Eco-evolutionary dynamics in host-virus systems

Dissertation

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Summary of the dissertation

In my thesis, I studied eco-evolutionary dynamics with the focus to advance this relatively novel research field. In general, I aim to develop a detailed mechanistic understanding of eco-evolutionary dynamics in host-virus systems and investigate the effects and important consequences of such dynamics in simple and increasingly complex food webs.

The first chapter of this thesis serves as a general introduction into eco-evolutionary dynamics. Here, I review most recent findings concerning the field of eco-evolutionary dynamics and propose several further research directions by identifying important gaps in our knowledge, which then served as the most important driver for my thesis work.

The second chapter addresses several missing links identified in chapter one; i) study eco-evolutionary dynamics with different types of biotic species interactions, ii) use systems with more than one evolving species, iii) use systems with more than one evolving trait, iv) combine empirical work with modeling. I test in great detail for eco-evolutionary dynamics in a host-virus system and combine this empirical work with mathematical modeling. This chapter shows the strength of combining experimental work with modeling. The results in this chapter show how ecology and evolution are tightly linked in coevolving populations. Furthermore, I discuss the mechanisms by which they both (ecology and evolution) affect one another and underline the important consequences of these eco-evolutionary dynamics for the predictability, stability and maintenance of variation in such populations.

In the third chapter, I extend the relatively simple host-virus community of chapter two with an additional player. As eco-evolutionary dynamics are not well understood in more complex systems, this approach enabled testing for increasing complexity in a controlled experimental design by comparing more complex systems with the relatively simple two species host-virus system. Here, I conclude that in contrast to the two species system, increasing complexity resulted in multiple indirect (ecological and evolutionary) effects, and in distinct eco-evolutionary dynamics. The direct and indirect interactions between ecology and evolution are important for the coexistence of multiple (competing) consumers and are thus crucial to understand the mechanisms driving community structure and diversity.

In the last chapter I take a different approach. As the results from the second chapter show a tight link between ecology and evolution, I investigate here the result of these eco-evolutionary dynamics on parallel and divergent evolution between replicate host populations

that coevolved with a virus. To do so, I test for parallel and divergent evolution between different replicate host populations on both the phenotypic and genomic level. I show that host populations evolve highly parallel based on their (resistance) phenotypes, but in contrast, diverge when looking at SNPs and INDELs (hereafter: small variants). With this I confirm that degrees of parallelism depend on the level of biological organization. However and most importantly, I show that these populations evolve parallel when looking at structural variation (duplication of large genomic region). Divergence observed when looking at small variants is a consequence of eco-evolutionary dynamics in these coevolving populations. The interactions between ecology and evolution result in strong effects of drift due to population bottlenecks and genetic hitchhiking of small variants caused by selective sweeps (of large genomic duplication). The combined effect of drift and genetic hitchhiking lead to an overall genomic divergence between populations based on small variants.

Zusammenfassung der Dissertation

In meiner Doktorarbeit untersuchte ich ökologisch - evolutionäre Dynamiken mit dem Fokus dieses relativ neue Forschungsgebiet voranzutreiben. Im Allgemeinen versuche ich ein detailliertes Verständnis dieser dynamischen Abläufe in einem Wirt-Virus-System zu entwickeln, und deren Auswirkungen und wichtigen Folgen in einfachen und immer komplexer werdenden Nahrungsnetzen zu untersuchen.

Das erste Kapitel dieser Arbeit dient als allgemeine Einführung in ökologisch - evolutionäre Dynamiken. Hier liefere ich eine Bewertung neuester Erkenntnisse und schlage mehrere weiterführende Forschungsrichtungen vor, die ich anhand der existierenden Lücken in unserem jetzigen Wissen identifiziere und die als wichtigste Treiber für meine Doktorarbeit dienten.

Das zweite Kapitel befasst sich mit einigen der Lüken, die ich in Kapitel I dargestellt habe: ökologisch - evolutionären Dynamiken mit verschiedenen Typen biotischer Interaktionen, Systeme in denen mehr als eine Art und mehrere Merkmale evolvieren und die Kombination empirischer Arbeit mit Modellierung. Im Detail, teste ich ökologisch - evolutionäre Dynamiken in einem Wirt-Virus-System und kombiniere diese empirische Arbeit mit mathematischen Modellierung. Dieses Kapitel zeigt die Stärke der Kombination von experimentellen Arbeiten mit Modellierung. Die Ergebnisse in diesem Kapitel zeigen weiterhin, wie eng Ökologie und Evolution in koevolvierenden Populationen verwoben sind. Darüber hinaus diskutiere ich die Mechanismen, mit denen sich beide (Ökologie und Evolution) gegenseitig beeinflussen und unterstreiche die wichtigen Folgen dieser ökologisch - evolutionären Dynamiken für die Vorhersehbarkeit, Stabilität und Aufrechterhaltung der Variation in solchen Populationen.

Im dritten Kapitel erweitere ich das relativ einfache Wirt-Virus-System von Kapitel II mit einem zusätzlichen Organismus. Das Verständnis von ökologisch - evolutionäre Dynamiken in komplexeren Systemen ist noch nicht hinreichend untersucht, durch den hier beschriebenen Ansatz ist es mir möglich ansteigende Komplexität in einem kontrollierten experimentellen System zu testen, indem komplexere Systeme mit dem einfachen Wits-Virus-System verglichen werden. Ich komme hierbei zu dem Schluss, dass im Gegensatz zum Wirt-Virus-System, ansteigende Komplexität zu multiplen (ökologischen und evolutionären) indirekten Effekten und zu veränderten ökologisch - evolutionäre Dynamiken führt. Die

direkten und indirketen Interaktionen zwischen Ökologie und Evolution sind wichtig für die Koexistenz von mulitplen (konkurrierenden) Konsumenten und somit entscheidend für das Verständnis der treibenden Mechanismen für die Struktur von Artengemeinschaften und deren Diversität.

