%$ ; choice of *S.cer.*:  $90.9 \pm 3.91\%$ ; development on *S.cer.*: choice of *K.lac.*:  $97.5 \pm 1.74\%$ ; choice of *S.cer.*:  $98.2 \pm 1.79\%$ ).

The influence of the chosen yeast species (*Kluyveromyces lactis*, *Saccharomyces cerevisiae* or none) and the yeast species consumed during larval development (*Kluyveromyces lactis* or *Saccharomyces cerevisiae*) (factors), as well as the interaction between the two factors on survival until eclosion of unparasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 31**. The results of the ANCOVA show that none of the factors had a significant influence on survival.

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**Table 31:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until eclosion of unparasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until eclosion of unparasitised <i>Drosophila melanogaster</i>	Chosen yeast	$F_{2,363} = 0.3274$	$p = 0.72101$
	Yeast for development	$F_{1,362} = 3.0007$	$p = 0.08408$
	Chosen yeast*Yeast for development	$F_{2,360} = 0.3939$	$p = 0.67471$

**Unparasitised larvae**, that were not allowed to choose (no choice/control larvae), but were randomly transferred either on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* to pursue their development, showed a significant difference in their **development time** until eclosion as adult flies between those feeding on *Kluyveromyces lactis* and those feeding on *Saccharomyces cerevisiae* ( $F_{1,83} = 40.464$ ;  $p < 0.0001$ ). Development time was longer in larvae feeding on *Kluyveromyces lactis* than in larvae feeding on *Saccharomyces cerevisiae* (development on *K.lac.*:  $10.1 \pm 0.14$ d; development on *S.cer.*:  $9.0 \pm 0.06$ d) (**Fig. 52B**).

Development time of unparasitised larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, significantly differed between those fed on *Kluyveromyces lactis* and those fed on *Saccharomyces cerevisiae* ( $F_{1,159} = 22.369$ ,  $p < 0.0001$ ). Their development on *Kluyveromyces lactis* took longer than that on *Saccharomyces cerevisiae* (development on *K.lac.*:  $9.7 \pm 0.11$ d; development on *S.cer.*:  $9.1 \pm 0.05$ d). There was also a significant difference in development time between larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, and no choice larvae, when both were transferred on *Kluyveromyces lactis* to pursue their development ( $F_{1,128} = 5.1066$ ,  $p = 0.02553$ ). Choice larvae exhibited a shorter development time on *Kluyveromyces lactis* than no choice larvae (choice larvae:  $9.7 \pm 0.11$ d; no choice larvae:  $10.1 \pm 0.14$ d). When both (choice and no choice larvae) had to feed on *Saccharomyces cerevisiae*, no significant difference in development time was found ( $F_{1,114} = 0.5636$ ,  $p = 0.4544$ ), and the larvae showed almost identical development times (choice larvae:  $9.1 \pm 0.05$ d; no choice larvae:  $9.0 \pm 0.06$ d).

Unparasitised larvae that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, also exhibited a significant difference in their development time between those feeding on *Kluyveromyces lactis* and those feeding on *Saccharomyces cerevisiae* ( $F_{1,103} = 10.450$ ,  $p < 0.01$ ). Their development time was longer when feeding on *Kluyveromyces lactis* than when feeding on *Saccharomyces cerevisiae* (development on *K.lac.*:  $9.7 \pm 0.12$ d; development on *S.cer.*:  $9.2 \pm 0.09$ d). There was also a significant difference in development time between larvae that had preferred *Saccharomyces cerevisiae* over *Kluy-*

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*veromyces lactis* and no choice larvae, when both were transferred on *Kluyveromyces lactis* to pursue their development ( $F_{1,96} = 4.7649$ ,  $p = 0.03148$ ). Choice larvae exhibited a shorter development time on *Kluyveromyces lactis* than no choice larvae (choice larvae:  $9.7 \pm 0.12$ d; no choice larvae:  $10.1 \pm 0.14$ d). When both (choice and no choice larvae) had to feed on *Saccharomyces cerevisiae*, no significant difference in development time was found ( $F_{1,90} = 1.765$ ,  $p = 0.1874$ ), and they exhibited similar development times (choice larvae:  $9.2 \pm 0.09$ d; no choice larvae:  $9.0 \pm 0.06$ d).

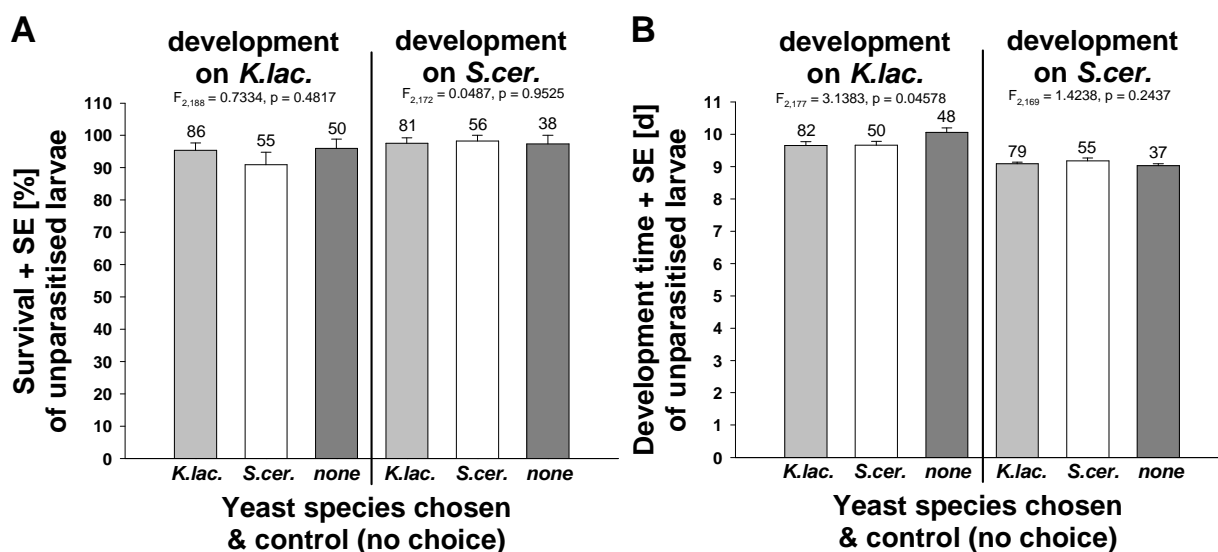
When choice larvae were compared with one another, there was no significant difference in development time between those that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae* and those that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, irregardless of whether larvae had fulfilled their development on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* (development on *K.lac.*:  $F_{1,130} = 0.0001$ ,  $p = 0.9932$ ; development on *S.cer.*:  $F_{1,132} = 1.0185$ ,  $p = 0.3147$ ). Their development times were very similar (development on *K.lac.*: choice of *K.lac.*:  $9.7 \pm 0.11$ d; choice of *S.cer.*:  $9.7 \pm 0.12$ d; development on *S.cer.*: choice of *K.lac.*:  $9.1 \pm 0.05$ d; choice of *S.cer.*:  $9.2 \pm 0.09$ d).

The influence of the chosen yeast species (*Kluyveromyces lactis*, *Saccharomyces cerevisiae* or none) and the yeast species consumed during larval development (*Kluyveromyces lactis* or *Saccharomyces cerevisiae*) (factors), as well as the interaction between the two factors on development time of unparasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 32**. The results of the ANCOVA show that the yeast species consumed during development had a global significant influence on development time. Additionally, the influence of the yeast species consumed during larval development differed among the yeast species chosen. The relative differences in development time between the yeasts consumed during development was, for example, higher in larvae that were not allowed to choose compared to those that had either chosen *Kluyveromyces lactis* or *Saccharomyces cerevisiae* (**Fig. 53B**).

**Table 32:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence development time of unparasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Development time of unparasitised <i>Drosophila melanogaster</i>	Chosen yeast	$F_{2,348} = 2.7744$	$p = 0.06378$
	Yeast for development	$F_{1,347} = 64.7271$	$p < 0.0001$
	Chosen yeast*Yeast for development	$F_{2,345} = 3.5838$	$p = 0.02881$

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**Fig. 52:** Results of the food choice experiment (III) with the yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae* and the subsequent crosswise transfer of larvae either to *Kluyveromyces lactis* (*K.lac.*) or *Saccharomyces cerevisiae* (*S.cer.*) for further development. Compared were *unparasitised larvae* that had been allowed to choose (choice: *K.lac.*: columns in light grey; *S.cer.*: columns in white) with unparasitised larvae that had not been allowed to choose (control/no choice larvae: *none*; columns in dark grey), but were randomly transferred either on *Kluyveromyces lactis* or on *Saccharomyces cerevisiae*. **A)** Survival until eclosion of unparasitised choice or no choice larvae, either developing on *Kluyveromyces lactis* or *Saccharomyces cerevisiae*. **B)** Development time until eclosion of unparasitised choice or no choice larvae, either developing on *Kluyveromyces lactis* or *Saccharomyces cerevisiae*. Shown are mean values + standard error (SE). Numbers above columns = number of replicates.

*Female adult flies* emerging from *unparasitised larvae*, that as larvae had not been allowed to choose (no choice/control larvae), but had been randomly transferred either on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* to pursue their development, showed a significant difference in their *dry weight* between those fed on *Kluyveromyces lactis* and those fed on *Saccharomyces cerevisiae* ( $F_{1,40} = 13.694$ ,  $p = 0.0006474$ ). Flies that as larvae had fed on *Saccharomyces cerevisiae* ( $0.4276 \pm 0.0166\text{mg}$ ) were heavier than those fed on *Kluyveromyces lactis* ( $0.3653 \pm 0.0085\text{mg}$ ) (**Fig. 53A**).

Female unparasitised larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, also exhibited a significant difference in their adult dry weight between those fed on *Kluyveromyces lactis* and those fed on *Saccharomyces cerevisiae* during their larval development ( $F_{1,80} = 16.808$ ,  $p < 0.0001$ ). Again, flies that as larvae had fed on *Saccharomyces cerevisiae* ( $0.4056 \pm 0.0087\text{mg}$ ) were heavier than those fed on *Kluyveromyces lactis* ( $0.3622 \pm 0.0061\text{mg}$ ). There was no significant difference in female adult dry weight between flies that as larvae had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, and no choice larvae, irregardless of the yeast species they had fed on to pursue

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their development (development on *K.lac.*:  $F_{1,66} = 0.0939$ ,  $p = 0.7603$ ; development on *S.cer.*:  $F_{1,54} = 1.584$ ,  $p = 0.2136$ ). When larvae had fed on *Kluyveromyces lactis*, choice larvae and no choice larvae had a similar dry weight as adult female flies (choice larvae:  $0.3622 \pm 0.0061\text{mg}$ ; no choice larvae:  $0.3653 \pm 0.0085\text{mg}$ ). No choice larvae were slightly heavier as adults than choice larvae, when fed on *Saccharomyces cerevisiae* (choice larvae:  $0.4056 \pm 0.0087\text{mg}$ ; no choice larvae:  $0.4276 \pm 0.0166\text{mg}$ ).

Female unparasitised larvae that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, also exhibited a significant difference in their adult dry weight between those fed on *Kluyveromyces lactis* and those fed on *Saccharomyces cerevisiae* during their larval development ( $F_{1,46} = 15.488$ ,  $p = 0.0002786$ ). Again, larvae that had fed on *Saccharomyces cerevisiae* exhibited a higher dry weight as adults than those that had fed on *Kluyveromyces lactis* (development on *K.lac.*:  $0.3354 \pm 0.0098\text{mg}$ ; development on *S.cer.*:  $0.4059 \pm 0.0160\text{mg}$ ). There was also a significant difference in female adult dry weight between flies that as larvae had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, and no choice larvae, though only when they had fed on *Kluyveromyces lactis* to pursue their development ( $F_{1,52} = 5.3337$ ,  $p = 0.02492$ ). When larvae had fed on *Saccharomyces cerevisiae*, female adult dry weight of choice larvae did not significantly differ from dry weight of flies that emerged from no choice larvae ( $F_{1,34} = 0.847$ ,  $p = 0.3639$ ). Female dry weight of no choice larvae was higher than that of choice larvae, when fed on *Kluyveromyces lactis* (choice larvae:  $0.3354 \pm 0.0098\text{mg}$ ; no choice larvae:  $0.3653 \pm 0.0085\text{mg}$ ), whereas flies were similarly heavy when fed on *Saccharomyces cerevisiae* (choice larvae:  $0.4059 \pm 0.0160\text{mg}$ ; no choice larvae:  $0.4276 \pm 0.0166\text{mg}$ ).

When larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, were compared with those that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, there was a significant difference in female dry weight when larvae had fed on *Kluyveromyces lactis* ( $F_{1,66} = 6.0318$ ,  $p = 0.01669$ ), but not when larvae had fed on *Saccharomyces cerevisiae* ( $F_{1,60} = 0.0003$ ,  $p = 0.9868$ ). Adult dry weight of larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae* was higher than adult dry weight of larvae that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, when larvae had fed on *Kluyveromyces lactis* during their development (choice: *K.lac.*:  $0.3622 \pm 0.0061\text{mg}$ ; choice: *S.cer.*:  $0.3354 \pm 0.0098\text{mg}$ ). With *Saccharomyces cerevisiae* as dietary yeast, dry weight was almost identical (choice of *K.lac.*:  $0.4056 \pm 0.0087\text{mg}$ ; choice of *S.cer.*:  $0.4059 \pm 0.0160\text{mg}$ ).



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**Male adult flies** emerging from *unparasitised larvae*, that as larvae had not been allowed to choose (no choice/control larvae), but had been randomly transferred either on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* to pursue their development, showed no significant difference in their **dry weight** between those fed on *Kluyveromyces lactis* and those fed on *Saccharomyces cerevisiae* ( $F_{1,42} = 2.649$ ,  $p = 0.1111$ ). Male adults that had fed on *Saccharomyces cerevisiae* during their larval development were slightly heavier than those that had fed on *Kluyveromyces lactis* (development on *K.lac.*:  $0.2736 \pm 0.0143$ mg; development on *S.cer.*:  $0.2984 \pm 0.0045$ mg) (**Fig. 53B**).

Male unparasitised larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, did also not exhibit a significant difference in their dry weight between those fed on *Kluyveromyces lactis* and those fed on *Saccharomyces cerevisiae* during their larval development ( $F_{1,71} = 2.2951$ ,  $p = 0.1342$ ), and were similarly heavy (development on *K.lac.*:  $0.2646 \pm 0.0068$ mg; development on *S.cer.*:  $0.2758 \pm 0.0029$ mg). A significant difference in male dry weight, however, occurred, between choice and no choice larvae when fed on *Saccharomyces cerevisiae* ( $F_{1,57} = 19.348$ ,  $p < 0.0001$ ), though not with *Kluyveromyces lactis* as dietary yeast species ( $F_{1,56} = 0.4065$ ,  $p = 0.5263$ ). Choice and no choice larvae were almost similarly heavy when fed on *Kluyveromyces lactis* (choice larvae:  $0.2646 \pm 0.0068$ mg; no choice larvae:  $0.2736 \pm 0.0143$ mg), but no choice larvae were heavier than choice larvae when fed on *Saccharomyces cerevisiae* (choice larvae:  $0.2758 \pm 0.0029$ mg; no choice larvae:  $0.2984 \pm 0.0045$ mg).

Male unparasitised larvae that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, did exhibit a significant difference in their dry weight between those fed on *Kluyveromyces lactis* and those fed on *Saccharomyces cerevisiae* during their larval development ( $F_{1,47} = 7.4003$ ,  $p = 0.009113$ ). Flies that as larvae had fed on *Saccharomyces cerevisiae* had a higher dry weight than those fed on *Kluyveromyces lactis* (development on *K.lac.*:  $0.2625 \pm 0.0050$ mg; development on *S.cer.*:  $0.2874 \pm 0.0070$ mg). In comparison to no choice larvae, there was no significant difference in male dry weight, irregardless of the dietary yeast species fed to them during their larval stage (development on *K.lac.*:  $F_{1,41} = 0.5242$ ,  $p = 0.4732$ ; development on *S.cer.*:  $F_{1,48} = 1.5674$ ,  $p = 0.2166$ ), and adults of choice and no choice larvae had an almost similar dry weight (development on *K.lac.*: choice larvae:  $0.2625 \pm 0.0050$ mg; no choice larvae:  $0.2736 \pm 0.0143$ mg; development on *S.cer.*: choice larvae:  $0.2874 \pm 0.0070$ mg; no choice larvae:  $0.2984 \pm 0.0045$ mg).

When larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, were compared with those that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, no significant difference in male dry weight of adult flies occurred, irregardless of the dietary yeast species fed to them as larvae (development on *K.lac.*:  $F_{1,55} = 0.0465$ ,  $p = 0.83$ ; development on *S.cer.*:  $F_{1,63} = 2.8253$ ,  $p = 0.09774$ ). Male adults were similarly

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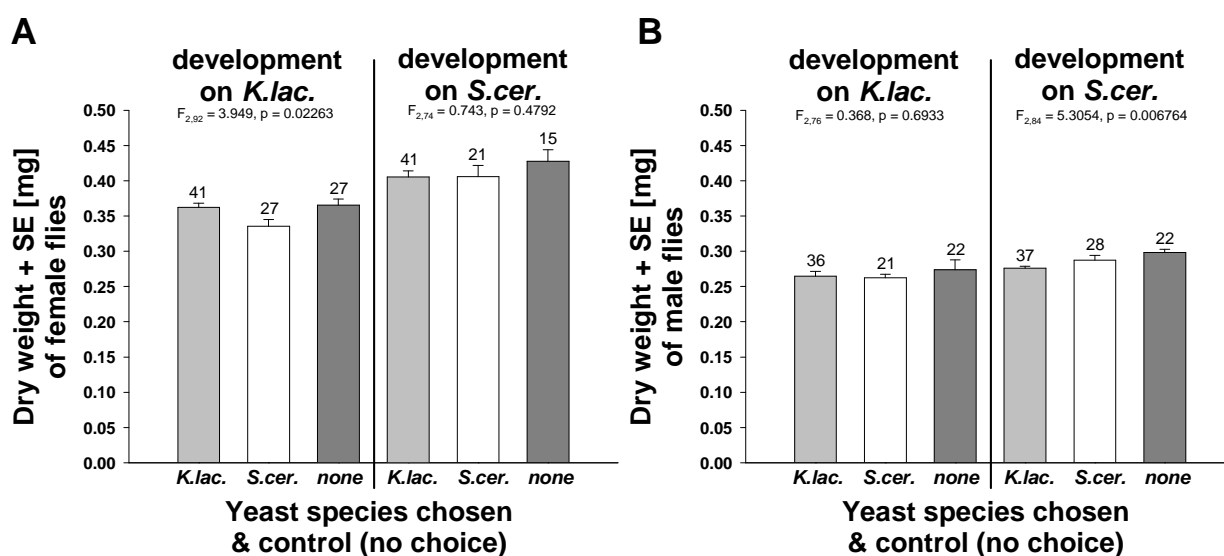
heavy in both cases (development on *K.lac.*: choice of *K.lac.*:  $0.2646 \pm 0.0068\text{mg}$ ; choice of *S.cer.*:  $0.2625 \pm 0.0050\text{mg}$ ; development on *S.cer.*: choice of *K.lac.*:  $0.2758 \pm 0.0029\text{mg}$ ; choice of *S.cer.*:  $0.2874 \pm 0.0070\text{mg}$ ).

The influence of the chosen yeast species (*Kluyveromyces lactis*, *Saccharomyces cerevisiae* or none), the yeast species consumed during larval development (*Kluyveromyces lactis* or *Saccharomyces cerevisiae*) and sex (factors), as well as the interaction between the three factors on adult dry weight of *Drosophila melanogaster* (response variable) can be seen in **Table 33**. The results of the ANCOVA show a global significant influence of the yeast consumed during development and sex on adult dry weight. Additionally, the influence of the yeast consumed during larval development on adult dry weight differed between male and female flies. For example, when larvae had chosen *Kluyveromyces lactis*, the relative difference in dry weight of male flies between the different yeast species consumed during larval development was lower than of female flies (**Fig. 54**). This did not only hold true for *Kluyveromyces lactis*, but also when *Saccharomyces cerevisiae* or no yeast species was chosen.

**Table 33:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence adult dry weight of *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Adult dry weight of <i>Drosophila melanogaster</i>	Chosen yeast	$F_{2,335} = 2.5226$	$p = 0.0818139$
	Yeast for development	$F_{1,334} = 35.6885$	$p < 0.0001$
	Sex	$F_{1,333} = 462.9018$	$p < 0.0001$
	Chosen yeast*Yeast for development	$F_{2,331} = 1.5711$	$p = 0.2093913$
	Chosen yeast*Sex	$F_{2,329} = 1.3882$	$p = 0.2509897$
	Yeast for development*Sex	$F_{1,328} = 14.2571$	$p = 0.0001895$
	Chosen yeast*Yeast for development*Sex	$F_{2,326} = 0.1659$	$p = 0.8471898$

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**Fig. 53:** Results of the food choice experiment (III) with the yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae* and the subsequent crosswise transfer of larvae either to *Kluyveromyces lactis* (*K.lac.*) or *Saccharomyces cerevisiae* (*S.cer.*) for further development. Compared were *unparasitised larvae* that had been allowed to choose (choice of *K.lac.*: columns in light grey; *S.cer.*: columns in white) with unparasitised larvae that had not been allowed to choose (control/no choice larvae: *none*; columns in dark grey), but were randomly transferred either on *Kluyveromyces lactis* or on *Saccharomyces cerevisiae*. **A)** Female dry weight of adult flies emerging from unparasitised choice or no choice larvae, either developing on *Kluyveromyces lactis* or *Saccharomyces cerevisiae*. **B)** Male dry weight of adult flies emerging from unparasitised choice or no choice larvae, either developing on *Kluyveromyces lactis* or *Saccharomyces cerevisiae*. Shown are mean values + standard error (SE). Numbers above columns = number of replicates.

*Parasitised larvae*, that were not allowed to choose (no choice/control larvae), but were randomly transferred either on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* to pursue their development, showed no significant difference in their *encapsulation ability* between those feeding on *Kluyveromyces lactis* and those feeding on *Saccharomyces cerevisiae* ( $F_{1,24} = 0.0$ ;  $p = 1.0$ ). Encapsulation ability was 0% in both of the yeast treatments (**Fig. 54**).

Parasitised larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, exhibited a significant difference in their encapsulation ability between those feeding on *Kluyveromyces lactis* and those feeding on *Saccharomyces cerevisiae* ( $F_{1,52} = 4.5025$ ,  $p = 0.03863$ ). Larvae fed on *Kluyveromyces lactis*, were more successful in encapsulating *Aso-bara tabida* offspring than those fed on *Saccharomyces cerevisiae* (development on *K.lac.*:  $29.6 \pm 8.96\%$ ; development on *S.cer.*:  $7.4 \pm 5.14\%$ ). There was also a significant difference in development time between larvae that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, and no choice larvae, when both were transferred on *Kluyveromyces lactis* to pursue their development ( $F_{1,40} = 11.978$ ,  $p = 0.001294$ ). Larvae that had pre-

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ferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, exhibited a higher encapsulation ability than no choice larvae (choice larvae:  $29.6 \pm 8.96\%$ ; no choice larvae:  $0.0 \pm 0.00\%$ ). When both (choice and no choice larvae) had to feed on *Saccharomyces cerevisiae*, no significant difference in development time was found ( $F_{1,36} = 1.8824$ ,  $p = 0.1786$ ), even though encapsulation ability in choice larvae was higher than in no choice larvae (choice larvae:  $7.4 \pm 5.14\%$ ; no choice larvae:  $0.0 \pm 0.00\%$ ).

Parasitised larvae that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, also exhibited a significant difference in their encapsulation ability between those feeding on *Kluyveromyces lactis* and those feeding on *Saccharomyces cerevisiae* ( $F_{1,27} = 7.047$ ,  $p = 0.01315$ ). Their encapsulation ability was higher when feeding on *Saccharomyces cerevisiae* than when feeding on *Kluyveromyces lactis* (development on *K.lac.*:  $0.0 \pm 0.00\%$ ; development on *S.cer.*:  $15.4 \pm 10.42\%$ ). There was also a significant difference in encapsulation ability between larvae that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis* and no choice larvae, when both were transferred on *Saccharomyces cerevisiae* to pursue their development ( $F_{1,22} = 4.4097$ ,  $p = 0.04742$ ). Larvae that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis* were more successful in encapsulating *Asobara tabida* offspring than no choice larvae (choice larvae:  $15.4 \pm 10.42\%$ ; no choice larvae:  $0.0 \pm 0.00\%$ ). When both (choice and no choice larvae) had to feed on *Kluyveromyces lactis*, no significant difference in encapsulation ability was found ( $F_{1,29} = 0.0$ ;  $p = 1.0$ ), and in both encapsulation ability was 0%.

When choice larvae were compared with one another, there was a significant difference in encapsulation ability between those that had preferred *Kluyveromyces lactis* over *Saccharomyces cerevisiae* and those that had preferred *Saccharomyces cerevisiae* over *Kluyveromyces lactis*, when both fulfilled their development on *Kluyveromyces lactis* ( $F_{1,41} = 12.911$ ,  $p = 0.0008673$ ). Larvae that had chosen *Kluyveromyces lactis* were more successful in encapsulating *Asobara tabida* offspring than those that had chosen *Saccharomyces cerevisiae* (choice of *K.lac.*:  $29.6 \pm 8.96\%$ ; choice of *S.cer.*:  $0.0 \pm 0.00\%$ ). When larvae had fed on *Saccharomyces cerevisiae* during their development, no significant difference in encapsulation ability occurred ( $F_{1,38} = 0.5562$ ,  $p = 0.4604$ ), and their encapsulation ability was more similar (choice of *K.lac.*:  $7.4 \pm 5.14\%$ ; choice of *S.cer.*:  $15.4 \pm 10.42\%$ ).

Data for **parasitised larvae of survival until pupariation** were not analysed, since choice larvae as well as no choice larvae exhibited a survival of 100% (data not shown), regardless of the dietary yeast species fed to them during their larval development.

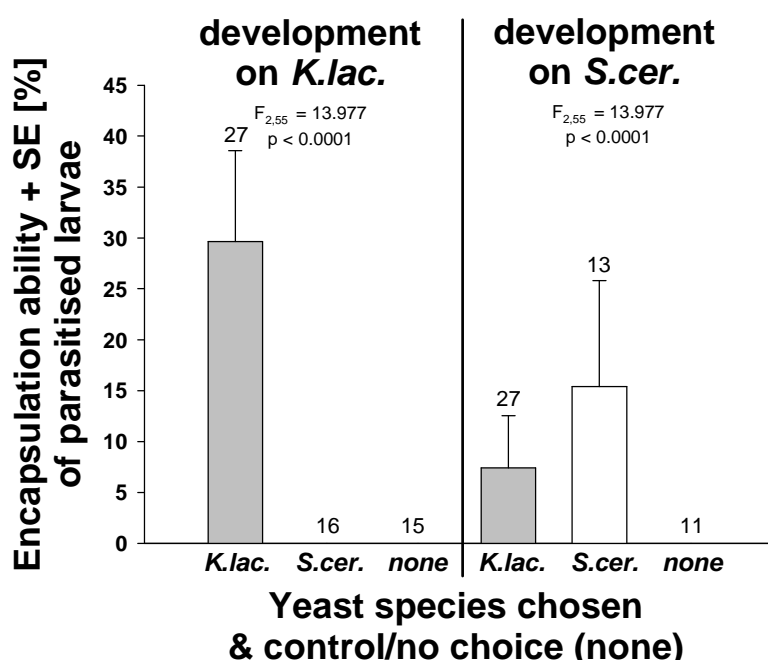
The influence of the chosen yeast species (*Kluyveromyces lactis*, *Saccharomyces cerevisiae* or none) and the yeast species consumed during larval development (*Kluyveromyces lactis* or *Saccharomyces cerevisiae*) (factors), as well as the interaction between the two

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factors on the encapsulation ability of parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 34**. The results of the ANCOVA show that the chosen yeast had a global significant influence on encapsulation ability. Additionally, the influence of the yeast species consumed during larval development on encapsulation ability depended on which yeast species had been chosen beforehand, as indicated by the significant influence of the interaction between chosen yeast and yeast consumed during development. Larvae exhibited a higher encapsulation ability when they were allowed to consume the yeast species they had chosen beforehand (**Fig. 55**).

**Table 34:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the encapsulation ability of parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Encapsulation ability of parasitised <i>Drosophila melanogaster</i>	Chosen yeast	$F_{2,106} = 7.1307$	$p = 0.001258$
	Yeast for development	$F_{1,105} = 2.2565$	$p = 0.136116$
	Chosen yeast*Yeast for development	$F_{2,103} = 5.0738$	$p = 0.007914$



**Fig. 54:** Results of the food choice experiment (III) with the yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae* and the subsequent crosswise transfer of larvae either to *Kluyveromyces lactis* (*K.lac.*) or *Saccharomyces cerevisiae* (*S.cer.*) for further development. Compared were *parasitised larvae* that had been allowed to choose (choice: *K.lac.* columns in light grey; *S.cer.*; columns in white) with parasitised larvae that had not been allowed to choose (control/no choice larvae: *none*; columns in dark grey; not detectable here due to encapsulation ability being 0%), but were randomly transferred either on *Kluyveromyces lactis* or on *Saccharomyces cerevisiae*. Shown are mean values + standard error (SE). Numbers above columns = number of replicates.

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**Table 35:** Overview over results of **Experiment III**. Comparison between choice and control/no choice larvae for the measured fitness traits. Significant results highlighted with grey background. Abbreviation of yeasts: *K.lac.* = *Kluyveromyces lactis*; *S.cer.* = *Saccharomyces cerevisiae*.

		<b>survival until eclosion</b>	<b>development time</b>	<b>female dry weight</b>	<b>male dry weight</b>	<b>encapsulation ability</b>
<b>control/no choice larvae</b>		survival on <i>K.lac.</i> as good as on <i>S.cer.</i>	longer development time on <i>K.lac.</i>	higher dry weight on <i>S.cer.</i>	similar dry weight on <i>K.lac.</i> and on <i>S.cer.</i>	similar encapsulation ability on <i>K.lac.</i> and on <i>S.cer.</i>
<b>choice larvae (<i>K.lac.</i>)</b>		survival on <i>K.lac.</i> as good as on <i>S.cer.</i>	longer development time on <i>K.lac.</i>	higher dry weight on <i>S.cer.</i>	similar dry weight on <i>K.lac.</i> and on <i>S.cer.</i>	higher encapsulation ability on <i>K.lac.</i>
<b>choice larvae (<i>K.lac.</i>) &amp; control larvae</b>	development on <i>K.lac.</i>	survival of choice larvae as good as of control larvae	shorter development time of choice larvae	similar dry weight of choice and control larvae	similar dry weight of choice and control larvae	higher encapsulation ability of choice larvae
	development on <i>S.cer.</i>	survival of choice larvae as good as of control larvae	similar development times of choice and control larvae	similar dry weight of choice and control larvae	higher dry weight of control larvae	similar encapsulation ability of choice and control larvae
<b>choice larvae (<i>S.cer.</i>)</b>		survival on <i>K.lac.</i> as good as on <i>S.cer.</i>	longer development time on <i>K.lac.</i>	higher dry weight on <i>S.cer.</i>	higher dry weight on <i>S.cer.</i>	higher encapsulation ability on <i>S.cer.</i>
<b>choice larvae (<i>S.cer.</i>) &amp; control larvae</b>	development on <i>K.lac.</i>	survival of choice larvae as good as of control larvae	shorter development time of choice larvae	higher dry weight of control larvae	similar dry weight of choice and control larvae	similar encapsulation ability of choice and control larvae
	development on <i>S.cer.</i>	survival of choice larvae as good as of control larvae	similar development times of choice and control larvae	similar dry weight of choice and control larvae	similar dry weight of choice and control larvae	higher encapsulation ability of choice larvae
<b>choice larvae (<i>K.lac.</i>) &amp; choice larvae (<i>S.cer.</i>)</b>	development on <i>K.lac.</i>	survival of choice larvae ( <i>K.lac.</i> ) as good as of choice larvae ( <i>S.cer.</i> )	similar development times of choice larvae ( <i>K.lac.</i> ) and of choice larvae ( <i>S.cer.</i> )	higher dry weight of choice larvae ( <i>K.lac.</i> )	similar dry weight of choice larvae ( <i>K.lac.</i> ) and choice larvae ( <i>S.cer.</i> )	higher encapsulation ability of choice larvae ( <i>K.lac.</i> )
	development on <i>S.cer.</i>	survival of choice larvae ( <i>K.lac.</i> ) as good as of choice larvae ( <i>S.cer.</i> )	similar development times of choice larvae ( <i>K.lac.</i> ) and of choice larvae ( <i>S.cer.</i> )	similar dry weight of choice larvae ( <i>K.lac.</i> ) and choice larvae ( <i>S.cer.</i> )	similar dry weight of choice larvae ( <i>K.lac.</i> ) and choice larvae ( <i>S.cer.</i> )	similar encapsulation ability of choice larvae ( <i>K.lac.</i> ) and choice larvae ( <i>S.cer.</i> )

## 7. Food choice of *Drosophila melanogaster* larvae

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### 7.4 Discussion

The results of the experiments I and II suggest that parasitised larvae show a tendency in exhibiting a yeast choice which differs from that of unparasitised larvae. This, at least to some extent, confirms the hypothesis, that parasitised *Drosophila melanogaster* larvae are able to detect qualitative differences of specific yeast species in reference to the larvae's altered physiological state, and that they self-select the particular yeast species accordingly, if given the choice. Yet, this tendency of parasitised larvae in exhibiting a yeast choice differing from that of unparasitised larvae, varied with the combination of yeast species offered and the time span after parasitisation that the food choice experiment was carried out.

Unfortunately, there is a dilemma in investigating food choice of parasitised larvae: There is no reliable information about the exact point in time, when parasitism actually has an effect on the physiology and behaviour of the host larva, neither about when the point in time after parasitism is reached, that a behavioural shift does not pay off any more (for the host larva), since the outcome of the host-parasite interaction has already been settled. Apart from that, these time points may vary distinctively between individual larvae. Thus, the 'parasitisation status' of parasitised larvae that took part in the food choice experiments, may have varied over a broad scale due to individual differences of larvae in their physiology. The defence process of encapsulation itself is reported to start at approx. 4-6hrs after parasitisation (WERTHEIM *et al.* 2005). This, however, refers to larvae, that have not been N-starved. Additionally to these variances in parasitised larvae, an individual variation in preferences seems to occur already in unparasitised larvae. The preferences exhibited by unparasitised larvae as described in the experiment 7.3 (a), were similar to those observed by DORSCH (2007) (larvae used in both studies stemmed from the same *Drosophila melanogaster* base population). When offered the yeast combination *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, larvae did not exhibit a preference for any of the two yeast species. When offered *Kluyveromyces lactis* in combination with *Metschnikowia pulcherrima*, larvae showed a strong preference for *Kluyveromyces lactis*, just as they showed a strong preference for *Saccharomyces cerevisiae*, when offered in the combination with *Metschnikowia pulcherrima*. Unparasitised larvae showed these similarities in food choice, despite being N-starved for a different number of hours in the studies described here. Thus, particular yeast species seem to elicit a steady and repeatable preference in *Drosophila melanogaster* larvae over a broad range of physiological conditions, especially in yeast combinations, where one of the yeast species more pronouncedly differs in quality and its impact on larval fitness, such as *Metschnikowia pulcherrima*. In the experiment described in 7.3 (b), however, where only the yeast combination *Kluyveromyces lactis* and *Saccharomyces cerevisiae* was tested after the time span of 24hrs after parasiti-

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sation, unparasitised larvae exhibited a strong preference for *Kluyveromyces lactis*. Apparently, yeast species exhibiting a similar quality and impact on fitness traits, as this seems to be the case for *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, produce a stronger variation in behavioural response as yeast species, which more pronouncedly differ in quality. Already in the experiment described in 7.3 (a), as well as in those carried out by DORSCH (2007), individual unparasitised larvae exhibited strongly differing preferences, which may be indicated by the higher standard errors in the results occurring in this yeast combination. Parasitised larvae, however, rather steadily exhibited a tendency to prefer *Kluyveromyces lactis* over *Saccharomyces cerevisiae*, even though a more or less high individual variation still occurred, and even though this preference was only statistically significant at the time span of 24hrs after parasitisation in both studies 7.3 (a) and (b). Especially in reference to the yeast combination *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, the tendency of parasitised larvae to prefer *Kluyveromyces lactis*, may make sense in our ‘human’ eyes, in two ways: Parasitised larvae could benefit from consuming *Kluyveromyces lactis* due to its beneficial impact on encapsulation ability, as observed in experiments presented earlier in this study (see chapters 5.2 and 5.3). Even though *Kluyveromyces lactis* and *Saccharomyces cerevisiae* seemed to be of equal quality, at least for unparasitised larvae, their impact on encapsulation ability in parasitised larvae differed. There is some evidence that parasitoids alter their hosts in ways beneficial to their own fitness (SLANSKY 1986). Assuming that this could also be the case in *Drosophila melanogaster* parasitised by *Asobara tabida*, the parasitoid may just as well benefit from host larvae feeding on *Kluyveromyces lactis*, since the impact of this yeast species on fitness traits of the parasitoid offspring (e.g. survival, development time) was more beneficial than that of *Saccharomyces cerevisiae* (see chapter 5.4). Thus, even if the behaviour of host larvae was ‘under parasitoid control’, the preference for *Kluyveromyces lactis* would still make sense. In order to discriminate between behavioural responses stemming from ‘decisions’ made by the host larva and those stemming from a manipulation by the parasitoid, a different experimental design would be necessary. Instead of letting *Asobara tabida* parasitise *Drosophila melanogaster* larvae, it would be necessary to implant a foreign object (nylon filament, oil droplet) into the haemocoel for the larva to encapsulate, in order to avoid potential manipulative impacts of a live parasitoid egg or larva.

At least in reference to the yeast combination *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, we may assume that the time periods after parasitisation larvae were forced to abstain from consuming any yeast at all, did not affect the impact of the yeast species on encapsulation ability. There was still the tendency of larvae feeding on *Kluyveromyces lactis* to exhibit enhanced encapsulation ability compared to those fed on *Saccharomyces cerevisiae* (see chapter 5.3). The N-starvation probably caused a kind of physiological ‘rest period’, both for the host larva in reference to its resource-craving immune defence, and



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for the development of the parasitoid offspring. In the other two yeast combinations including *Metschnikowia pulcherrima*, parasitised larvae (and developing parasitoids) probably face the dilemma that, although *Metschnikowia pulcherrima* may enhance encapsulation ability, it may have an unfavourable effect on other fitness traits (see chapter 5, as well as DORSCH 2007). The adverse impact on various fitness traits might be the explanation for the strong preference of unparasitised larvae for *Kluyveromyces lactis* over *Metschnikowia pulcherrima*. There does not seem to be a lot to win from the latter yeast species. This may also hold true for parasitised larvae, since at least at the two later time spans after parasitisation, they also exhibited a strong preference for *Kluyveromyces lactis*, which, at 24hrs after parasitisation, even significantly exceeded the preference of unparasitised larvae. The favourable impact of *Metschnikowia pulcherrima* on encapsulation ability may, however, pose a dilemma for parasitised larvae that could benefit from consuming this yeast species in reference to an enhanced immune defence against the intruder, but would suffer from the adverse effects on other fitness traits. This could be an explanation for the significant preference of parasitised larvae for *Metschnikowia pulcherrima* over *Kluyveromyces lactis* at the time span of 3hrs after parasitisation. Consuming *Metschnikowia pulcherrima* at this stage could still pay off for parasitised larvae. This potential benefit may, however, dwindle the more time has passed after parasitisation. Caterpillars could be shown to include toxic plants in their diet, enhancing their resistance to parasitoids, but only at the expense of reduced growth efficiency (SINGER *et al.* 2004). However, since *Kluyveromyces lactis* also seemed to have a beneficiary effect on encapsulation ability, there would actually, even for parasitised larvae, be no advantage in feeding on *Metschnikowia pulcherrima*. From the 'viewpoint' of *Asobara tabida*, it would not pay off at all, if host larvae fed on *Metschnikowia pulcherrima*, since this yeast species may enhance encapsulation ability and thus indirectly impede the parasitoid's development. On the other hand, development may be directly impeded by the yeast, as the results in chapter 5.4 suggest. When *Metschnikowia pulcherrima* was offered together with *Saccharomyces cerevisiae*, unparasitised larvae clearly preferred the latter, since there would again be nothing for them to win by feeding on *Metschnikowia pulcherrima*. That their preference for *Saccharomyces cerevisiae* got less pronounced at 6hrs and 24hrs after parasitisation, may be due to N-starving of larvae and their consequential tendency to be less choosy. Parasitised larvae could benefit more from consuming *Metschnikowia pulcherrima*, again due to its favourable impact on encapsulation ability. But, surprisingly, in the combination with *Saccharomyces cerevisiae*, which does not seem to have this favourable effect on immune defence, parasitised larvae nevertheless preferred this yeast over *Metschnikowia pulcherrima*. Merely at 24hrs after parasitisation, the preference ceased, which may again be due to N-starvation, just as in unparasitised larvae. This would, however, stand in contrast to the larvae's behavioural responses in the yeast combination *Kluyveromyces lactis* and

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*Metschnikowia pulcherrima*, where their preference for *Kluyveromyces lactis* got even more pronounced at 24hrs after parasitisation. N-hunger should have made them just as less choosy.