Im letzten Kapitel verfolge ich einen anderen Ansatz. Die Ergebnisse aus dem vorangegangenen Kapitel zeigen einen engen Zusammenhang zwischen Ökologie und Evolution, in diesem Kapitel untersuche ich vergleichend die aus den ökologisch - evolutionäre Dynamiken resultierende parallele und divergierende Selektion zwischen replizierten Wirts-Populationen die mit einem Virus koevolvieren. Um dies zu untersuchen teste ich parallele und divergierende Selektion zwischen replizierten Wirts-Population auf der phänotypischen und genotypischen Ebene. Ich zeige, dass die Evolution der Wirts-Populationen stark parallel verläuft, basierend auf den vorkommenden (Resistenz-) Phänotypen. Bei der Analyse von SNPs und INDELs zeigt sich aber divergierende Evolution. Hiermit kann ich bestätigen dass der Grad an Parallelität von dem zu betrachtendem Level (Phänotyp/Genotyp) abhängt. Jedoch komme ich zu dem wichtigen Ergebnis, dass die Populationen parallel evolvieren wenn man sich die strukturelle Variation (Duplizierung von großen genomischen Regionen) anschaut. Die beobachtete Divergenz in SNPs und INDELs ist eine Konsequenz aus den ökologisch - evolutionäre Dynamiken der Populationen. Die Interaktionen zwischen Ökologie und Evolution resultieren in einem starken Drift durch Bottlenecks in Populationen und genetischem Hitchhiking von SNPs und INDELs als Folge von Selective Sweeps der genomischen Duplikationen. Die kombinierten Effekte von Drift und genetischen Hitchhiking führen im großen zu einer Divergenz zwischen Populationen basierend auf SNPs und INDELs.

General Introduction

The classic view of evolutionary biology, where ecology drives evolutionary changes over relatively long time-scales has recently been expanded with the notion that evolutionary changes can often happen very fast (Thompson 1998). Furthermore, as increasingly more studies showed populations' potential to evolve rapidly (reviewed in i.e. Reznick & Ghalambor 2001; Schoener 2011; Koch *et al.* 2014), the traditional ecological approach to consider populations as genetically homogeneous without variation in traits had to be adjusted (Thompson 1998).

Indeed, populations can exhibit substantial genetic variation in traits through standing genetic variation, de novo mutations or gene flow (reviewed in Koch *et al.* 2014). This notion blurred the separation between the fields of ecology and evolution (Thompson 1998; Schoener 2011). When such rapidly evolving traits have an ecological effect on the population, community or ecosystem, they can alter the ecological processes that drive them (Yoshida *et al.* 2003; Hairston *et al.* 2005; Fussmann *et al.* 2007; Becks *et al.* 2010, 2012). As such, a dynamic interaction in both directions - ecology affecting evolution and evolution affecting ecology - exists, both driving and affecting one another on the same timescale (Fussmann *et al.* 2007; Pelletier *et al.* 2009; Post & Palkovacs 2009). This interaction between ecology and evolution has been termed eco-evolutionary dynamics, and is often referred to as 'the newest synthesis' (Schoener 2011).

Over the last decade, increasingly more research focused on this interface between ecology and evolution in order to understand the importance and role of eco-evolutionary dynamics. However, as the research field is relatively recent, the development of general concepts and an overall mechanistic understanding of eco-evolutionary dynamics are still on the way.

Eco-evolutionary dynamics: current questions and research motivation

In the first chapter of this thesis, I introduce the concepts of eco-evolutionary dynamics and review the most recent empirical and theoretical work in this research field. The main argument of this review article is that ecological and evolutionary dynamics are entangled and intertwined in many complex ways. They are important as they can both generate and maintain diversity within populations and they determine the stability of communities. However, I conclude that more studies are needed in order to tackle current gaps in our knowledge. Most empirical evidence for example, is coming from predator-prey systems

(Abrams & Matsuda 1997; Reznick & Ghalambor 2001; Yoshida *et al.* 2003; Becks *et al.* 2010, 2012; Kasada *et al.* 2014) with strong selection and where only one species and one trait can evolve. However, to develop a more general conceptual eco-evolutionary framework, eco-evolutionary dynamics needs to be studied with different types of species interactions. Moreover, empirical studies need to be combined with theoretical modeling in order to fully understand the complex ecological and evolutionary interactions and their consequences. These conclusions serve as the main drivers for the second chapter of this thesis work. More specifically, I developed a novel experimental model system that enables studying eco-evolutionary dynamics in detail in a host-virus system with coevolution. In this introduction, I will give an overview of the experimental model system with the organisms used, and shortly discuss the advantages in regard to studying eco-evolutionary dynamics with such an experimental set-up. Chapter two of the thesis discusses in detail the findings from this experimental model system. Furthermore, I combine these findings with mathematical modeling in order to test and confirm several predictions concerning eco-evolutionary dynamics in this host-virus system.

Another important conclusion from the review article (chapter one) is that research on eco-evolutionary dynamics mainly focused on just two interacting species and/or mostly followed species interactions over only a few generations. Natural communities are more complex, with many interacting species and thus possibly many evolving traits. Importantly, indirect and potentially delayed (over many generations) ecological and evolutionary effects in more complex systems can affect and alter these eco-evolutionary dynamics. Such effects are missed when species interactions are observed over short timescales (few generations) or in two species systems without the possibility of indirect effects. A general agreement on the importance of such eco-evolutionary dynamics is thus currently lacking. This is the main motivation for performing additional experiments with the host-virus system. In chapter three, I test how increasing complexity from the relatively simple two species host-virus system affects eco-evolutionary dynamics.