Since the potentially broad spectrum of qualitative differences of the yeast species offered in the food choice experiments is not known, the forces driving the food choice behaviour of parasitised larvae in the particular yeast combinations and the different time spans after parasitisation, remain highly speculative. Additionally, the behavioural response of larvae might have been influenced by both yeast species offered simultaneously, in that way, that larvae, perceiving the presence of both yeast species, assume, that they may eat from both of them at a certain time, but decide to consume that first, which at the given moment seems more attractive, and with the thought in mind “I may eat from the other one later”. Provided that there is a tendency of parasitised larvae to exhibit a differing behavioural response to the various yeast species compared to unparasitised larvae, the question arises, how larvae perceive the potential qualitative differences. As they move in and on their food, *Drosophila melanogaster* larvae, similar to many other dipteran especially cyclorhaphous larvae, perceive a number of food-associated stimuli, using a mixture of gustation and olfaction (COBB 1999). The anterior part of cyclorhaphous larvae is divided into two lobes, each of which carries two groups of papillae, the dorsal (or antennal) organ and the terminal (maxillary) organ (together called as antenno-maxillary complex). Confirmed by electrophysiology, the dorsal organs are stated to be the site of olfaction, the terminal organs for gustation (COBB 1999). Most of the larvae in the food choice experiment of the study presented here, made their choice in response to their olfactory stimulation. As could be observed in preliminary experiments, where the position of larvae during the experiment was checked after 5 minute time intervals, nearly all larvae stayed on the yeast patch they had decided to go first, until the hour given to them for decision making had passed. Only very few left the yeast patch within the hour to go on roving. Thus, the yeast species larvae chose to approximate according to their olfactory sensing, also was the one larvae decided to feed on, at least for as long as one hour. What kind of information do larvae perceive merely by the scent of the particular yeast species? Does the odour include information about its quality and effects on larval fitness traits? The fact that larvae may do so is suggested by results of food choice experiments with several different yeast species offered, as well as experiments on larval performance on these specific yeast species. COOPER (1960) observed that larvae of different species of *Drosophila* showed decided preferences when given the choice of several yeast species. Additionally COOPER (1960) as well as LINDSAY (1958) could show, that larvae tended to prefer those yeast species which best supported larval growth. This could also be shown for *Drosophila melanogaster* and the yeast species used in DORSCH (2007) and the present study (see chapter 5).

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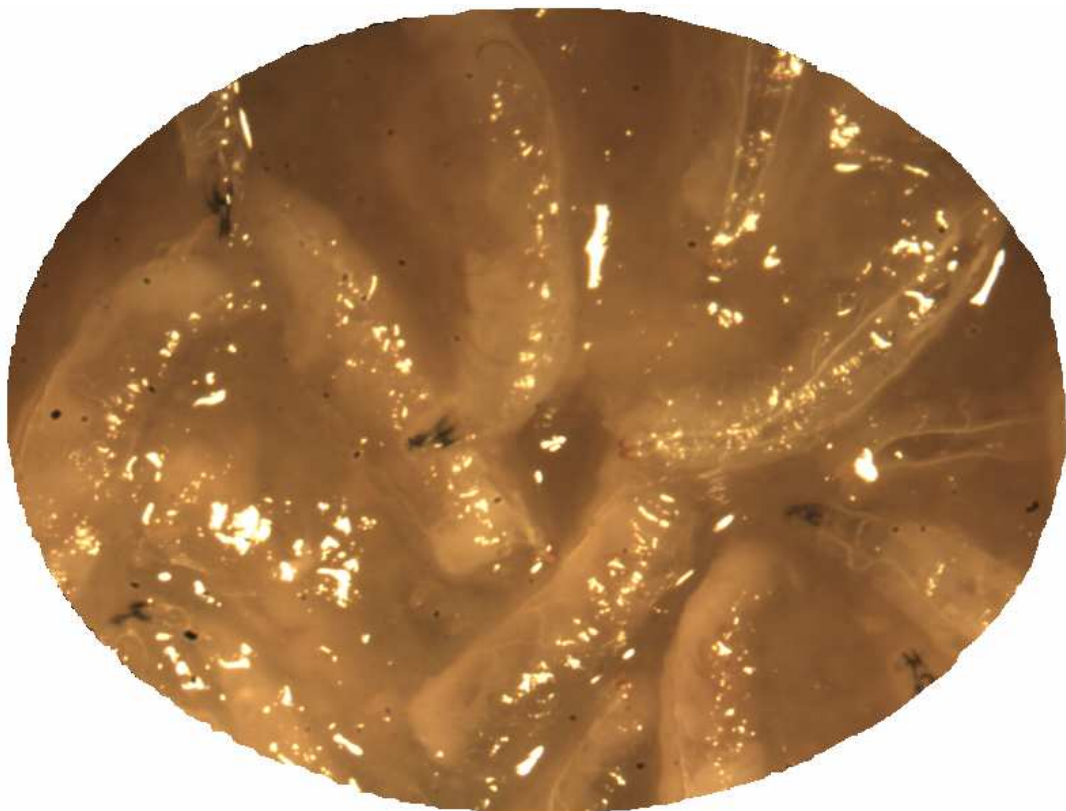
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The results described in chapter 7.3 (c) (Experiment III) add an explanatory piece to the puzzle of the observed variability in immune defences in general, and in reference to the encapsulation ability of larvae fed on *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, as well as the variability observed concerning the preference of larvae for either *Kluyveromyces lactis* or *Saccharomyces cerevisiae*. There seems to be a genetically determined preference in *Drosophila melanogaster* not for specific yeast species, but rather for particular qualitative features of dietary microorganisms. Even though the experimental larvae had not been confronted with any of the yeast species used in the experiments before (also not in their history as laboratory population, since they were always fed with ‘dead’ yeast hydrolysate), individual larvae exhibited preferences for a specific yeast species, without having had a gustatory contact to fuel their decision, but merely as a response of an olfactory stimulus. A naturally occurring intraspecific variation in olfactory response has been found in *Drosophila melanogaster* (COBB 1999). The individual responses to olfactory stimuli and the individual preferences of larvae may originate from genetically determined individual differences in tolerance and salubriousness of specific food ingredients. Hereby, the composition of a specific food item favourable to one larva needn’t be as favourable to another larva. Even though the two yeast species *Kluyveromyces lactis* and *Saccharomyces cerevisiae* are both comparably favourable to the development of (especially unparasitised) *Drosophila melanogaster* larvae in general, as could be shown in the chapters 5.2, 5.4 & 5.5 and by DORSCH (2007), it could be seen, above all in parasitised larvae, that *Kluyveromyces lactis* or *Saccharomyces cerevisiae*, respectively, may be more salubrious for and more beneficial to a specific fitness trait of one larva compared to another. This, however, may not hold true for any fitness trait. The survival of unparasitised as well as parasitised larvae for example, was just as high for all larval treatments, i.e. larvae that had preferred *Kluyveromyces lactis* and also fulfilled their development on this yeast species, did not exhibit a higher survival as larvae that had preferred *Saccharomyces cerevisiae* or larvae that were not allowed to choose any of the two yeast species. This was also the case for larvae that had preferred *Saccharomyces cerevisiae* and also pursued their development on this yeast species. In the other fitness traits that were measured, especially in female dry weight and encapsulation ability, the adequacy of a specific yeast species for individual larvae could be detected. Those larvae that could pursue their development on the yeast species they had preferred, performed better in reference to the measured fitness traits than larvae that were not allowed to choose, and especially than those larvae that had preferred the other of the two yeast species, but were forced to fulfil their development on the one they had not preferred. Despite a rather individual salubriousness of specific yeast species or components of these yeast species for individual larvae, particular yeast species seem to be salubrious for a larger number of larvae than others. This seems to be the case for *Saccharomyces cerevisiae*, since unparasitised larvae do in general seem to exhibit shorter

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development times and a higher female dry weight than when fed on *Kluyveromyces lactis*. Thus, this might be a rather 'robust' inherent quality of *Saccharomyces cerevisiae* to be able to yield these favourable effects. Especially in reference to encapsulation ability of parasitised larvae, however, the quality of neither *Saccharomyces cerevisiae* nor *Kluyveromyces lactis* seems to be particularly robust. Even though *Kluyveromyces lactis* was shown to have a favourable effect on encapsulation ability, just as *Saccharomyces cerevisiae* was shown not to have a similar effect (as seen in chapters 5.2 and 5.3), a high variability nevertheless occurred (as seen in the chapters 5.3, 5.4 and 5.5). Thus, particular *Saccharomyces cerevisiae* or *Kluyveromyces cerevisiae* lovers among *Drosophila melanogaster* larvae may only experience a beneficial impact on their encapsulation ability, when they are able to feed from and fulfil their development on the yeast of their liking.



## **8 Influence of dietary microorganisms on fitness traits of *Drosophila melanogaster* and the parasitoid *Leptopilina heterotoma***

### **8.1 Introduction**

Similar to the other important endoparasitoid of *Drosophila melanogaster* larvae in fermenting substrates, the braconid wasp *Asobara tabida*, offspring of *Leptopilina heterotoma* have to feed from host tissues to fulfil their development to adult wasps. The nutritional background of *Drosophila melanogaster* larvae to build up biomass may just as well influence the developmental success of *Leptopilina heterotoma* larvae feeding from this biomass. Hereby, *Leptopilina heterotoma* also has to face the immune defence of its host while feeding within the host's body cavity. In contrast to *Asobara tabida*, *Leptopilina heterotoma* possesses a well proven active mechanism to evade/suppress the immune defence of *Drosophila melanogaster*, being more effective than the passive mechanism of *Asobara tabida*, whereby the active mechanisms in *Asobara tabida* are still unclear (see chapter 3.2). Due to this immunosuppressive mechanism of female *Leptopilina heterotoma*, wild type *Drosophila melanogaster* larvae are only rarely reported to encapsulate *Leptopilina heterotoma* offspring (MORALES *et al.* 2005), even though encapsulation did occur in other studies (FELLOWES *et al.* 1999a; WALKER 1959).

May this lack of a successful immune defence against *Leptopilina heterotoma* by *Drosophila melanogaster* larvae create a modified condition in the interaction between *Leptopilina heterotoma* and *Drosophila melanogaster* in comparison to the interaction between *Asobara tabida* and *Drosophila melanogaster*? With regard to these potentially modified conditions, may the dietary components of *Drosophila melanogaster* larvae, in the form of different yeast species, still have a direct influence on the developmental success of *Leptopilina heterotoma* offspring, or possibly even an indirect influence via a modulation of the (usually poor) encapsulation ability of *Drosophila melanogaster*?

In a similar experiment as that described in chapter 5.4 carried out with *Asobara tabida*, the above questions should be tackled and preferably answered.

## ***8 Influence of dietary microorganisms on fitness traits of *Drosophila melanogaster* and the parasitoid *Leptopilina heterotoma****

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### **8.2 Material and methods**

#### **(a) *Experimental setup***

The bifactorial experiment comprised four yeast species (*Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae*) as first factor (treatment) and two different initial cell masses ( $10^{-10}$ g and  $10^{-8}$ g) of each of the yeast species as second factor (treatment), adding up to overall eight treatments. Parasitised as well as unparasitised larvae were exposed to the different treatments.

#### **(b) *Fly and parasitoid cultures***

See chapter 4.1.

#### **(c) *Preparation of media:***

See chapter 4.3.

#### **(d) *Preparation of larvae***

See chapter 4.4 and 4.5.

#### **(e) *Gaining of parasitised larvae***

For this experiment the observational method described in chapter 4.6.1 was used. One parasitoid each was allowed to parasitise approx. 16 larvae, of which two larvae each were transferred into each of the eight yeast treatments, directly after the parasitoid had finished the 16 parasitisations.

#### **(f) *Preparation of microorganisms***

For this experiment limited food conditions were prepared with  $10^{-10}$ g and  $10^{-8}$ g initial yeast cell mass. These two different cell masses were chosen after DORSCH (2007), who had tested fitness traits of unparasitised larvae for an initial yeast cell mass ranging from  $10^{-10}$ g till  $10^{-2}$ g.

#### **(g) *Experimental proceeding***

50µl each of the yeast suspensions containing a yeast cell mass of  $10^{-10}$ g and  $10^{-8}$ g respectively, were pipetted into the prepared test tubes filled with Banana medium II. Hereupon one larva each was transferred into the tubes with a fine brush and the tubes covered with dental rolls (HARTMANN). Test tubes containing one parasitised larva each as well as tubes with one unparasitised larva each (control tubes) were prepared. The tubes were incubated in a climate chamber at 25°C and a 16:8 L/D cycle. The development of unparasitised as well as parasitised larvae was followed until eclosion. Adult flies emerging from unparasitised larvae were dried and weighed. Adult flies emerging from parasitised larvae were dissected to look for the presence of a capsule. Female eclosed *Leptopilina heterotoma* adults were dissected to determine egg load, were dried and weighed, and the fat content was measured.

## 8 Influence of dietary microorganisms on fitness traits of *Drosophila melanogaster* and the parasitoid *Leptopilina heterotoma*

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### (h) Determination of encapsulation ability

After eclosion of adult flies emerging from parasitised larvae, the adults were killed in the freezer at a temperature of -20°C and stored there until dissection. When adults were dissected, the absence or presence of capsules was noted. Only those individuals were included in the analysis that contained a capsule (parasitised, successful defence/encapsulation). Unparasitised flies (not containing a capsule) and superparasitised flies (inhabiting more than one capsule) were excluded from the analysis.

### (i) Determination of egg load and fat content in *Leptopilina heterotoma*

See chapter 4.8.4

### (j) Statistical analysis

See chapter 4.9. The analysis was carried out with yeast species, initial yeast cell mass as well as parasitisation status (no, yes) and sex (female, male) as linear predictor.

## 8.3 Results

### (a) Unparasitised *Drosophila melanogaster* larvae:

There was a significant effect of the dietary yeast species on *survival until eclosion* of unparasitised larvae, though only at an initial yeast cell mass of  $10^{-8}$ g ( $10^{-10}$ g:  $F_{3,76} = 1.693$ ,  $p = 0.1756$ ;  $10^{-8}$ g:  $F_{3,76} = 29.073$ ,  $p < 0.0001$ ). At an initial yeast cell mass of  $10^{-10}$ g, adults having fed on *Kluyveromyces lactis* and *Saccharomyces cerevisiae*, both exhibited the highest survival (*K.lac.*:  $94.7 \pm 5.26\%$ ; *S.cer.*:  $90.00 \pm 6.88\%$ ), followed by those fed on *Metschnikowia pulcherrima* ( $85.0 \pm 8.2\%$ ), and *Pichia toletana* ( $70.0 \pm 10.5\%$ ). At an initial yeast cell mass of  $10^{-8}$ g, survival was again highest for larvae having fed on *Kluyveromyces lactis* and *Saccharomyces cerevisiae* (both  $100.00 \pm 0.00\%$ ), followed by those fed on *Pichia toletana* ( $85.0 \pm 8.2\%$ ), and *Metschnikowia pulcherrima* ( $25.0 \pm 9.9\%$ ) (**Fig. 55A**).

## 8 Influence of dietary microorganisms on fitness traits of *Drosophila melanogaster* and the parasitoid *Leptopilina heterotoma*

Survival was significantly affected by the initial yeast cell mass, when unparasitised larvae had fed on *Metschnikowia pulcherrima* ( $F_{1,38} = 14.867$ ,  $p < 0.001$ ) and on *Saccharomyces cerevisiae* ( $F_{1,38} = 5.468$ ,  $p = 0.02473$ ), but not when they had fed on *Kluyveromyces lactis* ( $F_{1,38} = 2.6827$ ,  $p = 0.1097$ ) and *Pichia toletana* ( $F_{1,38} = 1.2447$ ,  $p = 0.2716$ ).

The influence of dietary yeast species and initial cell mass (factors), as well as the interaction between the two factors on survival until eclosion of *Drosophila melanogaster* (response variable) can be seen in **Table 36**. The results of the ANCOVA show a significant influence of yeast species on survival. Additionally, the significant influence of the interaction between yeast and initial cell mass suggests that the influence of the initial yeast cell mass on survival until eclosion varies among the different yeast species. The relative difference in survival in larvae that had fed on *Kluyveromyces lactis*, for example, is much smaller than in larvae that had fed on *Metschnikowia pulcherrima* (**Fig. 55A**).

**Table 36:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until eclosion of *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until eclosion of <i>Drosophila melanogaster</i>	Yeast	$F_{3,156} = 13.2944$	$p < 0.0001$
	Initial cell mass	$F_{1,155} = 2.3242$	$p = 0.1295$
	Yeast*Initial cell mass	$F_{3,152} = 8.1972$	$p < 0.0001$

**Development time** of unparasitised larvae was also significantly affected by the dietary yeast species, and even at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,63} = 10.197$ ,  $p < 0.0001$ ;  $10^{-8}$ g: ( $F_{3,59} = 73.985$ ,  $p < 0.0001$ ). The initial yeast cell mass itself also had a significant influence on development time in all dietary yeast species (*K.lac.*:  $F_{1,36} = 5.4097$ ,  $p = 0.02577$ ; *M.pulch.*:  $F_{1,21} = 15.134$ ,  $p < 0.001$ ; *P.tol.*:  $F_{1,29} = 5.5269$ ,  $p = 0.02574$ ; *S.cer.*:  $F_{1,36} = 15.161$ ,  $p < 0.001$ ). Development time was usually shorter at an initial cell mass of  $10^{-8}$ g than at  $10^{-10}$ g, except when larvae had fed on *Metschnikowia pulcherrima*, where development time at  $10^{-8}$ g lasted longer than at  $10^{-10}$ g. Larvae fed on *Saccharomyces cerevisiae* exhibited the shortest development time at the higher yeast cell mass of  $10^{-8}$ g ( $9.7 \pm 0.1$ d), followed by larvae fed on *Kluyveromyces lactis* ( $10.3 \pm 0.2$ d), those fed on *Pichia toletana* ( $10.1 \pm 0.2$ d) and those fed on *Metschnikowia pulcherrima* ( $17.8 \pm 2.0$ d). At the lower yeast cell mass of  $10^{-10}$ g the order was the same, with larvae fed on *Saccharomyces cerevisiae* exhibiting the shortest development time ( $10.3 \pm 0.1$ d), followed by those fed on *Kluyveromyces lactis* ( $11.2 \pm 0.4$ d), *Pichia toletana* ( $11.1 \pm 0.4$ d) and finally *Metschnikowia pulcherrima* with the longest development time ( $13.1 \pm 0.6$ d) (**Fig. 55B**).

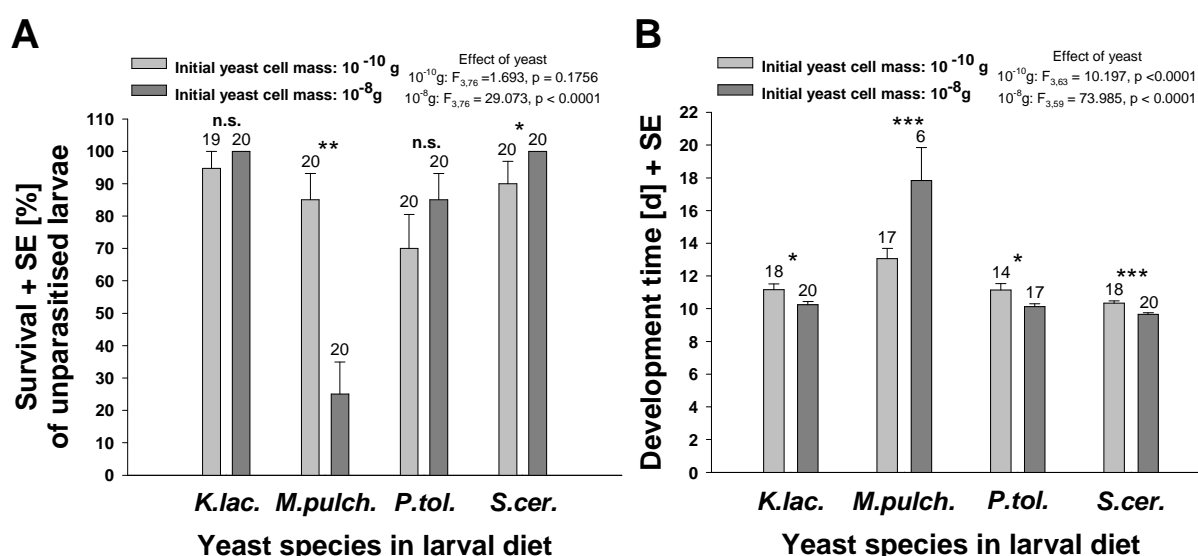


## 8 Influence of dietary microorganisms on fitness traits of *Drosophila melanogaster* and the parasitoid *Leptopilina heterotoma*

The influence of dietary yeast species and initial cell mass (factors), as well as the interaction between the two factors on development time of *Drosophila melanogaster* (response variable) can be seen in **Table 37**. The results of the ANCOVA show a significant influence of yeast species on development time. Additionally, the significant influence of the interaction between yeast and initial cell mass suggests that the influence of initial cell mass on development time varies among the different yeast species. The relative differences in development time between the two initial yeast cell masses are smaller in larvae that had fed on *Kluyveromyces lactis*, for example, than in larvae that had fed on *Metschnikowia pulcherrima* (**Fig. 55B**).

**Table 37:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence development time of *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Development time of <i>Drosophila melanogaster</i>	Yeast	$F_{3,126} = 53.2673$	$p < 0.0001$
	Initial cell mass	$F_{1,125} = 1.1348$	$p = 0.2889$
	Yeast*Initial cell mass	$F_{3,122} = 17.8827$	$p < 0.0001$



**Fig. 55:** A) *Survival* until eclosion (in %) and B) *Development time* (in days) of unparasitised *Drosophila melanogaster* larvae depending on the dietary yeast species and the initial yeast cell mass of each yeast species ( $10^{-10}$  and  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

## 8 Influence of dietary microorganisms on fitness traits of *Drosophila melanogaster* and the parasitoid *Leptopilina heterotoma*

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The *mesothorax length* of *female flies* that had emerged from unparasitised larvae was significantly influenced by the dietary yeast species at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,26} = 4.6219$ ,  $p = 0.01014$ ;  $10^{-8}$ g:  $F_{3,32} = 9.6585$ ,  $p < 0.001$ ). At the lower yeast cell mass, female flies that had as larvae fed on *Pichia toletana* exhibited the highest mesothorax length ( $1.0383 \pm 0.0114$ mm), followed by those fed on *Saccharomyces cerevisiae* ( $1.0348 \pm 0.0215$ mm), *Kluyveromyces lactis* ( $1.0300 \pm 0.0283$ mm) and *Metschnikowia pulcherrima* ( $0.8559 \pm 0.0872$ mm). At a yeast cell mass of  $10^{-8}$ g, flies having fed on *Kluyveromyces lactis* exhibited the highest mesothorax length ( $1.0696 \pm 0.0243$ mm), followed by those fed on *Saccharomyces cerevisiae* ( $1.0569 \pm 0.0161$ mm), *Pichia toletana* ( $1.0134 \pm 0.0287$ mm) and *Metschnikowia pulcherrima* ( $0.8196 \pm 0.0807$ mm) (**Fig. 56A**). Irregardless of the yeast species, no significant effect of yeast cell mass on the mesothorax length of female adult flies was found (*K.lac.*:  $F_{1,19} = 1.1368$ ,  $p = 0.2997$ ; *M.pulch.*:  $F_{1,6} = 0.0933$ ,  $p = 0.7704$ ; *P.tol.*:  $F_{1,12} = 0.6419$ ,  $p = 0.4386$ , *S.cer.*:  $F_{1,20} = 3.2618$ ,  $p = 0.08599$ ).

The *mesothorax length* of *male flies* that had emerged from unparasitised larvae was also significantly affected by the dietary yeast species, though only at an initial yeast cell mass of  $10^{-10}$ g ( $10^{-10}$ g:  $F_{3,34} = 3.4442$ ,  $p = 0.02736$ ;  $10^{-8}$ g:  $F_{3,23} = 2.4773$ ,  $p < 0.08678$ ; *M.pulch.* excluded:  $F_{2,29} = 1.41$ ,  $p = 0.2604$ ). Also in male flies, adults that had as larvae fed on *Pichia toletana*, exhibited the highest mesothorax length at an initial cell mass of  $10^{-10}$ g ( $0.9450 \pm 0.0140$ mm), followed by those fed on *Saccharomyces cerevisiae* ( $0.9241 \pm 7.5070 \times 10^{-3}$ mm), *Kluyveromyces lactis* ( $0.9158 \pm 0.0401$ mm) and *Metschnikowia pulcherrima* ( $0.8503 \pm 0.0217$ mm). At the higher yeast cell mass, those fed on *Saccharomyces cerevisiae* showed the highest mesothorax length ( $0.9430 \pm 0.0106$ mm), followed by those fed on *Pichia toletana* ( $0.8791 \pm 0.0250$ mm), *Kluyveromyces lactis* ( $0.8205 \pm 0.0471$ mm), and *Metschnikowia pulcherrima* ( $0.7616 \pm 0.0798$ mm) (**Fig. 56B**). Just as in female flies, the initial yeast cell mass did not have a significant effect on mesothorax length of male adult flies, irregardless of the yeast species they had fed on as larvae (*K.lac.*:  $F_{1,15} = 2.2753$ ,  $p = 0.1522$ ; *P.tol.*:  $F_{1,15} = 4.0705$ ,  $p = 0.0619$ , *S.cer.*:  $F_{1,14} = 2.2098$ ,  $p = 0.1593$ ). Data of flies that as larvae had fed on *Metschnikowia pulcherrima* were not analysed, since there were only two values at an initial yeast cell mass of  $10^{-8}$ g.

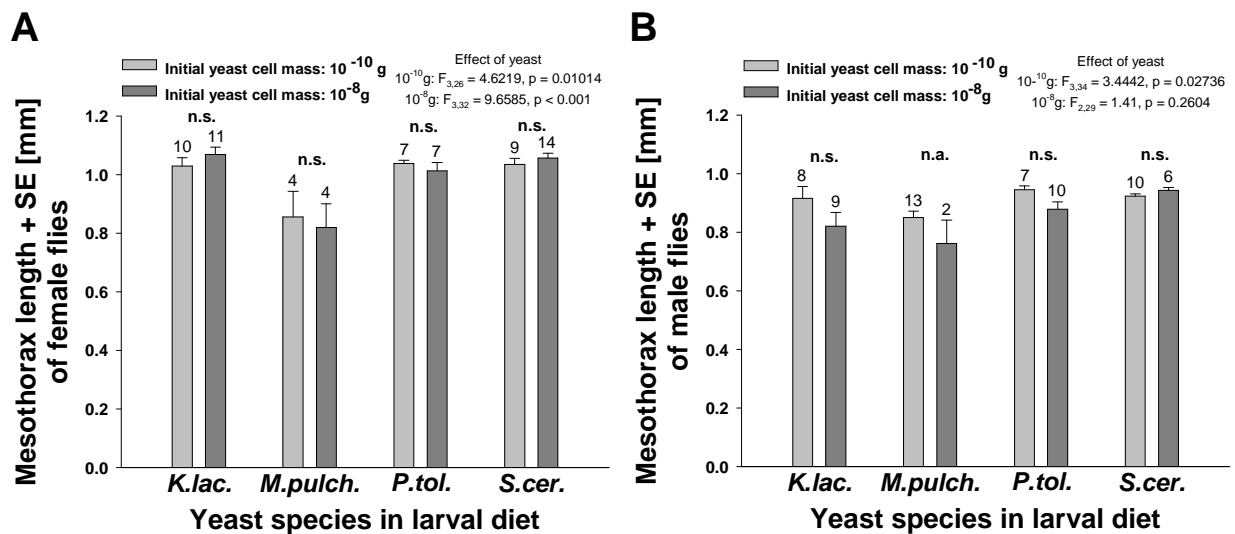
The influence of dietary yeast species, initial cell mass, sex (factors) as well as the interaction between the three factors on mesothorax length of adult *Drosophila melanogaster* (response variable) can be seen in **Table 38**. The results of the ANCOVA show a global significant influence of yeast species on mesothorax length. There is also a significant difference in mesothorax length between female and male flies. Female flies generally exhibited a higher mesothorax length than male flies. Additionally, the significant influence of the interaction between yeast species and sex shows that the influence of the dietary yeast species on mesothorax length of female or male adult flies differs significantly. Even though

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males generally exhibited a shorter mesothorax length, the relative differences in mesothorax length between female and male flies fed on the same yeast species varies among yeasts. The relative differences in female and male adult mesothorax length, was, for example, much smaller, when larvae had fed on *Metschnikowia pulcherrima* than when they had fed on *Saccharomyces cerevisiae* (Fig. 56).

**Table 38:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence mesothorax length of adult *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Mesothorax length of adult <i>Drosophila melanogaster</i>	Yeast	$F_{3,127} = 17.9457$	$p < 0.0001$
	Initial cell mass	$F_{1,126} = 0.9243$	$p = 0.33831$
	Sex	$F_{1,125} = 58.9813$	$p < 0.0001$
	Yeast*Initial cell mass	$F_{3,122} = 2.2917$	$p = 0.08173$
	Yeast*Sex	$F_{3,119} = 3.8282$	$p = 0.01170$
	Initial cell mass*Sex	$F_{1,118} = 3.6639$	$p = 0.05802$
	Yeast*Initial cell mass*Sex	$F_{3,115} = 0.9078$	$p = 0.43967$



**Fig. 56:** Mesothorax length (in mm) of adult *Drosophila melanogaster* emerging from unparasitised larvae. **A)** Mesothorax length of female flies and **B)** Mesothorax length of male flies, depending on the dietary yeast species and the initial yeast cell mass of each yeast species ( $10^{-10}$  and  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, n.a. = not analysed.

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The *dry weight* of *female flies* that had emerged from unparasitised larvae was significantly affected by the dietary yeast species at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,26} = 10.255$ ,  $p < 0.001$ ;  $10^{-8}$ g:  $F_{3,32} = 20.507$ ,  $p < 0.0001$ ). Female flies were generally heavier at an initial yeast cell mass of  $10^{-8}$ g compared to  $10^{-10}$ g, except of those fed on *Metschnikowia pulcherrima*, which eclosed as the lightest of all yeast treatments at the higher initial yeast cell mass ( $10^{-10}$ g:  $0.2405 \pm 0.0389$ mg;  $10^{-8}$ g:  $0.1715 \pm 0.0294$ mg). At both initial yeast cell masses, female flies were heaviest when as larvae having fed on *Kluyveromyces lactis* ( $10^{-10}$ g:  $0.3482 \pm 0.0113$ mg;  $10^{-8}$ g:  $0.3803 \pm 0.0135$ mg) and *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $0.3764 \pm 0.0117$ mg;  $10^{-8}$ g:  $0.3724 \pm 0.0126$ mg), followed by those fed on *Pichia toletana* ( $10^{-10}$ g:  $0.3480 \pm 0.0108$ mg;  $10^{-8}$ g:  $0.3670 \pm 0.0201$ mg) (**Fig. 57A**). In none of the yeast treatments did the initial yeast cell mass significantly affect female dry weight (*K.lac.*:  $F_{1,19} = 3.2533$ ,  $p = 0.08716$ ; *M.pulch.*:  $F_{1,6} = 2.0048$ ,  $p = 0.2066$ ; *P.tol.*:  $F_{1,12} = 0.6884$ ,  $p = 0.4229$ , *S.cer.*:  $F_{1,21} = 0.0483$ ,  $p = 0.8282$ ).

The *dry weight* of *male flies* that had emerged from unparasitised larvae was also significantly influenced by the dietary yeast species at an initial yeast cell mass of  $10^{-10}$ g ( $F_{3,34} = 10.345$ ,  $p < 0.0001$ ). A significant difference at  $10^{-8}$ g resulted only, when data for *M.pulch.* (only two values) were included in the analysis ( $F_{3,23} = 18.289$ ,  $p < 0.0001$ ; *M.pulch.* excluded:  $F_{2,22} = 2.1317$ ,  $p = 0.1425$ ). Males were affected by the dietary yeast species in a similar pattern as females: Again, male flies were generally heavier at an initial yeast cell mass of  $10^{-8}$ g compared to  $10^{-10}$ g, except of those fed on *Metschnikowia pulcherrima*, which eclosed as the lightest of all yeast treatments ( $10^{-10}$ g:  $0.2052 \pm 0.0126$ mg;  $10^{-8}$ g:  $0.1281 \pm 0.0342$ mg). At both initial yeast cell masses, male flies were heaviest when as larvae having fed on *Kluyveromyces lactis* ( $10^{-10}$ g:  $0.2774 \pm 0.0178$ mg;  $10^{-8}$ g:  $0.2671 \pm 0.0110$ mg) and *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $0.2784 \pm 6.0255 \times 10^{-3}$ mg;  $10^{-8}$ g:  $0.2888 \pm 0.0105$ mg), followed by those fed on *Pichia toletana* ( $10^{-10}$ g:  $0.2731 \pm 0.0074$ mg;  $10^{-8}$ g:  $0.2620 \pm 0.0052$ mg) (**Fig. 57B**). Similar to females, initial yeast cell mass had no significant influence on male dry weight when larvae had fed on *Kluyveromyces lactis* ( $F_{1,15} = 0.2561$ ,  $p = 0.6202$ ), *Pichia toletana* ( $F_{1,15} = 1.6023$ ,  $p = 0.2249$ ) and *Saccharomyces cerevisiae* ( $F_{1,14} = 0.8501$ ,  $p = 0.3721$ ). Again, data of flies that as larvae had fed on *Metschnikowia pulcherrima* were not analysed, since there were only two values at an initial yeast cell mass of  $10^{-8}$ g.

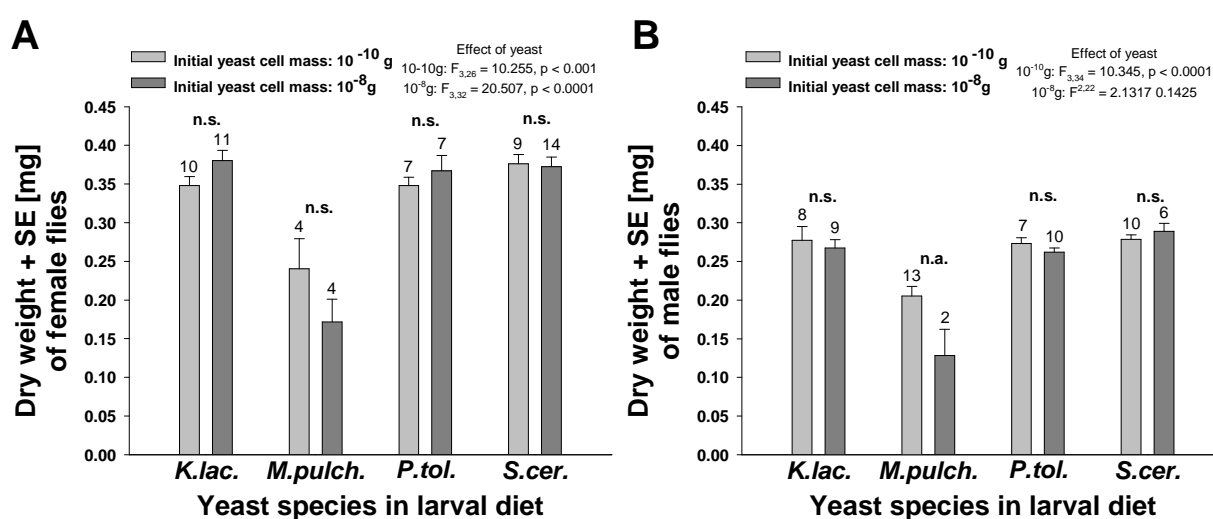
The influence of dietary yeast species, initial cell mass, sex (factors) as well as the interaction between the three factors on dry weight of adult *Drosophila melanogaster* (response variable) can be seen in **Table 39**. The results of the ANCOVA show a global significant influence of yeast species on dry weight and a significant difference in dry weight between female and male flies. Female flies were generally heavier than male flies. Additionally,

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the significant influence of the interaction between yeast species and initial cell mass shows that the influence of initial cell mass on dry weight depended on the yeast species in question. The relative differences in female as well as male dry weight between the two initial yeast cell masses are greater in larvae that had fed on *Metschnikowia pulcherrima*, for example, than in larvae that had fed on *Saccharomyces cerevisiae* (Fig. 57).

**Table 39:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence dry weight of adult *Drosophila melanogaster*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Dry weight of adult <i>Drosophila melanogaster</i>	Yeast	$F_{3,127} = 64.2441$	$p < 0.0001$
	Initial cell mass	$F_{1,126} = 0.0428$	$p = 0.8364$
	Sex	$F_{1,125} = 123.1521$	$p < 0.0001$
	Yeast*Initial cell mass	$F_{3,122} = 7.7716$	$p < 0.0001$
	Yeast*Sex	$F_{3,119} = 2.2910$	$p = 0.0818$
	Initial cell mass*Sex	$F_{1,118} = 1.4631$	$p = 0.2289$
	Yeast*Initial cell mass*Sex	$F_{4,115} = 0.9983$	$0.41154$



**Fig. 57:** Dry weight (in mg) of adult *Drosophila melanogaster* emerging from unparasitised larvae. **A)** Dry weight of female flies and **B)** Dry weight of male flies, depending on the dietary yeast species and the initial yeast cell mass of each yeast species ( $10^{-10}$  and  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, n.a. = not analysed.