In chapter four I focus again on the two species host-virus system. One of the conclusions regarding eco-evolutionary dynamics in this system (chapter two) is that they affect the outcome and trajectories of coevolving populations. In this chapter, I investigate how the dynamical interaction between ecology and evolution affect the repeatability of coevolution. To do so, I test whether antagonistic coevolution between host and virus result in parallel or divergent evolution between identical populations. More specifically, I investigate whether

and how parallelism and divergence observed on the phenotypic level of host resistance differs based on their genomes (small variants and structural variation).

A novel model system to study eco-evolutionary dynamics

Studying eco-evolutionary dynamics requires an experimental set-up that allows for detailed quantification of many parameters (such as population sizes and evolutionary changes) at regular time-points during experiments (with small time intervals between sampling points). Furthermore, to study the interaction between ecology and evolution, it is important that the observed dynamics are purely driven by the species interactions itself, without any experimental manipulation. Moreover, these interactions need to be studied over long time spams and many generations. To meet all these requirements, I choose to work with continuous cultures (chemostats; introduced below) using micro-organisms.

Model organisms

The recent isolation of a large dsDNA virus that infects eukaryotic algae provides the perfect model organisms to study eco-evolutionary dynamics with antagonistically coevolving host and virus for several reasons. First, as these organisms are fairly recent isolated from nature, much is unknown about their ecological interactions, and virtually nothing is known in the context of antagonistic coevolution between the host (algae) and virus. Thus, studying eco-evolutionary dynamics with these organisms is beneficial as it simultaneously advances our knowledge about algae and virus interactions, which are numerous in nature and assumed to have great effects on ecosystems (Fuhrman 1999; Suttle 2000; Suttle 2007). Second, the algal host is eukaryotic, which makes this study system fairly unique in terms of great population sizes and relatively fast generation times. Moreover, the scientific literature is rich with studies and examples of antagonistic coevolution with prokaryotic hosts (bacteria and bacteriophages), making it interesting to compare findings between eukaryotic and prokaryotic microbial hosts.

a) *Chlorella variabilis*

Chlorella variabilis is a eukaryotic fresh water microalgae belonging to the family of green algae. This alga has only been observed to reproduce asexually by mitosis and has an entirely

haploid life-cycle. For this work, I use a strain of the alga (NC64A) that can be infected by a large dsDNA virus.

b) Paramecium bursaria chlorella virus 1 (PBCV-1)

Chlorella virus belongs to the Phycodnaviridae virus family. Key characteristics of this virus are that they are remarkably large (~ 0,2 μm), have a lytic cycle and have an icosahedral capsid structure containing the 330-kbp long dsDNA genome. PBCV-1 is a prototype in the Phycodnaviridae family because it is most studied and the first from which the whole genome was sequenced. The PBCV-1 strain replicates in the algal host (NC64A) and has an infectious cycle that is comparable to bacteriophages. The infection cycle (Vanetten *et al.* 1983; Van Etten & Meints 1999) of PBCV-1 starts by attachment to the Chlorella NC64A cell wall and subsequent digestion of the cell wall at the attachment point using enzymes present in the capsid. Cell wall digestion is followed by DNA entry into the host cell. Virus DNA replication starts and new virus particles are assembled in the virus assembly center. The virus has a lytic life cycle, thus virus particles (~ 300 PFUs) are released from the host cell by cell lysis and killing of the host cell (6-8h after attachment).

c) *Brachionus calyciflorus*

Brachionus calyciflorus is a fresh water planktonic rotifer. These rotifers are filter feeders and use cilia to create and direct a water current towards the mouth opening. Food particles (phytoplankton and organic matter) are ingested and digested in the stomach. *Brachionus calyciflorus* reproduces by cyclical parthenogenesis, but can be maintained in the lab as obligate asexual. One asexual clonal line of this rotifer species is used as a predator for *chlorella variabilis* in chapter 3 of the thesis.

Continuous flow-through systems (chemostats)

Continuous flow-through systems are the ideal experimental set-up to study eco-evolutionary dynamics. Ones such experiments start, they can run for an unlimited amount of time without external manipulation by researchers. This stands is in contrast with serial transfer experiments, where populations are transferred at regular time-intervals into new cultures with fresh resources. In the latter case, experimental populations undergo population bottlenecks imposed by the researcher at every transfer and resource concentrations are not constant (Barrick & Lenski 2013). Thus, observed dynamics (evolutionary and ecological dynamics) in such systems are not solely driven by the interactions between species or with

their environment. Artificial bottlenecks for example, can change the interaction strength between and within species, making it difficult to assess the resulting eco-evolutionary dynamics.

Thus in this work, I study the interactions between hosts and virus (and in some cases with additional predators) in chemostat systems (Figure 1). These are closed and sterile systems with a continuous in-flow of nutrients (necessary for algal growth), whereas the same volume is continuously removed. These chemostats have sampling ports that can be used to add the organisms to the system (algae, virus or rotifers) and from which samples can be taken for the assessment of several parameters of interest (e.g. population size). I use the chemostat set-up in combination with time-shift experiments (Gaba & Ebert 2009). Samples taken over the course of the experiments can be saved and stored, serving as a (living) fossil record. These fossil records can be used later to assess evolutionary changes by challenging organisms from different time-points to each other (time-shift experiments). Thus, using a combination of chemostats and time-shift experiments enables me to follow detailed ecological and evolutionary dynamics simultaneously, which is necessary to study eco-evolutionary dynamics.