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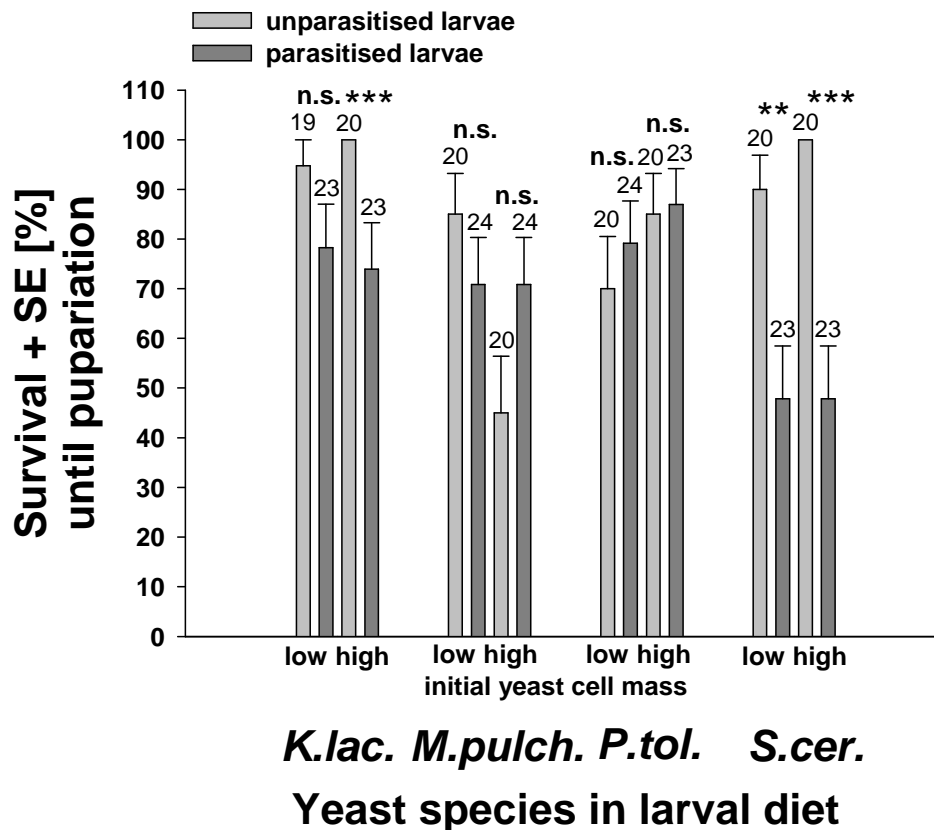
(b) *Parasitised Drosophila melanogaster* larvae: The dietary yeast species fed to parasitised *Drosophila melanogaster* larvae shortly after parasitisation had a significant effect on the *survival until pupariation* of parasitised larvae, though merely at an initial yeast cell mass of  $10^{-8}$ g ( $10^{-10}$ g:  $F_{3,90} = 2.1388$ ,  $p = 0.1009$ ;  $10^{-8}$ g:  $F_{3,89} = 2.7943$ ,  $p = 0.04487$ ). Survival of parasitised larvae until pupation was highest for larvae that had fed on *Pichia toletana* at both initial yeast cell masses, though it was again higher at the higher yeast cell mass ( $10^{-10}$ g:  $79.2 \pm 8.5\%$ ,  $10^{-8}$ g:  $87.0 \pm 7.2\%$ ), followed by larvae fed on *Kluyveromyces lactis* ( $10^{-10}$ g:  $78.3 \pm 8.8\%$ ,  $10^{-8}$ g:  $73.9 \pm 9.4\%$ ), those fed on *Metschnikowia pulcherrima* ( $10^{-10}$ g/ $10^{-8}$ g:  $70.8 \pm 9.5\%$ ) and finally and surprisingly those fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g/ $10^{-8}$ g:  $47.8 \pm 10.7\%$ ) (**Fig. 58**). The initial yeast cell mass did not affect survival until pupariation significantly, irregardless of the yeast species (*K.lac.*:  $F_{1,44} = 0.1144$ ,  $p = 0.7368$ ; *M.pulch.*:  $F_{1,46} = 0$ ,  $p = 1.0$ ; *P.tol.*:  $F_{1,45} = 0.488$ ,  $p = 0.4884$ ; *S.cer.*:  $F_{1,44} = 0$ ,  $p = 1.0$ ).

When comparing *survival until pupariation* of both unparasitised as well as parasitised larvae, so did the dietary yeast species only have a significant influence when larvae had fed on *Kluyveromyces lactis* at an initial cell mass of  $10^{-8}$ g ( $10^{-10}$ g:  $F_{1,40} = 2.409$ ,  $p = 0.1285$ ;  $10^{-8}$ g:  $F_{1,41} = 14.888$ ,  $p = 0.000396$ ), and when they had fed on *Saccharomyces cerevisiae*, irrespective of the initial yeast cell mass ( $10^{-10}$ g:  $F_{1,41} = 8.9829$ ,  $p = 0.004612$ ;  $10^{-8}$ g:  $F_{1,41} = 34.007$ ,  $p < 0.0001$ ). When fed on the other yeast species, no significant difference in survival until pupariation between unparasitised and parasitised larvae resulted (*M.pulch.*:  $10^{-10}$ g:  $F_{1,41} = 1.3983$ ,  $p = 0.2438$ ,  $10^{-8}$ g:  $F_{1,42} = 2.8962$ ,  $p = 0.09618$ ; *P.tol.*:  $10^{-10}$ g:  $F_{1,41} = 0.4652$ ,  $p = 0.4989$ ,  $10^{-8}$ g:  $F_{1,41} = 0.0325$ ,  $p = 0.858$ ) (**Fig. 58**). Survival until pupariation in unparasitised larvae was similar to survival until eclosion, except for larvae that had fed on *Metschnikowia pulcherrima* at an initial yeast cell mass of  $10^{-8}$ g. Here,  $45.0 \pm 11.41\%$  pupariated, whereas only  $25.0 \pm 9.93\%$  reached the adult stage (see **Fig. 55A**).

The influence of dietary yeast species, initial cell mass, parasitisation status (yes, no) (factors) as well as the interaction between the three factors on survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae (response variable) can be seen in **Table 40**. The results of the ANCOVA show a global significant influence of yeast species on survival until pupariation. Additionally, survival of unparasitised larvae differed significantly from that of parasitised larvae. The significant influence of the interaction between yeast and parasitisation on survival suggests that unparasitised larvae experienced a different influence of the dietary yeast species on survival than parasitised larvae. The relative differences in survival between unparasitised and parasitised larvae were, for example, much smaller when larvae had fed on *Pichia toletana* than when they had fed on *Saccharomyces cerevisiae* (**Fig. 58**). These yeast-specific differences additionally depended on the initial yeast cell mass, shown by the significant influence of the interaction

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between yeast, initial cell mass and parasitisation status. This can be seen by comparing survival on *Metschnikowia pulcherrima* and *Saccharomyces cerevisiae*: At both initial yeast cell masses the relative difference in survival between unparasitised and parasitised larvae fed on *Metschnikowia pulcherrima* is smaller than those fed on *Saccharomyces cerevisiae*. Additionally, however, the difference in survival of larvae fed on *Metschnikowia pulcherrima* changes algebraic sign, since at the initial yeast cell mass of  $10^{-10}$ g, survival of unparasitised larvae is higher than that of parasitised larvae, at an initial cell mass of  $10^{-8}$ g it is vice versa. When larvae had fed on *Saccharomyces cerevisiae*, survival was always higher in unparasitised larvae (Fig. 58).



**Fig. 58:** Survival (in %) until pupariation of unparasitised as well as parasitised *Drosophila melanogaster* larvae, depending on the dietary yeast species and the initial yeast cell mass of each yeast species (**low** =  $10^{-10}$ g, **high** =  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*\* p < 0.01, \*\*\* p < 0.001.

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**Table 40:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival until pupariation of unparasitised and parasitised *Drosophila melanogaster* larvae. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival until pupariation of <i>Drosophila melanogaster</i>	Yeast	$F_{3,342} = 3.8133$	$p = 0.010385$
	Initial cell mass	$F_{1,341} = 0.0196$	$p = 0.888799$
	Parasitisation	$F_{1,340} = 10.7099$	$p = 0.001178$
	Yeast*Initial cell mass	$F_{3,337} = 1.9514$	$p = 0.121135$
	Yeast*Parasitisation	$F_{3,334} = 9.3290$	$p < 0.0001$
	Initial cell mass*Parasitisation	$F_{1,333} = 0.0887$	$p = 0.766009$
	Yeast*Initial cell mass*Parasitisation	$F_{3,330} = 2.9210$	$p = 0.034142$

The **encapsulation ability** of parasitised *Drosophila melanogaster* larvae was zero in all treatments, since no capsules were found in the few flies that had emerged, irregardless of yeast species and initial yeast cell mass.

### (c) *Leptopilina heterotoma*:

A significant effect of the dietary yeast species of *Drosophila melanogaster* larvae on the **survival** of *Leptopilina heterotoma* could be found, though merely at an initial yeast cell mass of  $10^{-8}$ g ( $10^{-10}$ g:  $F_{3,90} = 1.6848$ ,  $p = 0.1759$ ;  $10^{-8}$ g:  $F_{3,89} = 3.3601$ ,  $p = 0.02227$ ). Survival of *Leptopilina heterotoma* was highest when their host larvae had fed on *Pichia toletana*, and this at both initial yeast cell masses ( $10^{-10}$ g:  $79.2 \pm 8.5\%$ ,  $10^{-8}$ g:  $87.0 \pm 7.2\%$ ). It was lowest, when host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g/ $10^{-8}$ g:  $47.8 \pm 10.7\%$ ). Just as in *Saccharomyces cerevisiae*, survival was hardly affected by the initial yeast cell mass when host larvae had fed on *Kluyveromyces lactis*, and was in general still comparably high ( $10^{-10}$ g:  $60.9 \pm 10.4\%$ ,  $10^{-8}$ g:  $68.2 \pm 10.2\%$ ). When host larvae had fed on *Metschnikowia pulcherrima*, survival of *Leptopilina heterotoma* was still rather high at an initial yeast cell mass of  $10^{-10}$ g ( $10^{-10}$ g:  $66.7 \pm 9.8\%$ ), but quite low at an initial yeast cell mass of  $10^{-8}$ g ( $10^{-8}$ g:  $50.0 \pm 10.4\%$ ). Irregardless of the yeast species fed to the larvae, there was no significant effect of the initial yeast cell mass on survival of *Leptopilina heterotoma* (*K.lac.*:  $F_{1,44} = 0.0893$ ,  $p = 0.07665$ , *M.pulch.*:  $F_{1,46} = 1.3214$ ,  $p = 0.2563$ , *P.tol.*:  $F_{1,45} = 0.488$ ,  $p = 0.4884$ , *S.cer.*:  $F_{1,44} = 0$ ,  $p = 1.0$ ) (**Fig. 59A**).

The influence of dietary yeast species and initial cell mass (factors) as well as the interaction between the two factors on survival of *Leptopilina heterotoma* (response variable) can be seen in **Table 41**. The results of the ANCOVA show a global significant influence of yeast species on survival.



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**Table 41:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence survival of *Leptopilina heterotoma*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Survival of <i>Leptopilina heterotoma</i>	Yeast	$F_{3,183} = 4.4267$	$p = 0.004982$
	Initial cell mass	$F_{1,182} = 0.0324$	$p = 0.857427$
	Yeast*Initial cell mass	$F_{3,179} = 0.6216$	$p = 0.601917$

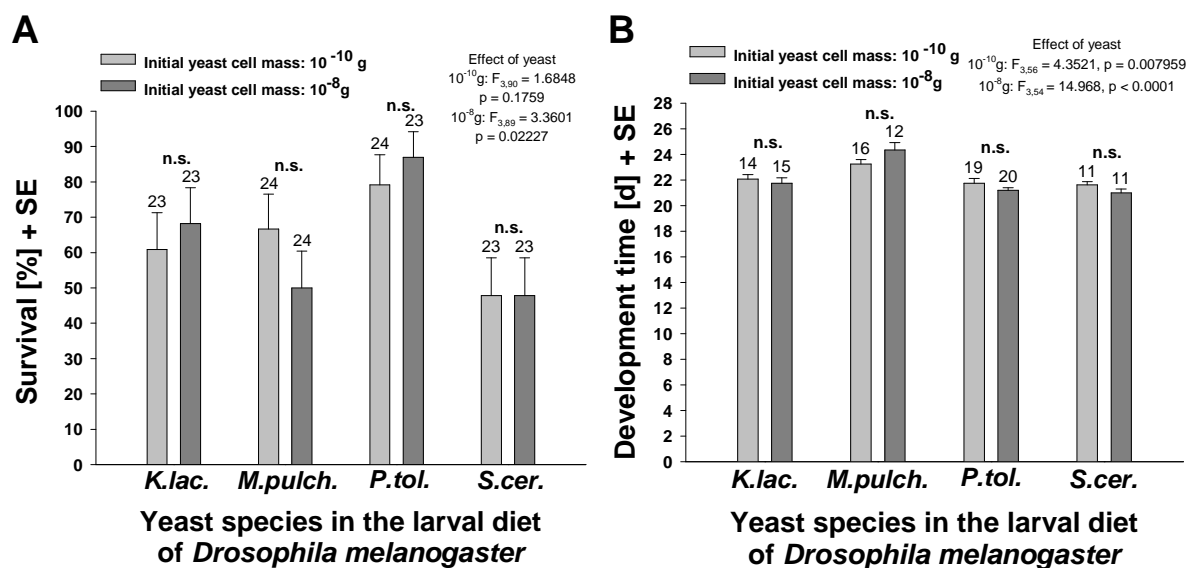
The dietary yeast species fed to *Drosophila melanogaster* larvae significantly affected the *development time* of *Leptopilina heterotoma* at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,56} = 4.3521$ ,  $p = 0.007959$ ;  $10^{-8}$ g:  $F_{3,54} = 14.968$ ,  $p < 0.0001$ ). Irregardless of the yeast species, development time of *Leptopilina heterotoma* was always shorter at an initial cell mass of  $10^{-8}$ g than at  $10^{-10}$ g. It was shortest when host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $21.6 \pm 0.2$ d,  $10^{-8}$ g:  $21.0 \pm 0.3$ d), followed by *Pichia toletana* ( $10^{-10}$ g:  $21.7 \pm 0.4$ d,  $10^{-8}$ g:  $21.2 \pm 0.2$ d), and by *Kluyveromyces lactis* ( $10^{-10}$ g:  $22.1 \pm 0.4$ d,  $10^{-8}$ g:  $21.7 \pm 0.4$ d). Development time was longest, when host larvae had fed on *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $23.3 \pm 0.3$ d,  $10^{-8}$ g:  $24.3 \pm 0.6$ d). The initial yeast cell mass did not have a significant effect on development time of *Leptopilina heterotoma* (*K.lac.*:  $F_{1,27} = 0.3606$ ,  $p = 0.5532$ ; *M.pulch.*:  $F_{1,26} = 2.856$ ,  $p = 0.1030$ ; *P.tol.*:  $F_{1,37} = 1.612$ ,  $p = 0.2121$ ; *S.cer.*:  $F_{1,20} = 2.6834$ ,  $p = 0.1170$ ) (**Fig. 59B**).

The influence of dietary yeast species and initial cell mass (factors) as well as the interaction between the two factors on development time of *Leptopilina heterotoma* (response variable) can be seen in **Table 42**. The results of the ANCOVA show a global significant influence of yeast species on development time.

**Table 42:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence development time of *Leptopilina heterotoma*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Development time of <i>Leptopilina heterotoma</i>	Yeast	$F_{3,114} = 17.4649$	$p < 0.0001$
	Initial cell mass	$F_{1,114} = 0.3626$	$p = 0.54832$
	Yeast*Initial cell mass	$F_{3,110} = 2.1929$	$p = 0.09294$

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**Fig. 59:** A) Survival (in %) and B) Development time (in days) of *Leptopilina heterotoma* depending on the dietary yeast species and the initial yeast cell mass of each yeast species ( $10^{-10}$  and  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant.

The body size of female *Leptopilina heterotoma*, measured as *hind tibia length*, was significantly influenced by the yeast species in the diet of *Drosophila melanogaster* larvae, irrespective of the initial yeast cell mass ( $10^{-10}$ g:  $F_{3,31} = 8.7632$ ,  $p = 0.0002341$ ;  $10^{-8}$ g:  $F_{3,25} = 3.5855$ ,  $p = 0.02777$ ). Only when larvae had fed on *Kluyveromyces lactis*, did also initial yeast cell mass have a significant effect on hind tibia length (*K.lac.*:  $F_{1,11} = 6.9982$ ,  $p = 0.02277$ ; *M.pulch.*:  $F_{1,16} = 0.2208$ ,  $p = 0.6448$ ; *P.tol.*:  $F_{1,19} = 0.6426$ ,  $p = 0.4327$ ; *S.cer.*:  $F_{1,10} = 2.2155$ ,  $p = 0.1675$ ). Hind tibiae in *Leptopilina heterotoma* whose host larvae had fed on *Kluyveromyces lactis* were significantly shorter at an initial yeast cell mass of  $10^{-8}$ g ( $0.5682 \pm 0.0136$ mm) compared to  $10^{-10}$ g ( $0.6045 \pm 0.0068$ mm). Hind tibiae were longest when host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $0.6227 \pm 0.0056$ mm;  $10^{-8}$ g:  $0.6087 \pm 0.0080$ mm), and shortest when host larvae had fed on *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $0.5625 \pm 0.0109$ mm;  $10^{-8}$ g:  $0.5525 \pm 0.0207$ mm). When host larvae had fed on *Pichia toletana*, hind tibia length was nearly similarly long at both initial yeast cell masses with intermediate values ( $10^{-10}$ g:  $0.5992 \pm 0.0074$ mm;  $10^{-8}$ g:  $0.5922 \pm 0.0052$ mm) (Fig. 60A). Hind tibiae of female *Leptopilina heterotoma* were always shorter, when host larvae had fed on an initial yeast cell mass of  $10^{-8}$ g compared to  $10^{-10}$ g.

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The influence of dietary yeast species and initial cell mass (factors) as well as the interaction between the two factors on hind tibia length of female *Leptopilina heterotoma* (response variable) can be seen in **Table 43**. The results of the ANCOVA show a global significant influence of yeast species on hind tibia length.

**Table 43:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence hind tibia length of female *Leptopilina heterotoma*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Hind tibia length of female <i>Leptopilina heterotoma</i>	Yeast	$F_{3,60} = 10.1533$	$p < 0.0001$
	Initial cell mass	$F_{1,59} = 3.9523$	$p = 0.0517$
	Yeast*Initial cell mass	$F_{3,56} = 0.6978$	$p = 0.5573$

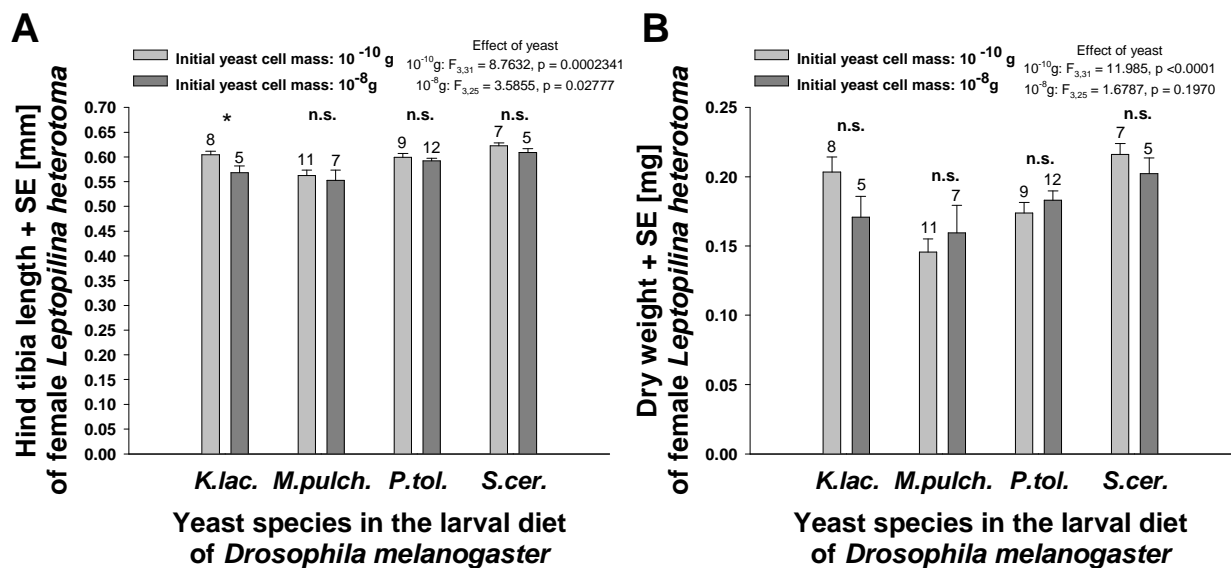
Body size of female *Leptopilina heterotoma*, measured as **dry weight**, was less influenced by the yeast species in the diet of *Drosophila melanogaster* larvae than hind tibia length. There was only a significant difference in dry weight of female *Leptopilina heterotoma* at an initial yeast cell mass of  $10^{-10}$ g ( $10^{-10}$ g:  $F_{3,31} = 11.985$ ,  $p < 0.0001$ ;  $10^{-8}$ g:  $F_{3,25} = 1.6787$ ,  $p = 0.1970$ ), whereby yeast cell mass itself did not affect dry weight significantly, regardless of the dietary yeast species (*K.lac.*:  $F_{1,11} = 3.2006$ ,  $p = 0.1011$ ; *M.pulch.*:  $F_{1,16} = 0.5041$ ,  $p = 0.4879$ ; *P.tol.*:  $F_{1,19} = 0.8371$ ,  $p = 0.3717$ ; *S.cer.*:  $F_{1,10} = 1.1372$ ,  $p = 0.3113$ ). *Leptopilina heterotoma* females were heaviest, when host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $0.2162 \pm 0.0077$ mg;  $10^{-8}$ g:  $0.2023 \pm 0.0111$ mg), followed by *Kluyveromyces lactis* ( $10^{-10}$ g:  $0.2034 \pm 0.0109$ mg;  $10^{-8}$ g:  $0.1707 \pm 0.0152$ mg) and *Pichia toletana* ( $10^{-10}$ g:  $0.1739 \pm 0.0076$ mg;  $10^{-8}$ g:  $0.1832 \pm 0.0067$ mg), and finally *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $0.1456 \pm 0.0095$ mg;  $10^{-8}$ g:  $0.1595 \pm 0.0197$ mg) (**Fig. 60B**). Dry weight of female *Leptopilina heterotoma* was higher at an initial yeast cell mass of  $10^{-10}$ g compared to  $10^{-8}$ g, when their host larvae had fed either on *Kluyveromyces lactis* or *Saccharomyces cerevisiae*, but vice versa for the other two yeast species.