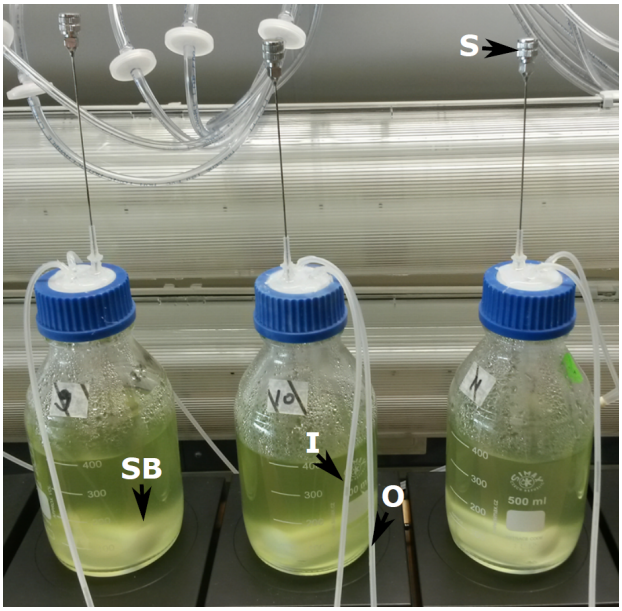

Figure 1 | Chemostats. Chemostats used in this work consist out of 500 ml glass bottles containing 400 ml of sterile medium. Fresh nutrients are delivered with a continuous inflow of new medium (I) and the same amount of volume is removed through a continuous outflow (O). Sterile fresh air is supplied continuously (I) over the surface of the medium. Samples can be taken using sampling ports (S) on top of the chemostat, ensuring the chemostat systems remain sterile. The cultures are mixed continuously with magnetic stirring bars (SB) and are placed in front of a light source (switched of for the purpose of the picture).

Research aims

Chapter one

In this chapter, I introduce the basic framework of eco-evolutionary dynamics, review most recent empirical and theoretical work in the research field and identify current gaps in our knowledge.

Chapter two

Eco-evolutionary dynamics are well established and many imperial examples illustrate the important link between ecology and evolution at one time-scale. However, eco-evolutionary dynamics have not been studied explicitly with antagonistic coevolving populations. In this chapter, I test for such interactions between ecology and evolution in a host-virus system. Furthermore, I aim to show the consequences of eco-evolutionary dynamics for antagonistic coevolution and use a mathematical model to test several predictions.

Chapter three

In this chapter I study the effects of increasing complexity (more biotic interactions) onto eco-evolutionary dynamics. Here, I extend previous host-virus system by adding a predator for the host. Doing so, I aim to evaluate cascading direct and indirect ecological and evolutionary consequences of increasing complexity and investigate their effects in an eco-evolutionary context.

Chapter four

In the last chapter, I investigate whether antagonistic coevolution between the algal host and virus result in parallel or divergent evolution between different replicate populations. I aim to link detailed information about eco-evolutionary dynamics in these populations to explain patterns of parallelism or divergence over different levels of biological organization (phenotype, genes, small variants and large structural variation).

CHAPTER ONE
Published review

frontiers in
ECOLOGY AND EVOLUTION

REVIEW ARTICLE
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Why rapid, adaptive evolution matters for community dynamics

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Evolution on contemporary timescales has recently been recognized as an important driver for ecological change. It is now well established that evolutionary change can affect the interactions between species within a few generations and that ecological interactions may influence the outcome of evolution in return. This tight link between ecology and evolution is of fundamental importance as it can determine the stability of populations and communities, as well as the generation and maintenance of diversity within and among populations. Although these eco-evolutionary dynamics and feedbacks have now been demonstrated many times, we are still far away from understanding how often they occur in nature. We summarize recent findings on eco-evolutionary dynamics, with a focus on consumer-resource interactions, from theory and empirical research. We identify gaps in our knowledge and suggest future research directions to provide a mechanistic understanding and predictive capability for community and ecosystem responses to environmental change.

Keywords: eco-evolutionary dynamics, eco-evolutionary feedback, rapid evolution, species interaction, consumer-resource

INTRODUCTION

Since the realization that evolutionary processes can be relatively fast, the traditional notion to consider evolutionary biology and ecology as two independent fields has changed dramatically. Although it is known that ecological change can drive evolutionary processes through natural selection, the interplay of ecology and evolution as a dynamic interaction in both directions and on contemporary timescales, has only recently been considered. In ecology, populations are usually considered to be genetically homogeneous and without variation in traits. Evolutionary processes are traditionally considered to be too slow to interact directly with ecological change. Initial theoretical models predicted the potential of rapid evolution to drive the entanglement of evolutionary and ecological dynamics (Abrams and Matsuda, 1997). Now, increasingly more studies underline the idea that populations can exhibit substantial genetic variation in traits that affect population dynamics (Tessier et al., 2000; Lankau and Strauss, 2007; Franks and Weis, 2008; Johnson, 2011; Yang et al., 2012; Novy et al., 2013), and population dynamics can alter the strength and direction of selection within a few generations (Yoshida et al., 2003; Becks et al., 2010, 2012). This confirms the paradigm that demographic and evolutionary changes are ultimately entangled (Ford, 1949; Pimentel, 1961, 1968).

The importance of this tight interaction between ecological and evolutionary change on one timescale has been emphasized in several studies and recent review articles (Fussmann et al., 2007; Pelletier et al., 2009), which has also been named the "newest synthesis" (Schoener, 2011). However, we currently cannot tell how changes in this interaction affect our ability to predict ecological and evolutionary trajectories. Are there ecological processes that are more likely to be affected by evolution within a few generations? And in return, are there ecological processes and species interactions that are more likely to promote rapid evolution? How widespread is the occurrence of a continuous feedback between ecological and evolutionary change? At present we simply do not know. We argue here that a key requirement to answer these questions is to account for higher complexity with more biotic interactions and different agents of selection. Integrating community ecology into the framework of eco-evolutionary dynamics and vice versa allows accounting for time-lagged and cascading effects across different trophic levels. We use simple consumer-resource systems here for explaining the conceptual framework of rapid evolution and eco-evolutionary dynamics before we discuss examples and consequences for more complex systems and dynamics.