The influence of dietary yeast species and initial cell mass (factors) as well as the interaction between the two factors on dry weight of female *Leptopilina heterotoma* (response variable) can be seen in **Table 44**. The results of the ANCOVA show a global significant influence of yeast species on dry weight.

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**Table 44:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence dry weight of female *Leptopilina heterotoma*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Dry weight of female <i>Leptopilina heterotoma</i>	Yeast	$F_{3,60} = 9.8467$	$p < 0.0001$
	Initial cell mass	$F_{1,59} = 0.0797$	$p = 0.7788$
	Yeast*Initial cell mass	$F_{3,56} = 1.7931$	$p = 0.1590$



**Fig. 60:** A) *Hind tibia length* (in mm) and B) *Dry weight* (in mg) of *Leptopilina heterotoma* depending on the dietary yeast species and the initial yeast cell mass of each yeast species ( $10^{-10}$  and  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ .

The dietary yeast species of *Drosophila melanogaster* host larvae significantly influenced the *relative fat content* of female *Leptopilina heterotoma* at both initial yeast cell masses ( $10^{-10}$ g:  $F_{3,31} = 6.8974$ ,  $p = 0.001086$ ;  $10^{-8}$ g:  $F_{3,25} = 4.4695$ ,  $p = 0.01206$ ). When larvae had fed on *Kluyveromyces lactis*, initial yeast cell mass also had a significant effect on relative fat content ( $F_{1,11} = 7.0525$ ,  $p = 0.02236$ ), but not when host larvae had fed on one of the other three yeast species (*M.pulch.*:  $F_{1,16} = 1.192$ ,  $p = 0.2911$ ; *P.tol.*:  $F_{1,19} = 2.6641$ ,  $p = 0.1191$ ; *S.cer.*:  $F_{1,10} = 0.1785$ ,  $p = 0.6816$ ). Relative fat content was highest in female *Leptopilina heterotoma* when their host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $0.3040 \pm 0.0069$ mg/mg;  $10^{-8}$ g:  $0.3107 \pm 0.0166$ mg/mg), followed by *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $0.2713 \pm 0.0089$ mg/mg;  $10^{-8}$ g:  $0.3086 \pm 0.0411$ mg/mg) and *Kluyveromyces lactis* ( $10^{-10}$ g:  $0.2566 \pm 0.0094$ mg/mg;  $10^{-8}$ g:  $0.3071 \pm 0.0191$ mg/mg), and finally by

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*Pichia toletana* ( $10^{-10}$ g:  $0.2463 \pm 0.0093$ mg/mg;  $10^{-8}$ g:  $0.2209 \pm 0.0115$ mg/mg) (**Fig. 61A**) The relative fat content of *Leptopilina heterotoma* females was usually higher at an initial yeast cell mass of  $10^{-8}$ g compared to  $10^{-10}$ g, except when host larvae had fed on *Pichia toletana*.

The influence of dietary yeast species and initial cell mass (factors) as well as the interaction between the two factors on the relative fat content of female *Leptopilina heterotoma* (response variable) can be seen in **Table 45**. The results of the ANCOVA show a global significant influence of yeast species on relative fat content.

**Table 45:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the relative fat content of female *Leptopilina heterotoma*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Relative fat content of female <i>Leptopilina heterotoma</i>	Yeast	$F_{3,60} = 7.8762$	$p = 0.0001795$
	Initial cell mass	$F_{1,59} = 1.2313$	$p = 0.2719001$
	Yeast*Initial cell mass	$F_{3,56} = 2.2031$	$p = 0.0977980$

Neither the yeast species fed to *Drosophila melanogaster* host larvae nor the initial yeast cell mass offered to host larvae, had a significant influence on the **number of eggs** carried by freshly hatched *Leptopilina heterotoma* virgins (effect of yeast: at  $10^{-10}$ g:  $F_{3,31} = 1.7693$ ,  $p = 0.1735$ ; at  $10^{-8}$ g:  $F_{3,25} = 2.7592$ ,  $p = 0.06328$ ; effect of initial yeast cell mass: *K.lac.*:  $F_{1,11} = 0.6724$ ,  $p = 0.4296$ ; *M.pulch.*:  $F_{1,16} = 0.2024$ ,  $p = 0.6589$ ; *P.tol.*:  $F_{1,19} = 0.2415$ ,  $p = 0.6287$ , *S.cer.*:  $F_{1,10} = 1.4612$ ,  $p = 0.2545$ ) (but see results of the ANCOVA presented later in the text). The highest number of eggs were carried by *Leptopilina heterotoma* females whose host larvae had fed on *Saccharomyces cerevisiae* ( $10^{-10}$ g:  $97.0 \pm 14.60$ ;  $10^{-8}$ g:  $121.0 \pm 9.20$ ), followed by those whose host larvae had consumed *Kluyveromyces lactis* ( $10^{-10}$ g:  $82.3 \pm 11.89$ ;  $10^{-8}$ g:  $67.8 \pm 12.08$ ) and *Pichia toletana* ( $10^{-10}$ g:  $71.1 \pm 17.00$ ;  $10^{-8}$ g:  $82.3 \pm 15.18$ ), and finally those, whose host larvae had fed on *Metschnikowia pulcherrima* ( $10^{-10}$ g:  $56.9 \pm 7.56$ ;  $10^{-8}$ g:  $50.6 \pm 13.04$ ) (**Fig. 61B**). When host larvae had fed on *Pichia toletana* or *Saccharomyces cerevisiae*, respectively, the number of eggs carried by *Leptopilina heterotoma* was higher at an initial yeast cell mass of  $10^{-8}$ g compared to  $10^{-10}$ g. In the other two yeast species it was vice versa.

The influence of dietary yeast species and initial cell mass (factors) as well as the interaction between the two factors on the number of eggs of female *Leptopilina heterotoma* (response variable) can be seen in **Table 46**. The results of the ANCOVA show a global significant influence of yeast species on the number of eggs. This result differs from that yielded by the GLM-analysis with only one factor, namely yeast as linear predictor. By

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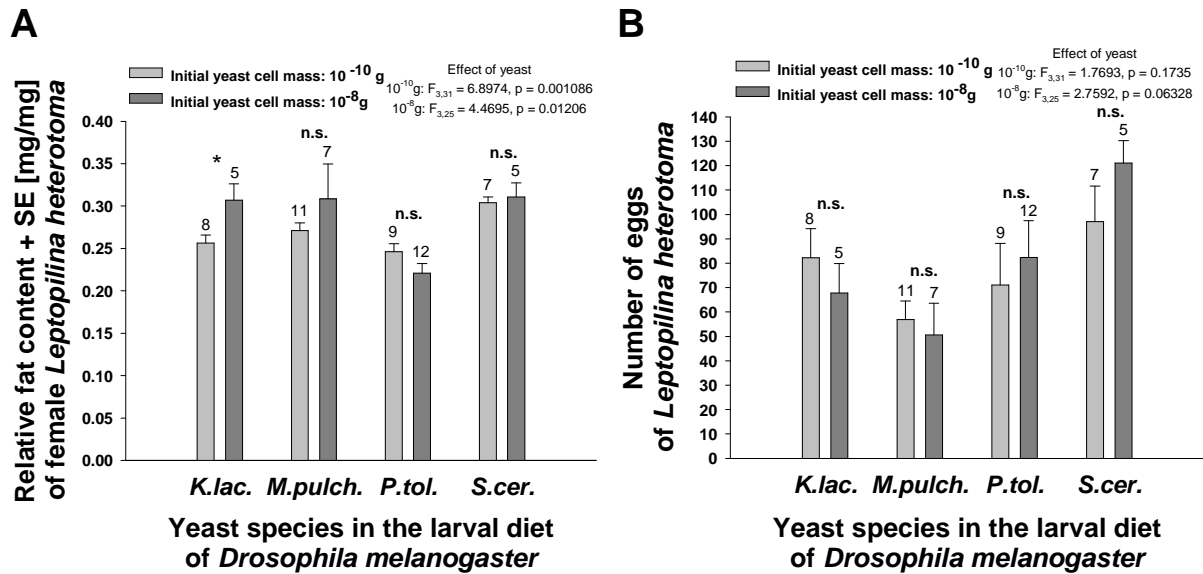
adding the covariate ‘initial yeast cell mass’ into the model, the variation in number of eggs due to the covariate is removed, thus, providing a more precise analysis.

Even though a linear regression did not yield any significant correlation between the number of eggs and the size parameters hind tibia length and dry weight (see later in the text & **Fig. 62**), the absolute number of eggs was corrected for a potential influence of size parameters which may have been concealed by the different yeast treatments. For this, the absolute number of eggs of each *Leptopilina heterotoma* female was divided by the hind tibia length of the same female on the one hand and by the dry weight of the individual female on the other hand. The graphs very much resembled that of the absolute number of eggs and are therefore not shown here. The results of the ANCOVAs (see **Table 46**), however, were different in the way, that the significant influence of the dietary yeast species on the number of eggs disappeared.

**Table 46:** Results of the Analysis of Covariance (ANCOVA) on factors and factor interactions that influence the number of eggs of female *Leptopilina heterotoma*. Interactions between factors are indicated by \*.

Measured fitness trait	Factors and interactions	F-value	p-value
Absolute number of eggs of female <i>Leptopilina heterotoma</i>	Yeast	$F_{3,60} = 4.1413$	$p = 0.01012$
	Initial cell mass	$F_{1,59} = 0.0241$	$p = 0.87722$
	Yeast*Initial cell mass	$F_{3,56} = 0.5658$	$p = 0.63981$
Number of eggs per hind tibia length	Yeast	$F_{3,60} = 2.5567$	$p = 0.0643$
	Initial cell mass	$F_{1,59} = 0.0332$	$p = 0.8560$
	Yeast*Initial cell mass	$F_{3,56} = 0.9757$	$p = 0.4108$
Number of eggs per dry weight	Yeast	$F_{3,60} = 0.7950$	$p = 0.5018$
	Initial cell mass	$F_{1,59} = 0.0648$	$p = 0.7999$
	Yeast*Initial cell mass	$F_{3,56} = 0.7502$	$p = 0.5268$

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**Fig. 61:** A) *Relative fat content* (in mg per mg dry weight) and B) *Number of eggs* of *Leptopilina heterotoma*, depending on the dietary yeast species and the initial yeast cell mass of each yeast species ( $10^{-10}$  and  $10^{-8}$ g). Shown are mean values + standard error (SE). Numbers above columns = number of replicates. Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*. Significance levels: n.s. = not significant, \*  $p < 0.05$ .

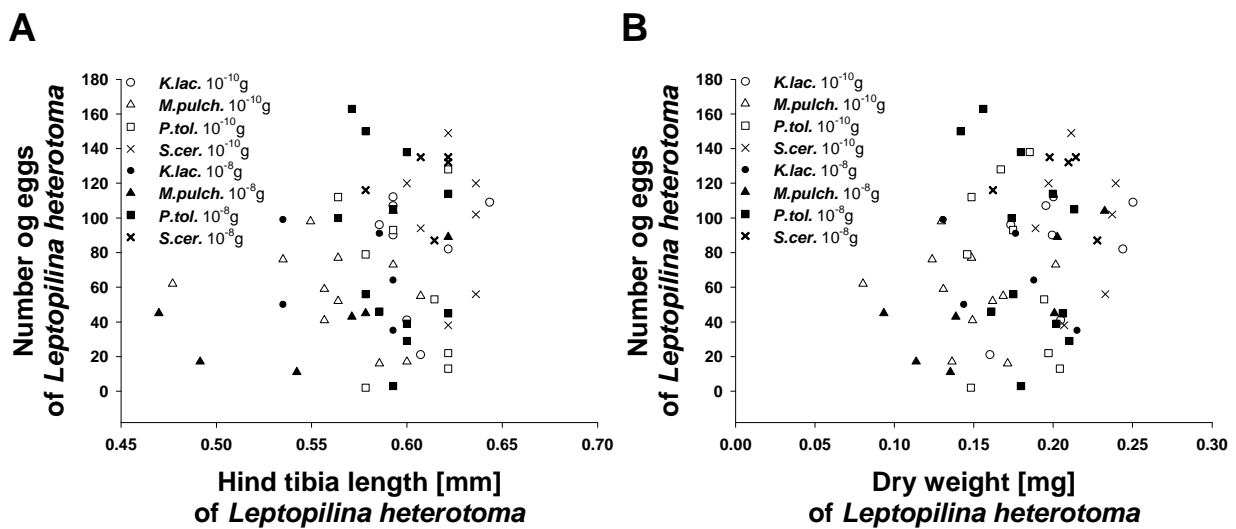
Linear regressions were carried out to test for potential significant positive or negative correlations between the fitness traits body size (as hind tibia length and dry weight), absolute fat content and number of eggs. The number of eggs carried by *Leptopilina heterotoma* females (dependent variable) was not significantly correlated to any of the measured body size parameters (independent variables), hind tibia length ( $R^2 = 0.03491$ ,  $F_{1,62} = 3.279$ ,  $t = 1.811$ ,  $p = 0.07502$ ), or dry weight ( $R^2 = 0.03802$ ,  $F_{1,62} = 3.49$ ,  $t = 1.868$ ,  $p = 0.06648$ ) (**Fig. 62A & B**). There was, however, a significant positive correlation between the absolute fat content of *Leptopilina heterotoma* females and hind tibia length ( $R^2 = 0.2788$ ,  $F_{1,62} = 25.36$ ,  $t = 5.036$ ,  $p < 0.0001$ ) (**Fig. 63A**). A multiple regression between the absolute fat content (dependent variable) and the number of eggs as well as hind tibia length (independent variables) did not reveal any correlation between absolute fat content and number of eggs (**Fig. 63B & Table 47**).

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**Table 47:** Multiple regression of number of eggs and hind tibia length on absolute fat content of *Leptopilina heterotoma* over all dietary yeast species and both initial yeast cell masses ( $10^{-10}$ g and  $10^{-8}$ g).  $F_{(2,63)} = 12.935$ ,  $R^2 = 0.275$ ,  $p < 0.0001$ .

Character	Coefficient	Std. error	t-value
Constant	$-8.87 \times 10^{-2}$	0.028	-3.154
Number of eggs	$3.361 \times 10^{-5}$	0.000	0.809
Hind tibia length (mm)	0.229	0.049	4.713***

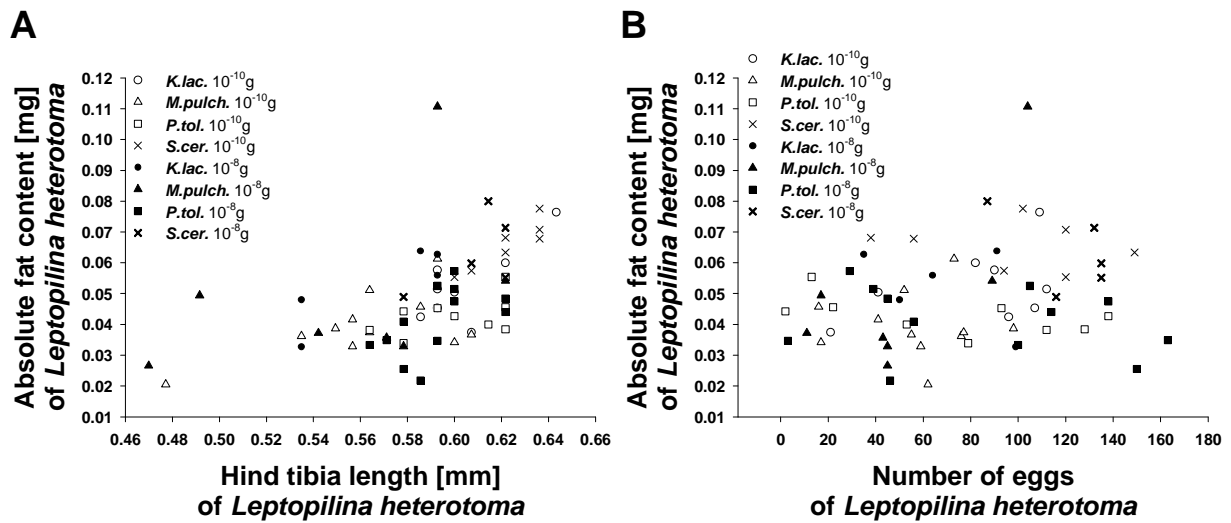
\*\*\* $p < 0.0001$



**Fig. 62:** Scatter plots of individual values of **A**) Hind tibia length (in mm) versus number of eggs and **B**) Dry weight (in mg) versus number of eggs of female *Leptopilina heterotoma* over all dietary yeast species and both initial yeast cell masses ( $10^{-10}$ g and  $10^{-8}$ g). Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*, as well as *K* = *Kluyveromyces lactis* and *S* = *Saccharomyces cerevisiae*.



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**Fig. 63:** Scatter plots of individual values of **A)** Hind tibia length (in mm) versus absolute fat content (in mg) and **B)** Number of eggs versus absolute fat content (in mg) of female *Leptopilina heterotoma* over all dietary yeast species and both initial yeast cell masses ( $10^{-10}$  g and  $10^{-8}$  g). Abbreviations of yeast species: *K.lac.* = *Kluyveromyces lactis*, *M.pulch.* = *Metschnikowia pulcherrima*, *P.tol.* = *Pichia toletana*, *S.cer.* = *Saccharomyces cerevisiae*, as well as *K* = *Kluyveromyces lactis* and *S* = *Saccharomyces cerevisiae*.

### 8.4 Discussion

The results may allow drawing the following conclusions: (i) The different dietary yeast species had no influence on the (dis)ability of *Drosophila melanogaster* to fend off parasitoid offspring of *Leptopilina heterotoma*. Similar to *Asobara tabida* (ii) most fitness traits measured in *Leptopilina heterotoma* were influenced by the yeast species in the diet of its host larvae, (iii) not all measured fitness traits of *Leptopilina heterotoma* may experience a similar favourable or unfavourable influence of a specific dietary yeast species, (iv) the initial yeast cell mass meant to represent limited and intermediate conditions for the development of *Drosophila melanogaster* larvae, very rarely had an influence on fitness traits of *Leptopilina heterotoma*. (v) With regard to the influence of yeast quality, *Leptopilina heterotoma* showed a less pronounced tendency to perform better on host larvae which had consumed the yeast species favourable for their own larval and adult fitness traits. The ‘better performance’ was more pronouncedly fitness-trait specific (no ‘overall’ better performance).

Unparasitised *Drosophila melanogaster* larvae showed a similar response to the different dietary yeast species and the two initial yeast cell masses (meant to represent limited and intermediate conditions) as has already been shown in chapter 5.4 in the experiment carried out with *Asobara tabida*.