CONCEPTUAL FRAMEWORK

We use the term rapid evolution to describe changes in heritable trait distribution or allele frequency within a population over a few generations (c.f. microevolution). This trait variation may arise from the emergence of novel genotypes, gene flow and genetic mixing. Our definition of rapid evolution also includes selection on standing genetic variation in populations. Many of the best examples of rapid evolution are indeed from populations with standing genetic variation, where populations can rapidly evolve by changing genotype frequencies (lineage sorting)

or by genetic mixing (Turcotte et al., 2011; Agrawal et al., 2012; Cameron et al., 2013). With this definition, we are less strict than those used by other authors. Thompson (1998) and Hairston et al. (2005) define rapid evolution as a process that simultaneously alters the ecological trajectory; however, this definition makes the strong assumption that changes of the ecological dynamics can be observed. Rapid evolutionary change can, however, also result in simply maintaining a *status quo* by sustained directional selection (Merilä et al., 2001).

Much of our mechanistic understanding of eco-evolutionary dynamics is based on predator-prey systems comprising microbial organisms. These enable multi-generational experiments in the laboratory that can be directly compared to predictions made by theoretical models. As an example of how rapid evolution and ecological dynamics are entangled, we compare trait and population dynamics of a model predator-prey system. Theoretical and empirical literature on the ecology of predator-prey interactions is vast and these systems are the best-studied examples for the tight link between ecological and evolutionary dynamics on one timescale (Abrams and Matsuda, 1997; Reznick et al., 2001; Yoshida et al., 2003; Becks et al., 2010, 2012). In classic ecological predator-prey systems, the entire process of death and birth is solely driven by the densities of the prey and predator, which result in standard ecological one-quarter lag predator-prey cycles (**Figure 1A**). However, when the prey population exhibits diversity—whether from *de novo* mutation, gene flow or standing genetic variation—within traits that affect its susceptibility to predation, the prey population can evolve rapidly in response. This rapid evolutionary response can have major effects on the ecological dynamics of the predator-prey system and result in a number of different types of dynamics, including steady state, chaos, or limit cycles. In these cases, it is not only the densities of predators and prey that drive the system dynamics, but also the changes in trait distributions that directly affect birth and death rates (**Figure 1B**). These eco-evolutionary dynamics, with a tight link between ecological and rapid evolutionary change, are often complex and interactions can go into both directions (**Table 1**): rapid evolution affecting ecological dynamics, or ecological change affecting rapid evolution.

In some cases, eco-evolutionary dynamics can result in a loop where ecological and evolutionary change continuously feedback into each other and produce for example almost out-of-phase predator-prey population cycles (**Figure 1C**). This means that there is a continuous change in the importance of predator and prey densities (ecology) and of the trait distributions (evolution) affecting birth and death of predator and prey. These eco-evolutionary feedbacks are a distinct subset of eco-evolutionary dynamics in that they specifically refer to reciprocally interacting ecological and evolutionary processes (Palkovacs and Post, 2008; Post and Palkovacs, 2009), rather than simply considering the effects of ecology on evolution, or less often, the effects of evolution on ecology. Eco-evolutionary feedbacks are characterized by fluctuating selection which leads to oscillating population densities as different traits are favored at different time points (**Figure 1C**). This maintains trait variation and can allow the diversity of organisms that bear these traits to persist. Thus, one of the most important consequences of eco-evolutionary feedbacks is that the alteration of population and community dynamics results in the maintenance of diversity.

In this review, we discuss examples that document how, together, rapid evolution and ecological change result in eco-evolutionary feedbacks or dynamics, and what implications these feedbacks have on communities. We summarize recent findings from field studies, experiments and theory with the aim to identify processes where the close interaction between ecological and evolutionary dynamics can, within a few generations, play a major role in determining the ecological and evolutionary trajectories. We focus on recent research involving consumer-resource interactions to identify important next steps that could help reveal the conditions under which the tight link between rapid evolutionary change and ecological dynamics matters for the stability and persistence of communities, as well as for the maintenance of diversity.

CONSUMER-RESOURCE INTERACTIONS

Eco-evolutionary feedbacks have been primarily investigated in predator-prey communities, since the strong selection exerted by predation drives evolution rapidly enough to enable synchrony of evolutionary and ecological dynamics (Abrams, 2000). Abrams and Matsuda (1997) used a model to show that when a prey species evolves a defended genotype at a cost of a lower growth rate, classic predator-prey dynamics exhibiting a typical quarter-phase lag (**Figure 1A**) are shifted toward longer cycles, where predator and total prey cycle out of phase (**Figure 1C**). These out-of-phase cycles are indicative of eco-evolutionary feedbacks and were first highlighted experimentally by Yoshida et al. (2003) using plankton communities comprising the alga *Chlorella vulgaris* and the rotifer *Brachionus calyciflorus*. The algal population consisted of several genotypes differing in their degree of edibility, with a trade-off of lower growth rate for increased defense (Yoshida et al., 2004). These dynamics have also been observed, but in more detail, in a community with *Chlamydomonas reinhardtii* and the same rotifer species (Jones et al., 2009; Becks et al., 2010, 2012). Key to this system is that the algal prey population consists of genetically variable individuals (Valiadi and Becks unpublished data) with a trade-off between defense against rotifer predation (by growing in colonies) and competitive ability for nutrients. Rapid evolution within the prey population, as a response to predation (i.e., changes in the frequencies of defended and undefended prey type), determines the dynamics of the predator-prey system and whether or not the polymorphism of defended and undefended prey types is maintained. These experiments confirmed the predictions of a mathematical model (**Figure 2**) where the prey (algae) evolves a defense when predation is intense but loses this defense (and gains competitive ability) when the predators (rotifers) are scarce and prey are abundant (**Figures 2B,D**). Rapid evolution of the prey results in sustained oscillations of the community and trait dynamics, as well as the maintenance of the initial trait diversity.

Eco-evolutionary feedbacks in predator-prey systems can give rise to a number of different types of dynamics depending on food web complexity, the efficiency, and cost of prey defense (Yoshida et al., 2007; Jones et al., 2009; Tien and Ellner, 2012), and the amount of functional variation initially present in the system

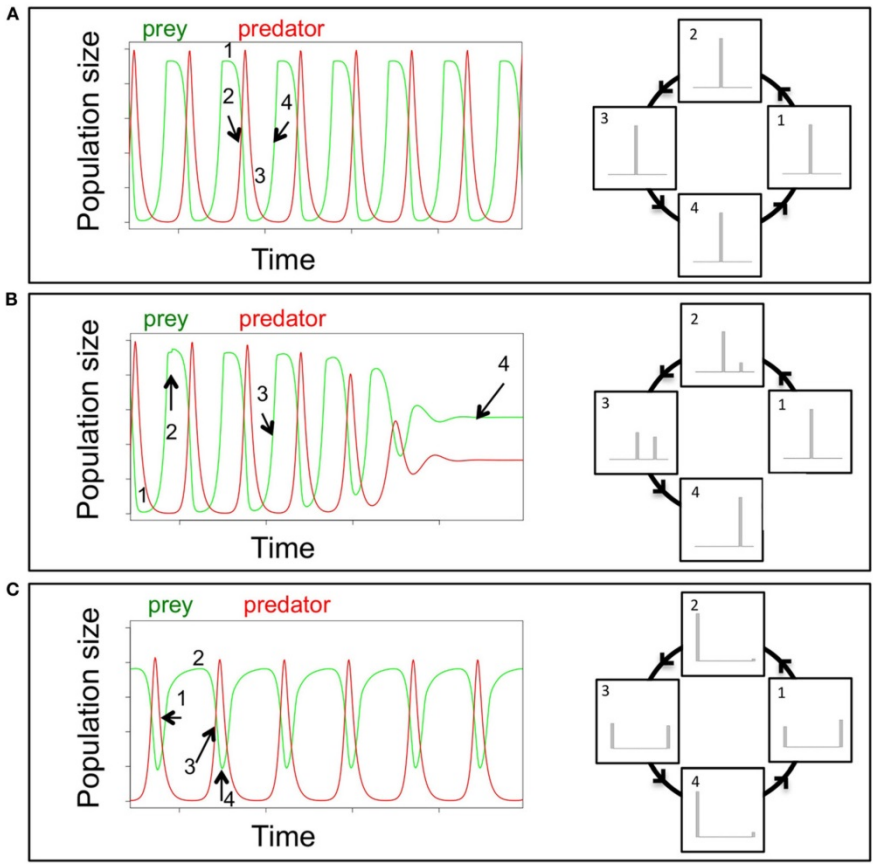

FIGURE 1 | Conceptual framework for the effects of rapid evolution on the quantitative dynamical behavior of a predator-prey system shown as population dynamics (left) and the corresponding trait distribution of a defense against the predator within the prey population (right). **(A)** For a cyclical predator-prey system without evolution, the predator and prey cycle with a phase shift of a quarter of a period. High prey densities (time point 1) lead to growth of the predator population, which simultaneously results in high death rates of the prey and decreasing prey densities (time point 2). As an outcome of low prey densities, the predator populations' growth rate becomes negative (time point 3) and the resulting low density of the predator allows the prey population to rapidly grow again, as long as the predator population stays low (time point 4). As the classical ecology case does not consider evolution and trait variation, the trait distribution does not change over time with changes in prey and predator densities (numbers in left and right columns are corresponding time points). **(B)** The introduction of trait variation in the prey population by the emergence of a new prey type that is defended against predation stabilizes the dynamics from cycles to steady state dynamics. Introduction of a new phenotype in the prey population, either through *de novo* evolution or gene flow that, for example, reduces consumption by the predator (i.e., a "defended" prey), could result in a change of the quantitative dynamics of the system. A newly introduced defended prey type has (time point 2), in the presence of the predator, a lower death rate compared to the undefended prey and its frequency increases over time through several predator-prey cycles (time point 3). Increasing defended prey results in less efficient predation as well as reduced growth and population sizes of the predator and the undefended prey goes extinct and the predator-prey dynamics switch to steady state dynamics (time point 4). Thus, the evolution in the prey population, shown as trait distribution in the right column of **Figure 1B**, has a direct effect on the ecological dynamics of predator and prey. **(C)** For the case where the defense level against predation is very efficient but comes at a cost of a low competitive ability, a full eco-evolutionary feedback can be observed. The growth rates of the two prey types then depend on the density of the prey, while the density of the predator and its ability to feed on the two different preys determines the preys' death rates. The relative impact of the two processes continuously changes, driven by the changes in prey and predator densities, which in turn are driven by changing frequencies of the two prey types. Here, low rotifer densities select for undefended prey (time point 2), which in return results in an increase in predator densities (time point 3). With high predator densities, the defended prey increases in frequency, driving the predator to low densities again (time point 4). As a result, the overall dynamics of the predator-prey system differs drastically from classical predator-prey dynamics: the system cycles, but cycles are much longer than classic consumer-resource cycles (**Figure 1A**) and almost out of phase. This represents a full eco-evolutionary feedback loop where evolutionary change (changes in the trait distribution, right column **Figure 1C**) affects the ecological dynamics (density of the predator), which in return drives the evolutionary change (the circle in the right column is closed) (Palkovacs and Post, 2008; Post and Palkovacs, 2009).

Table 1 | Sample of recent (2010–2014) studies on eco-evolutionary dynamics.