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In larvae, that in this experiment had been parasitised by *Leptopilina heterotoma*, however, none of the dietary yeast species that had been shown to beneficially influence encapsulation ability of *Drosophila melanogaster* against *Asobara tabida* offspring, had an effect on immune defence. At limited and intermediate food conditions, even *Drosophila melanogaster* larvae parasitised by *Asobara tabida* did not show a significant difference in their encapsulation ability depending on the dietary yeast species they had consumed (see chapter 5.4). A significant influence of the dietary yeast species mainly occurred when yeasts had been offered at *ad libitum* conditions (see chapter 5.2, 5.3 and 5.5). Nevertheless, in an experiment that had been carried out in the same fashion as the one presented in chapter 5.2, *Drosophila melanogaster* larvae had not been shown capable of encapsulating *Leptopilina heterotoma* offspring even at *ad libitum* yeast conditions (experiment and data not shown in this study). Wild type *Drosophila melanogaster* larvae in general are reported to only rarely encapsulate eggs of *Leptopilina heterotoma* (MORALES *et al.* 2005). Due to the rather aggressive mechanism of *Leptopilina heterotoma* to evade or suppress the immune defence of *Drosophila melanogaster* (see chapter 3.2), dietary compounds - at least in the form of specific dietary yeast species - do not seem to have the power of influencing this mechanism and of enabling *Drosophila melanogaster* larvae to withstand this mechanism. The immunosuppressive mechanism of *Leptopilina heterotoma* is reported to act so fast and effective that infection results in a nearly complete failure of attacked *Drosophila melanogaster* larvae to mount an immune transcriptional response, as if the larvae did not recognise they were infected (SCHLENKE *et al.* 2007). This highly effective venom is also supposed to be largely responsible for the ability of *Leptopilina heterotoma* to attack a broad host-range (SCHLENKE *et al.* 2007).

Despite this lack in influence of dietary yeast species on the immune defence of *Drosophila melanogaster* against *Leptopilina heterotoma* offspring (at least concerning 'visible' encapsulation ability), offspring of *Leptopilina heterotoma* turned out to experience a partly substantial influence on their developmental success posed by the different dietary yeast species. The yeast-specific influence on survival of parasitised *Drosophila melanogaster* larvae was more pronounced (and here significant) in larvae parasitised by *Leptopilina heterotoma* than those parasitised by *Asobara tabida*, as indicated by a significant interaction between yeast and parasitisation status (see **Table 10** in chapter 5.4 for the experiment with *Asobara tabida* and **Table 40** for *Leptopilina heterotoma*). This was mainly due to the significant difference in survival between unparasitised and parasitised larvae that had fed on *Saccharomyces cerevisiae*. In the experiment carried out with *Asobara tabida*, there was already a tendency of *Saccharomyces cerevisiae* of not being as favourable a diet to parasitised as to unparasitised larvae. Just as in larvae parasitised by *Asobara tabida*, the survival of larvae parasitised by *Leptopilina heterotoma* was generally lower than survival of unparasitised larvae. This may again have been due to the venom

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injected into the host haemocoel during oviposition. This venom of *Leptopilina heterotoma* is reported of having several deleterious effects on infected host larvae (SCHLENKE *et al.* 2007). Even though the different yeast species don't seem to influence the host's immune defence, they nevertheless seem to modulate other deleterious effects of the venom injected by *Leptopilina heterotoma*. This modulation may not directly act upon the venom, but rather benefit the host (and therefore actually the parasite, since an enhanced host survival would in the end merely benefit the parasitoid) by helping to pay the cost of pathophysiological effects of the parasitisation. Again, the question certainly remains, which specific components of the different yeast species may act in this way and may act better, than for example, components posed by *Saccharomyces cerevisiae*.

In contrast to *Asobara tabida*, *Leptopilina heterotoma* did not exhibit such a convincing tendency to perform better in host larvae that had consumed a yeast species which favoured the host's own fitness traits. In *Asobara tabida*, nearly all measured fitness traits (survival, development time, hind tibia length, dry weight and number of eggs) were positively influenced by *Kluyveromyces lactis*, a yeast species which also had a positive influence on fitness traits of *Drosophila melanogaster*. Merely relative fat content in female *Asobara tabida* was lower when host larvae had consumed *Kluyveromyces lactis* (lower than e.g. with *Saccharomyces cerevisiae*). This may, however, have been due to a trade-off between number of eggs and relative fat content, even if the trade-off could not be detected by a multiple regression (see chapter 5.4). In contrast, all measured fitness traits in *Asobara tabida* were unfavourably influenced by *Metschnikowia pulcherrima*, which also had a negative influence on fitness traits of *Drosophila melanogaster*. *Saccharomyces cerevisiae* (besides *Kluyveromyces lactis* the most favourable diet for *Drosophila melanogaster*) also proved to be a favourable diet for *Asobara tabida* fitness traits followed by *Pichia toletana*, which usually had an intermediate influence on *Drosophila melanogaster* fitness traits. In *Leptopilina heterotoma*, the pattern was less consistent. Even though most of the measured fitness traits (development time, hind tibia length, dry weight, relative fat content and number of eggs) experienced a favourable influence by *Saccharomyces cerevisiae*, the important fitness trait 'survival' was worst when host larvae had fed on *Saccharomyces cerevisiae* (see **Fig. 59A**). *Metschnikowia pulcherrima*, which had proved unfavourable for *Drosophila melanogaster* and *Asobara tabida*, also had a negative influence on some of the measured fitness traits in *Leptopilina heterotoma* (development time, hind tibia length, dry weight and number of eggs), but survival was comparably high (at least at an initial cell mass of  $10^{-10}$ g), as well as relative fat content. The yeast species *Pichia toletana* with a more or less intermediate influence on fitness traits of *Drosophila melanogaster* and *Asobara tabida* proved a beneficial diet for most of the measured fitness traits and was mostly as favourable as *Kluyveromyces lactis*. Seemingly, the influence of dietary yeast species on *Leptopilina heterotoma* is more fitness-trait spe-

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cific than in *Asobara tabida*, so that it is difficult to judge, which yeast species may have an overall beneficial or unfavourable influence on the performance of *Leptopilina heterotoma*. Why are the responses in *Leptopilina heterotoma* less consistent than those in *Asobara tabida*? Maybe the presumably more deleteriously acting venom of *Leptopilina heterotoma* affects metabolism of *Drosophila melanogaster* in so many different ways, that no single yeast species is capable of meeting all metabolic demands and pose an overall favourable food. According to BOULÉTREAU & WAJNBERG (1986), who compared the two sympatric cynipid wasps *Leptopilina boulardi* and *Leptopilina heterotoma*, host suitability is supposed to be strongly influenced by nutritional factors. A comparison between the two parasitoid species led to contrary conclusions depending on whether well-fed or crowded hosts [poorly fed hosts] were considered. Abundant resources favoured the establishment of *Leptopilina heterotoma*, exhibiting a better development in well fed hosts than *Leptopilina boulardi* (BOULÉTREAU & WAJNBERG 1986). The authors, however, merely modulated the amount of food (with yeast offered as Baker's yeast) consumed by the host larvae. Yet, regarding the different yeast species, it is hard to judge, whether a host is poorly or well-fed, since yeast quality seems to have a substantial influence on the nutritional status of host larvae. Additionally, and maybe surprisingly, parasitoid fitness traits were mainly influenced by yeast quality, even though besides yeast quality also the initial yeast cell mass offered, strongly influenced performance of *Drosophila melanogaster*. Probable reasons for the lack of influence of initial yeast cell mass on parasitoid fitness have already been discussed in chapter 5.4. With regard to the qualitative differences among the various yeast species that may grow on decaying fruits as well as the different amount of yeast biomass that may be present, a general pattern concerning the influence of the nutritional status of *Drosophila melanogaster* host larvae on the performance of its parasitoids, and especially *Leptopilina heterotoma*, can hardly be drawn.

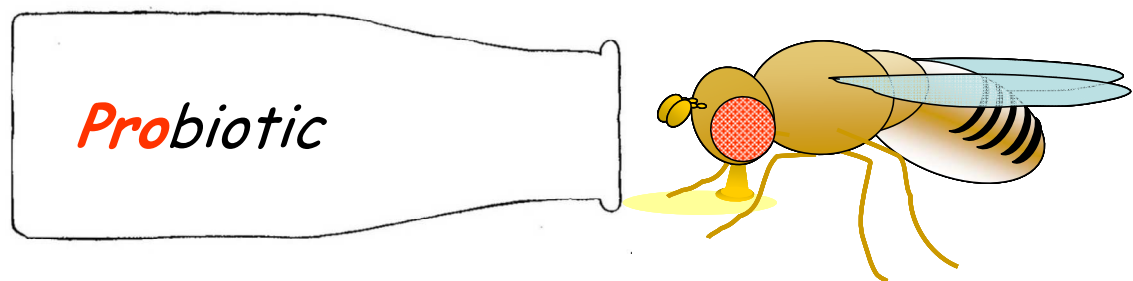
Just as in *Asobara tabida*, no trade off between reproduction (number of eggs) and survival (fat reserves as absolute fat content) was detected in *Leptopilina heterotoma*. Possible reasons for the lack of a trade off or an apparent lack of a trade off has already been discussed in chapter 5.4. The same reasons may also apply for *Leptopilina heterotoma*.

Maybe it should not be too surprising to find a rather high variability in responses of different fitness traits (of *Drosophila melanogaster* as well as its parasitoids) to specific dietary yeast species. After a dietary shift from cornmeal medium to pure banana, statistically significant changes in gene expression patterns with differences between food sources could be detected in *Drosophila melanogaster*, by using complementary DNA microarrays (CARSTEN *et al.* 2005). Since the different dietary yeast species may vary substantially in nutritional content and their nutritional value, genes associated with various biochemical pathways may be expressed differentially in response to consumption of specific yeast spe-

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cies. The biochemical pathways may lead to a differential phenotypic expression of the various fitness traits, so that one may experience a favourable, the other a less favourable influence, especially against the background of the diverse tissue- and organic-specific effects diet may have on an organism (RUDEN *et al.* 2005).



### 9 Final discussion and conclusions

The importance of microorganisms for the development, maintenance and sustenance of life on earth can hardly be overestimated and may not be approached by any other organisms. The global ecosystem depends on the activities of microorganisms, and the association and interaction of microorganisms with other organisms (fungi, plants, animals) (for example as gut microbiota in many animals), is supposed to play a role in adaptation and evolution of higher organisms (ZILBER-ROSENBERG & ROSENBERG 2008). Since the “father of immunology” ILYA METCHNIKOV, who theorised that ingestion of healthy bacteria found in fermented foods could counteract toxic bacteria and was the key to good health, investigating the microbiota and the immune system have increased significantly in recent years (HUFFNAGLE & NOVERR 2008). Even though a vast number of microbiota may be initiators of diseases, microbiota may also pose a major positive regulatory force for immune responses (NOVERR & HUFFNAGLE 2004). As a saprophagous insect that fulfils its development on decaying organic matter, *Drosophila melanogaster* encounters a plethora of different microorganisms. Especially yeast species pose the most important food source for larval as well as adult *Drosophila melanogaster*. Do these metabolically highly diverse microbial species influence life history traits of *Drosophila melanogaster* and its interaction with the parasitoid wasps *Asobara tabida* and *Leptopilina heterotoma*? The dietary yeast species *Cryptococcus albidus*, *Kluyveromyces lactis*, *Metschnikowia pulcherrima*, *Pichia toletana* and *Saccharomyces cerevisiae*, found in association to *Drosophila melanogaster* and chosen as model microbial species, could be shown to influence a number of life history traits of unparasitised as well as parasitised *Drosophila melanogaster* larvae and emerging adults, as well as the parasitoids *Asobara tabida* and *Leptopilina heterotoma* (chapters 5 and 8). The differing nutritional quality posed by the different yeast species as well as the quantity in which the yeast species had been offered to *Drosophila melanogaster* larvae, differentially influenced survival and development time of unparasitised larvae, survival and encapsulation ability of parasitised larvae as well as fitness traits such as body size (measured as mesothorax length) and body weight (measured as dry weight) of adult flies emerging from unparasitised larvae. Some yeast species could be shown to have an overall favourable influence on the performance of *Drosophila melanogaster* (such as *Kluyveromyces lactis* and *Saccharomyces cerevisiae*), some to have an intermediate influence (*Cryptococcus albidus* and *Pichia toletana*) as well as an unfavourable influence (*Metschnikowia pulcherrima*). The favourable or unfavourable influence depended on the amount of biomass offered to the larvae. At *ad libitum* conditions, nearly all dietary yeast species were comparably beneficial to unparasitised larvae, with the only exception of *Metschnikowia pulcherrima*, where larvae usually experienced a comparably long development time.

Unparasitised as well as parasitised larvae could be shown to olfactorily perceive different yeast species and prefer to consume those favourable for their own larval and adult life history traits. When given the choice, unparasitised larvae exhibited different and partly distinct preferences for the different yeast species (DORSCH 2007 and chapter 7). Hereby it turned out, that larvae tended to show a preference for those yeast species which had been shown to favour their larval development and adult fitness traits (*Kluyveromyces lactis* and *Saccharomyces cerevisiae*), and to reject those, that had been shown to be rather unfavourable for larval and adult performance (*Metschnikowia pulcherrima*). When yeasts were offered at intermediate or limited conditions (different initial yeast cell biomasses were offered), the responses of the measured life history traits to the different yeast species showed a higher within-treatment variation and got more inconsistent (chapter 5.4 and 8). Not all measured fitness traits were either only positively or only negatively influenced by a specific yeast species. Instead, some traits were favourably, some less favourably influenced. Additionally, some of the yeast species that had turned out as an overall favourable microbial diet at *ad libitum* conditions, such as *Saccharomyces cerevisiae*, were less beneficial compared to the other yeast species at limited conditions. The relation between initial yeast cell mass offered and the response in the measured fitness traits was not always of linear character. This could be seen in *Metschnikowia pulcherrima*, whose influence was even less favourable when offered at intermediate conditions (intermediate cell mass) compared to limited conditions (low cell mass).

The frequently observed positive influence of a mixed diet compared to a diet composed of only one or few dietary factors, could not be observed in *Drosophila melanogaster* with a diet composed of more than one yeast species (chapter 5.5). This, however, depended on which yeast species were offered in combination. For example, when the highly beneficial yeast species *Kluyveromyces lactis* was offered in combination with the less beneficial yeast species *Metschnikowia pulcherrima*, the influence on *Drosophila melanogaster* fitness traits was partly even less favourable than the influence of *Metschnikowia pulcherrima* alone. Besides initial yeast cell mass also ambient temperature affected the influence of the different yeast species on *Drosophila melanogaster* performance, with some yeast species being more favourable at the lower, some at the higher ambient temperature (chapter 5.5). This was, however, again often trait-specific.

In parasitised larvae, some yeast species (*Kluyveromyces lactis* and *Metschnikowia pulcherrima*) were shown to exhibit a beneficial influence on the immune defence (measured as encapsulation ability) of *Drosophila melanogaster* against offspring of the parasitoid *Asobara tabida*, when offered at *ad libitum* conditions (chapters 5.2, 5.3 and 5.5). The underlying mechanism of this phenomenon was tried to be revealed by determining the number of haemocytes circulating in the haemolymph of *Drosophila melanogaster* larvae (chapter 6), since an enhanced encapsulation ability is supposed to be related to the number

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of circulating haemocytes which play a major part in the encapsulation process (ESLIN & PRÉVOST 1995). As model yeasts, *Kluyveromyces lactis*, that had been shown to enhance encapsulation ability, as well as *Saccharomyces cerevisiae*, that had not been shown to influence encapsulation ability as positively (see chapters 5.2 and 5.3), were chosen. The number of haemocytes circulating in the haemolymph of unparasitised as well as parasitised *Drosophila melanogaster* larvae that had fed on *Kluyveromyces lactis* did not differ significantly from that of larvae that had fed on *Saccharomyces cerevisiae*, suggesting that the number of haemocytes may not be responsible for the observed differences in encapsulation ability. The within-treatment variation, however, was extremely high. This high variation may hint at inter-individual differences in the influence of specific yeast species on the encapsulation ability of parasitised larvae. Larvae that had been given the possibility to fulfil their development on the yeast species they had preferred in a prior food choice experiment, turned out to exhibit an enhanced encapsulation ability, irregardless of whether they had chosen and fulfilled their development on *Kluyveromyces lactis* or *Saccharomyces cerevisiae* (chapter 7). This may suggest, a genetic or epigenetic disposition of individual larvae to tolerate and profit from specific yeast species or their metabolic products. Some yeast species may hereby be more salubrious to a larger number of individuals (such as *Kluyveromyces lactis*) than other yeast species (e.g. *Metschnikowia pulcherrima*). *Kluyveromyces lactis*, for example, still seemed to pose a more favourable influence on encapsulation ability than *Saccharomyces cerevisiae*, despite the observed inter-individual variation. The positive influence of *Kluyveromyces lactis* was more pronounced and occurred more frequently (chapters 5.2, 5.3 and 5.5), and parasitised larvae showed a significant preference for *Kluyveromyces lactis* when offered in combination with *Saccharomyces cerevisiae* (chapter 7). The results also suggested that the nutritional demands of parasitised larvae may not be similar to those of unparasitised larvae. Yeast species that had turned out to be rather unfavourable for the performance of unparasitised larvae, proved to positively influence traits of parasitised larvae, such as encapsulation ability that was favourably influenced by *Metschnikowia pulcherrima*. At limited food conditions, the significant differences in influence of the dietary yeast species on encapsulation ability was evened out and immune defence was generally comparably low. Immune defence of *Drosophila melanogaster* against offspring of *Leptopilina heterotoma* was not influenced at all by the different dietary yeast species, probably due to the aggressive mechanism of *Leptopilina heterotoma* to evade immune defences of its host (among other things VLPs that destroy host lamellocytes). The usually higher mortality of parasitised compared to unparasitised larvae, irregardless of whether they had been parasitised by *Asobara tabida* or *Leptopilina heterotoma*, most likely due to the venom injected by the parasitoids during oviposition, nevertheless experienced a modulation by the different dietary yeast species. This suggests a specific influence of different dietary yeast species on virulence factors (e.g.



venoms) of the parasitoids. It may also suggest that the beneficial influence of some yeast species on encapsulation ability of *Drosophila melanogaster* against *Asobara tabida* offspring may not necessarily have been due to an influence on the number of circulating haemocytes, but also on other components of the cellular immune defence (e.g. Prophenoloxidase-activity) or on virulence factors (adhesiveness of eggs, venom) of the parasitoid.

Besides the influence of dietary yeast species on the immune defence of *Drosophila melanogaster* (at least against *Asobara tabida*) and hereby its resistance against parasitoids, as well as virulence factors of the parasitoid, several life history traits of *Asobara tabida* and *Leptopilina heterotoma* were also significantly influenced (chapters 5.4, 5.5 and 8). Yet, no general pattern could be observed, whether parasitoids perform better on hosts in good or in bad nutritional condition. It was mainly *Asobara tabida* which showed a tendency to perform better in hosts which had consumed the yeast species favourable for the host's own larval or adult life history traits. The qualitative differences between yeast species were hereby of main importance. Neither *Asobara tabida* nor *Leptopilina heterotoma* showed a convincing response to the different initial yeast cell masses, i.e. intermediate or limited conditions. This may probably be due to the parasitoid's development being slightly temporally shifted to that of its host, so that *Drosophila melanogaster* may either have had the chance of compensating for nutritional losses at the beginning of its development, or that yeast biomass had increased before *Drosophila melanogaster* larvae or parasitoid larvae could experience serious (yeast-) starvation. Parasitoids could, thus, be neither positively (if parasitoids perform better in starved hosts) nor negatively (if parasitoids perform better in well-fed hosts) influenced in their performance by initial yeast cell mass.

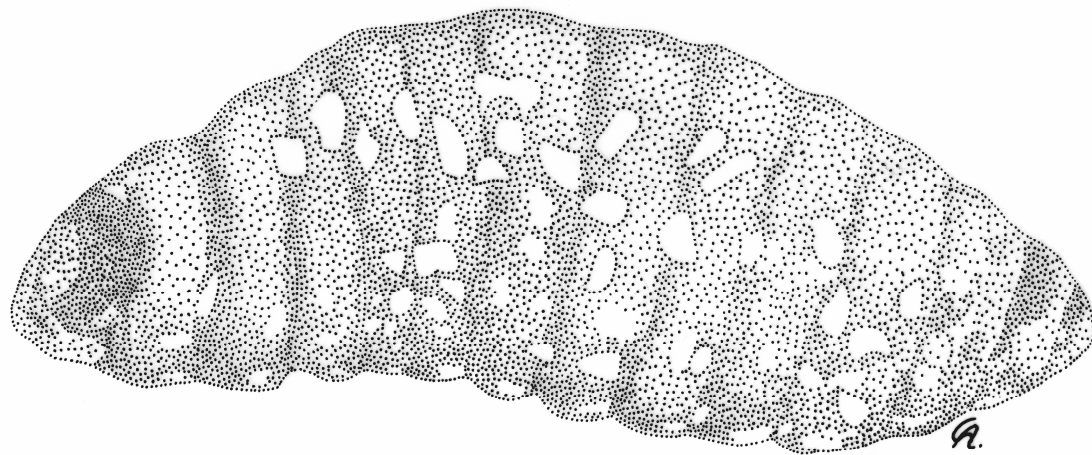
Especially in *Leptopilina heterotoma* the responses of the various measured life history traits to the different dietary yeast species was rather inconsistent. There was no overall favourable or unfavourable influence on all measured traits by one specific yeast species, with some traits being positively, some less positively influenced by the same yeast species. This may be due to the presumably more deleteriously acting venom of *Leptopilina heterotoma*, which may affect metabolism of *Drosophila melanogaster* in so many different ways, that no single yeast species may be capable of meeting all metabolic demands and pose an overall beneficial food.

The results strongly suggest that the microbial species inhabiting the same ephemeral resource as *Drosophila melanogaster* may profoundly influence the outcome of the interaction between *Drosophila melanogaster* and its parasitoids *Asobara tabida* and *Leptopilina heterotoma*, both by modulating host immune defences as well as parasitoid life history traits. The microbial environment of this host-parasitoid interaction may even have influenced or still do influence the evolutionary processes forming the interaction between host

## 9 *Final discussion and conclusions*

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and parasite. Evidence suggests that substantial inter-individual variability may exist with regard to the nutritious value of dietary compounds. Some dietary compounds in the form of yeast species and their metabolic products may be more adequate and salubrious for one larva compared to another, probably due to genetic and epigenetic dispositions. The high variability in the responses of *Drosophila melanogaster* and its parasitoids may also be due to the fact that the food itself, composed of live microbial organisms, exhibits a substantial variability in its physiology and thus its nutritional value for higher trophic levels.



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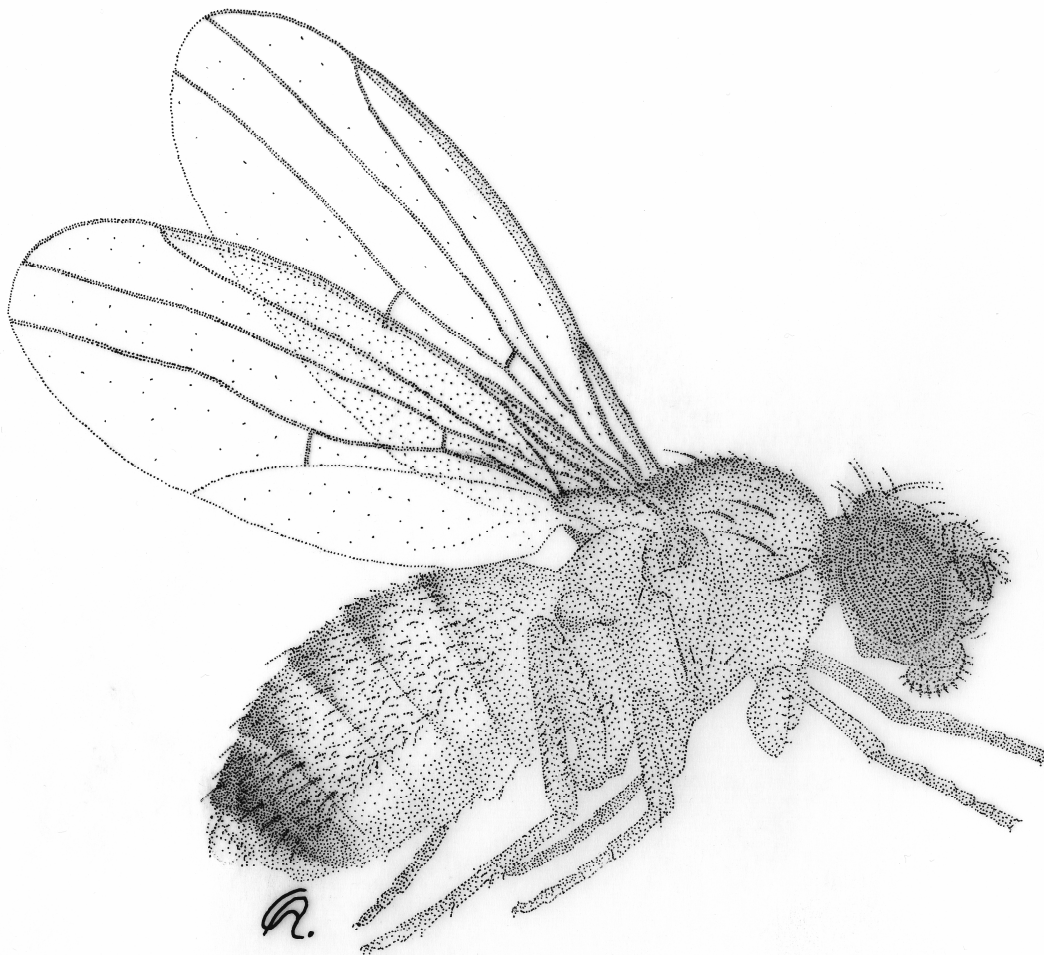
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Illustration of the life cycle of *Drosophila melanogaster*

Picture source: Cover illustration of *Science*, Vol. 297, No. 5590, 2002

## 12 Curriculum vitae

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Date of birth: March 12, 1972

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1978 – 1982	Elementary School in Mertingen, Germany
1982 – 1991	Grammar School at Gymnasium Donauwörth, Germany
1991 – 1992	Voluntary work in the social sector (hospital): “Freiwilliges Soziales Jahr” at the Waldkrankenhaus in Erlangen, Germany
1992 – 1994	Vocational education as Medical Laboratory Assistant in Erlangen, Germany
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1996 – 2003	Studies in Biology at the Christian-Albrechts-University in Kiel
2003	Diploma Thesis; Title: Ausgewählte Aspekte zur Ökologie von <i>Filago vulgaris</i> Lam. in Schleswig-Holstein (Selected aspects about the ecology of <i>Filago vulgaris</i> Lam. in Schleswig-Holstein)
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2005 – 2008	Occupational activity as research assistant at the Zoological Institute, Animal Ecology, University of Kiel and doctoral thesis

## 13 Glossar

**Adaptive immunity:** Defence mechanism of a multicellular organism against pathogens and parasites which are recognised by randomly generated receptors. The receptors have a narrow specificity. Specific immunity is often used as a synonym, even though there is evidence that specificity and memory may not only occur in adaptive immunity. It is supposed to occur in jawed vertebrates only.

**ANCOVA:** Short for 'Analysis of covariance'. Statistical model with one continuous response variable and one or more factors (independent variables/quantitative predictors). ANCOVA tests whether certain factors have an effect on the response variable after removing the variance due to one of the factors (covariate). The inclusion of covariates can increase statistical power since it accounts for some of the variability of the response variable and thus increases the ratio of variance explained by the independent variables.

**Arrhenotoky:** Arrhenotoky is a common mode of reproduction in the Hymenoptera. Haploid males arise from unfertilised eggs, receiving a single set of maternal chromosomes, whereas diploid females arise from fertilised eggs and receive both maternal and paternal chromosomes.

**Bateman's principle:** Theory founded by A. J. Bateman after which sexual selection is supposed to favour promiscuity in males rather than females as a result of differences in parental investment in gametes (anisogamy). In males, mating rate seems to be a major determinant for fitness (therefore mostly females are a limiting resource), whereas in females longevity is supposed to be a major determinant, since they have to make greater physiological sacrifices for the production of each surviving offspring.

**Coevolution:** Reciprocal evolutionary change in interacting species driven by natural selection.

**Chorion:** Hard, protective outer part of an insect egg. The insect egg generally consists of two layers, the outer chorion, that may show an elaborate sculpturing and carry diverse appendages, and the inner vitelline membrane.

**Critical weight/size:** A certain weight/size threshold in (*Drosophila*) larvae, after which feeding no longer affects the time course to pupation.

**Detoxification-limitation hypothesis:** The hypothesis suggests that the ability of herbivores to eliminate plant secondary metabolites, largely determines which plants and how much of them they can eat.

**Dietary self-selection:** According to WALDBAUER & FRIEDMAN (1991), the behaviour of dietary self-selection observed in animals over all taxa, is a continuous regulation of food intake, involving frequent shifts between different foods, whereby the choice of foods or nutrients are non-random and beneficiary to fitness.

**Ectoparasite:** Parasites that develop and live on their host's exterior.

**Encapsulation:** Cellular innate immune response where multiple haemocytes bind to larger invaders, like parasitoids and nematodes that cannot be phagocytosed by a single cell.

**Endoparasite:** Parasites that develop and live within internal organs or tissues of their host.

**Genotype-by-environment interaction:** When distinct genotypes vary in the degree to which their phenotypes are affected by environmental conditions they are subject to a genotype-by-environment interaction.

**Genotype-by-genotype interaction:** The traits of a relationship between host and parasite are determined by the genotype of the host and the parasite, and the outcome of a host-parasite interaction is determined by the specific combination of the host and the parasite genotype.

**GLM:** Short for 'Generalised linear model'. This statistical model is a generalisation of regression models founded on the least squares method. It relates the random distribution of the dependent variable measured in the experiment (response variable) to the systematic (non-random) and independent variable (factor, linear predictor) through a function called link function.

**Gregarious parasitoid:** Parasitoids in which several larvae (more than one and up to several thousand) share one host and are able to successfully complete their development.

**Hamilton-Zuk hypothesis:** According to the hypothesis of BILL HAMILTON & MARLENE ZUK (1982), under certain circumstances females should choose males on the basis of secondary sexual traits. The full expression of these traits is hereby limited by parasite infection and the condition of the traits might indicate genetic resistance to parasites. Females choose males with exaggerated secondary traits in order to obtain resistance genes for their offspring by mating with those males. The hypothesis thus predicts that within species one should find a negative correlation between parasite load and male brightness on the one hand, and mating success on the other.

**Idiobiont:** Parasitoids that prevent their host's further development after parasitisation (by permanent paralysis) and feed merely on the resources available at oviposition.

**Immunohandicap hypothesis:** The hypothesis explains the frequently observed phenomenon that in vertebrates, immune response and resistance to infection appear to be consistently lower in males compared to females, by suggesting that an increased level of testosterone in males, increases mating success but suppresses the immune response.

**Innate immunity:** Defence mechanism of a multicellular organism against pathogens and parasites which are recognised by receptors encoded in the germline. The receptors are hereby supposed to have a broader specificity. Evidence, however, suggests that specificity and memory also occur in innate immunity. It is supposed to occur in all metazoans.

**Kairomone:** a chemical that conveys information between two species, one of which is the signaler, the other the receiver of information. If the receiver alone benefits from the information transfer, the chemical is called a kairomone.

**Koinobiont:** Parasitoids that allow further development of their host after parasitisation and only start to feed invasively and destructively until their host either pupates or becomes an adult.

**Macroparasites:** Parasites that grow but do not multiply in their host and produce specialised infective stages that are released to infect new hosts.

**Microparasites:** Parasites that are small and often intracellular and multiply directly within their host where they are often extremely numerous.

**Minimal viable weight/size:** A certain weight/size threshold at which (*Drosophila*) larvae can develop into adults if food is completely withdrawn.

**Mycocins:** Extracellular agents of yeasts having fungicidal or fungistatic action.

**Optimal diet theory:** The theory predicts that foragers should prefer prey that yield more energy per unit handling time. As the abundance of higher value prey increases, lower value prey should be dropped from the diet. Foragers should obey a quantitative threshold rule for when specific prey types should be included or excluded from an optimal diet.

**Optimal foraging theory:** Theory after which organisms forage in such a way as to maximise their energy intake per time unit.

**Ovigeny:** Timing of egg maturation in a female parasitoid.



**Ovigeny index:** Proportion of the potential lifetime complement of eggs that is mature upon emergence of a female parasitoid. In a purely proovigenic parasitoid, ovigeny index would thus be 1, in a purely synovigenic parasitoid ovigeny index would be 0.

**Ovipositor:** Specialised structure in many female insects used for laying eggs. The sclerites that make up the ovipositor are homologous to parts of the legs primitively associated with the 8<sup>th</sup> and 9<sup>th</sup> abdominal segments.

**Parasite:** An organism that lives in or on another living organism (the host) deriving its nutrients from it (usually one or a very few host individuals), showing some degree of structural adaptation to it and normally causing it some harm.

**Parasitoid:** Usually an insect whose larvae develop by feeding on the bodies of other arthropods, usually also insects and the larval feeding results in the death of the parasitoid's host. A parasitoid also usually has a free-living adult stage.

**Pathogenicity:** Qualitative capacity of an infectious agent to inflict a disease on an organism (qualitative property).

**Pathogen associated molecular patterns (PAMPs):** Conserved microbial structures or products of microbial metabolism that the innate immunity is capable of recognising. They are unique to microorganisms (also non-pathogenic ones) and are not present in the host.

**Pattern recognition receptors (PRRs):** Receptors encoded in the germ line that bind to molecules (e.g. peptidoglycans and glucans) associated with microbes.

**Preference-performance hypothesis:** Originally formulated in plant-insect research (also as oviposition-preference-offspring-performance hypothesis), the hypothesis states that oviposition-preference patterns of insects correspond to host suitability for offspring development because females are assumed to maximise their fitness by ovipositing on high-quality hosts. The selection of oviposition sites in females is assumed to be determined by the quality of the oviposition site for offspring development, and that there is a positive correlation between oviposition preference and offspring performance. The hypothesis has been applied in plant insect, algae-amphipod, host-parasite and prey-predator systems, but also in other fields.

**Prebiotics:** Non-digestible food ingredients that, during their way through the intestinal tract of an organism, beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of microorganismal species already resident in the intestinal tract, and thus attempt to improve host health.

**Probiotics:** Living microorganisms (bacteria or yeasts), that exert a beneficial effect on the health of the host when ingested in adequate amounts, by modulating mucosal or systemic immunity and improving nutritional and microbial balance in the intestinal tract.

**Proovigeny:** All eggs of a female parasitoid are mature at the time of eclosion of the adult female.

**Red-Queen-Hypothesis:** This hypothesis was originally devised by Leigh Van Valen in 1973: "For an evolutionary system, continuing development is needed just in order to maintain its fitness relative to the systems it is co-evolving with". I.e., in order to stay extant, both evolutionary systems that are tightly engaged in coevolved interaction must continuously evolve, since fitness increases in one evolutionary system tend to result in fitness decreases in the other system. Thus, the only way to maintain relative fitness is to improve in a similar way. The name for this hypothesis is based on the reflection uttered by the Red Queen to Alice in Lewis Carroll's novel *Through the looking glass* (1872): "Now, here, you see, it takes all the running you can do, to keep in the same place". In 1982 Graham Bell proposed a version of the Red Queen Hypothesis explaining why the

phenomenon of sexual reproduction is so widespread among organisms despite the two-fold advantage of asexual reproduction. This version is especially interwoven with host-parasite coevolution. According to the hypothesis, hosts and parasites are considered to be engaged in an antagonistic arms race, i.e. a reciprocal coadaptation in which the evolving parties continually respond and counterrespond to the selection pressures imposed by each other. Parasite genotypes most successful in attacking the most common host genotypes and reducing their fitness will be favoured by natural selection. Rare host genotypes, in turn, enjoy a fitness advantage that declines as they become more common. After a time lag, the former rare host genotypes are expected to be attacked by a parasite genotype able to infect the new hosts. This time-lagged frequency-dependent selection against common host genotypes may lead to cycling of both host and parasite allele frequencies. Parasites are expected to evolve rapidly so as to infect disproportionately any genotype that becomes common and thereby drive it down in frequency. This sets up an oscillation in which parasite gene frequencies track the gene frequencies of their host. Mathematical models have shown that this may favour sexual reproduction in host populations, since via sexual reproduction and the maintenance of genetic diversity connected to it hosts are better able to keep up the arms race with their rapidly evolving parasites. The hypothesis not only explains how parasites can maintain sexual reproduction, but it is also relevant for the evolution of characters such as mating systems, pathogen virulence, host resistance and the maintenance of genetic diversity in natural populations.

**Resistance:** Traits that prevent infection or limit its extent.

**Self-medication (Zoopharmacognosy):** Process by which wild animals select and use specific plants with medicinal properties for the treatment and prevention of diseases.

**Solitary parasitoid:** Parasitoids in which only one larva is able to successfully complete development on a host. If more than one egg is laid into a host by one or more female parasitoids, the larvae compete and only one survives.

**Somatic hypermutation:** Process by which receptors of the immune system are diversified, allowing the immune system to adapt its response to new elicitors that may occur during the lifetime of an organism.

**Superparasitism:** Deposition of a clutch of eggs (the clutch may consist of a single egg) on a host that has already been parasitised by a member of the same species.

**Synovigeny:** Egg maturation occurs after eclosion of the female parasitoid.

**Tolerance:** Traits that do not reduce or eliminate infection, but instead reduce or offset its fitness consequences.

**Trade-off:** A linkage between traits that constrains the simultaneous evolution of two or more traits. Trade-off can be of physiological kind, meaning that within a single individual giving more resource to one trait implies that less will be allocated to the other.

**Vibrotaxis:** Use of mechanical, vibratory stimuli for host location by female parasitoids.

**Virulence:** Relative degree of damage (loss of fitness) done to a host by the pathogen (quantitative property).

## *A fairy tale*

An evolutionary ecologist's fairy tale, told by Stephen C. Stearns  
[The Evolution of Life Histories, 1992]



### *The Plum*

*Pushed by a gust of wind, a plum falls to the ground and splits. Into a few drops of juice that enter the cleft, yeast cells topple from the plum's skin. Bacteria float in. The yeast and bacteria multiply. Within an hour, the first faint odours of ethanol waft out, and soon a female fruit fly lands. She walks back and forth along the cleft, pausing frequently to probe with her mouth parts, then lays nine eggs in an irregular row and departs. Many other females visit the plum. The pearly eggs, driven into the flesh of the fruit by ovipositors, gleam in the light reflected from the ground. The two filaments with which each egg breathes stick like arms into the air. A black ground beetle stumbles into the plum and probes along the cleft, eating every egg it touches. A day later, the remaining eggs begin to hatch. The larvae wander for a few hours over the rotting plum, following concentrations of bacteria and yeast. As they grow, they shed their tough skin and expand into the soft membranes beneath. More females visit the cleft. Large flies and beetles*

## *A fairy tale*

suck the fermenting brew. The population of larvae near the cleft grows dense, but the first-laid larvae move on. On the lower side of the fruit, a fungal mycelium invades, feeding on the juices, excreting antibiotics. It clears a growing zone in which a fly larva would starve. The lower side of the fruit turns brown and liquefies. After five days, the first-laid larvae move up out of the flesh and seek well-aerated hiding places among the folds of skin. Forming tough brown cylinders, they pupate. A small wasp alights and drives her needle-like ovipositor repeatedly through the skin of the plum. Every time its tip enters a pupa, she lays one egg. Wasp larvae hatch and devour their hosts. A few pupae hatch during the night of the twelfth day after the plum fell. When they split the pupal skins and emerge, their bodies are plump and white, their wings are soft and crumpled, and they weigh about two milligrams. They move tentatively while their cuticle hardens and their wings expand. Their bodies darken. In the afternoon, they fly out. The next day one of them, a female, finds a fallen apple thick with flies and beset by slugs. She lands on the splotched skin and listens. Soon she hears the characteristic wing beats of a male. She approaches. He sees her and dances to the side, waving his wings. She turns. He mounts and mates, riding her for several minutes after the sperm are transferred. During the night, she lays thirty eggs on the apple. She moves on, each day laying twenty or thirty eggs into various fruit. Every two or three days, she mates again, and the sperm most recently received fertilize the next eggs laid. Twenty-one days after she left the plum, having laid more than 400 eggs, she slips. A wing gets mired in fruit sap; a beetle eats her. At the plum, the fungus, having sucked the fruit dry, sends up yellow cups of spores. Some laggard, undernourished fly larvae pupate eighteen days after they were laid. A few escape the wasps and hatch five days later. They weigh about one milligram, and the females among them can lay no more than ten eggs per day. They fly off to find mates, food, and sites to deposit their eggs. Twenty metres away, a fly settles on a crack in the skin of a newly fallen plum.



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