References	Level of organization	Type of study	Type of interaction/ecological driver	Organism(s)
ECOLOGY DRIVES RAPID EVOLUTION				
Agrawal et al., 2012	Community	Experiment (field manipulation)	Consumer-resource, plant-herbivore	Common evening primrose (*Oenothera biennis*), Insects
Burton et al., 2010	Community	Theory	Three-trait trade-off model, range expansion, biological invasion	Model species
Kelehear et al., 2012	Community	Experiment	Host-parasite, range expansion	Nematode lungworm (*Rhabdias pseudosphaerocephala*), Cane toad (*Rhinella marina*)
Swain, 2011	Population	Experiment (long-term study)	Over-harvesting, exploitation	Fish (e.g., Atlantic cod, *Gadus morhua*)
Thériault et al., 2011	Population	Theory	Eco-genetic modeling, fisheries-induced rapid evolution	Brook charr (*Salvelinus fontinalis*)
Turley et al., 2013	Community	Experiment (long-term, field manipulation)	Plant-herbivore	Sorrel plant (*Rumex acetosa*), Common rabbit (*Oryctolagus cuniculus*)
RAPID EVOLUTION DRIVES ECOLOGY				
Agrawal et al., 2013	Community	Field experiment	Plant-herbivore	Evening primrose (*Oenothera biennis*), Seed predator moth (*Mompha brevivittella*)
Bassar et al., 2010	Ecosystem	Experiment	Predator-prey and ecosystem structure and function	Trinidadian guppies (*Poecilia reticulata*)
Cameron et al., 2013	Population	Experiment	Rapid evolution driven by density-dependent competition	Soil mite (*Sancassania berlesei*)
Coulson et al., 2011	Population	Theory and Field data	Environmental change on life history and population dynamics	Wolf (*Canis lupus*)
Friman et al., 2014	Community	Experiment	Predator-prey and competition	Bacteria (*Pseudomonas fluorescens*), Protist (*Tetrahymena thermophile*)
Hairston et al., 2005; Ellner et al., 2011	Population, Community	Theory	Predator-prey, environmental change	Theoretical (based on Abrams and Matsuda), Medium ground finch (*Geospiza fortis*), Freshwater copepod (*Onychodiaptomus sanguineus*)
Terhorst et al., 2010	Community	Experiment	Predator-prey	Mosquito larvae (*Wyeomyia smithii*), Protozoa *(Colpoda* sp.)
Turcotte et al., 2011	Population	Experiment	Rapid evolution on population growth	Green peach aphid (*Myzus persicae*)
Walsh et al., 2012	Ecosystem	Experiment	Predator-prey and ecosystem function	Zooplankton *(Daphnia dentifera),* Phytoplankton community
ECO-EVOLUTIONARY FEEDBACKS				
Becks et al., 2010, 2012	Community	Theory and Experiment	Predator-prey	Chlorophyte alga (*Chlamydomonas reinhardtii*), Rotifer (*Brachionus calyciflorus*)
Ellner and Becks, 2011	Community	Theory	Predator-prey	Based on chlorophyte alga (*Chlamydomonas reinhardtii*), Rotifer (*Brachionus calyciflorus*)
Farkas et al., 2013	Community	Field observations and Theory	Eco-evolutionary feedback in consumer-resource community in spatial context	Stick insect (*Timema cristinae*), Plants *(Adenostoma fasciculatum, Ceanothus spinosus*)
Hanski, 2011	Ecosystem	Field observations	Eco-evolutionary dynamics in metapopulations	Glanville fritillary butterfly (*Melitaea cinxia*)
Sanchez and Gore, 2013	Population	Theory and Experiment	Eco-evolutionary feedback between allele frequency of cooperative gene and population size	Microbial yeast, *Saccharomyces cerevisiae*
Turcotte et al., 2011	Population	Experiment	Eco-evolutionary feedback loop between evolution and population density	Green peach aphid (*Myzus persicae*)
Yamamichi et al., 2011	Community	Theory	Predator-prey	Inspired by chlorophyte alga (*Scenedesmus* and *Desmodesmus*), Rotifers

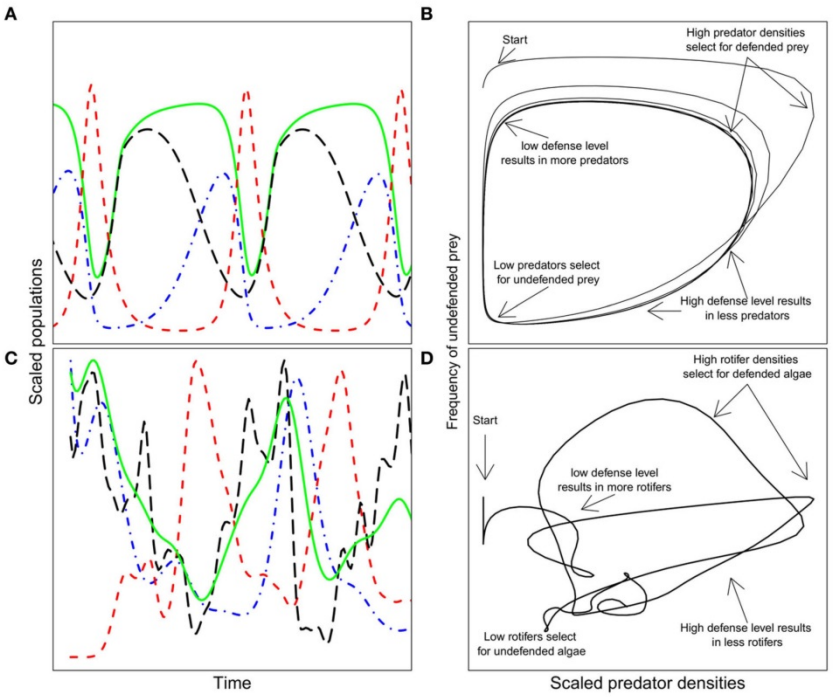

FIGURE 2 | Details of eco-evolutionary feedback dynamics in a predator-prey system from model predictions and experiments (after Becks et al., 2010, 2012). (A) Scaled population sizes of the predator, the total prey, defended, and undefended prey; total prey: solid green line, red dashed line: predator, blue long-dashed line: undefended prey, black dash-dotted line: defended prey. **(B)** Frequency of the undefended prey as a function of predator densities and the major ecological and evolutionary drivers at different time points. The inevitable lag in response to selection and changes in population densities create then the long out of phase cycles. **(C,D)** are experimental data from a rotifer-algae system. For visualization reasons, only the fitted lines are shown.

(Becks et al., 2010). Yoshida et al. (2007) used algae-rotifer and bacteria-phage communities to demonstrate how cryptic cycles can occur when there is rapid evolution in prey defense traits, but the cost of this defense is not high enough to force significant competition among prey genotypes. Instead of observing population cycles in both the predator and prey, only the predator cycled while the algal population appeared to remain constant. This was because the rapid evolutionary cycling of prey genotypes within the population allowed for essentially constant total prey number, even while the predator population fluctuated. Again, this allows trait variation in the prey population to be maintained. Conversely, low levels of prey defense result in steady state dynamics instead of an eco-evolutionary feedback, because in this case, effective defense drives the predator to low levels, but not low enough to allow coexistence of the undefended prey type (Jones et al., 2009). As a consequence of the steady-state dynamics between the predator and prey, prey diversity is not maintained.

Studies of consumer-resource systems considering rapid evolution in both the predator and prey have revealed more complex evolutionary and population dynamics than those only considering rapid evolution in the prey populations (Jones et al., 2009; Tirok et al., 2011). A most striking outcome is that time periods of cycling predator and prey alternate with time periods of intermittency. Important to this dynamic is that during the latter time periods, trait variation is maintained and the next burst of cycles is not the result of new mutations, but rather of temporal changes in the dominance of different prey and predator types. Similar complex eco-evolutionary feedbacks were observed in a predator-prey food chain that was extended to include an intermediate predator (Ellner and Becks, 2011). Allowing for evolution of a costly defense against neither, one or both predators, the authors found that the increased number of interactions did not mask, but rather accentuated the eco-evolutionary dynamics. Long out-of-phase cycles and even chaotic dynamics were observed; with both resulting in the maintenance of the initial diversity in prey defense traits. Many studies have discovered rapid, adaptive evolution in prey populations exposed to novel or increased predation but they usually do not follow the consequences for predator-prey dynamics or potential for a full eco-evolutionary feedback. For example, a protozoan prey evolved a defense against predation by mosquito larvae by growing faster but to a smaller cell size, resulting in a change in the predator effect size within a few generations

(Terhorst et al., 2010). In this study, the reduced effect size of the predator does consequently change the predator's grazing rate and thus the strength of selection. However, whether this might change the direction of selection (from prey being defended to being competitive) and consequently change the evolutionary trajectory was not tested.

These examples emphasize the significance of rapid evolution and eco-evolutionary feedbacks in consumer-resource systems, within the context of community alterations, like invasions and expansions (Facon et al., 2006; Kinnison and Hairston, 2007; Burton et al., 2010; Jones and Gomulkiewicz, 2012). They also illustrate that the relative importance of variation within and among different traits might differ over time depending on the ecological dynamics. It is this dynamic, reciprocal, and often time-lagged cascading interaction between the ecological and evolutionary processes that makes its understanding so challenging.

NATURAL COMMUNITIES

The great challenge now facing evolutionary ecologists is to apply what we have shown by models and laboratory experiments to the natural world. This is far from trivial in complex ecosystems, especially considering that the same dynamics are often a result of very different processes. The main findings from the rotifer-algae chemostat systems, i.e., that genetic variation can alter ecological dynamics, have now been corroborated by field and mesocosm studies using the Trinidadian guppy *Poecilia reticulata*. In this model system, varying levels of predation underlie the rapid evolution of morphological (e.g., body size), life history (e.g., age at sexual maturity), and behavioral (e.g., anti-predator) traits in the prey (Magurran et al., 1992; Reznick et al., 1996, 1997, 2001; Kemp et al., 2009). In addition to linking the rapid evolutionary change with the varying levels of predation, it has been shown that the differentially adapted guppies make different use of resources, which can have cascading effects throughout the entire ecosystem. For example, Bassar et al. (Bassar et al., 2010; Ellner et al., 2011) found that guppy evolution indirectly affects decomposition rates and levels of benthic organic matter, while other ecosystem processes such as gross primary production and total nitrogen flux were only affected by ecological changes (i.e., intraspecific density). Similar results were obtained in another mesocosm study using the stickleback *Gasterosteus aculeatus*, to test for divergent effects on ecosystem function by fish with differentially-adapted foraging strategies (Harmon et al., 2009). Diversification into specialized benthic and limnetic feeders had profound effects on algae biomass and productivity by creating a positive feedback between dissolved organic carbon and algal productivity. Both studies showed the consequences of rapid evolution for several, often cascading, ecological and ecosystem processes. However, they do not allow for making further predictions on how ecological and evolutionary dynamics might change after the initial/short-term effect (in both cases, the mesocosm experiments lasted less than one fish generation). It is this long-term effect and the potential feedback that should be of our utmost interest for future studies as it can have far-reaching consequences for community dynamics and genetic diversity.

A series of studies comparing lakes with either landlocked or anadromous alewife (*Alosa pseudoharengus*) populations show how the ecological and evolutionary dynamics of communities and ecosystems might be dramatically altered by an initial ecological change. Landlocked alewives exert a constantly high grazing pressure on the zooplankton population throughout the year. Over time, the zooplankton population in these lakes evolved to be smaller and grow slower (Palkovacs and Post, 2008; Post et al., 2008; Walsh and Post, 2011). The changes in zooplankton had further evolutionary consequences for the alewives as they are suggested to have rapidly evolved smaller gape width and gill-raker spacing (Palkovacs et al., 2008; Palkovacs and Post, 2009). At the same time, these modifications in zooplankton and alewife populations increased phytoplankton biomass and lowered net primary production (Post et al., 2008; Walsh et al., 2012). A lower net primary production could have a large effect on ecosystem structure and energy flow; and could also, in theory, lead to a full eco-evolutionary feedback on the zooplankton dynamics. Conversely, anadromous alewife populations do not exert a constantly high grazing pressure throughout the year and thus, have not undergone the same evolutionary and ecological changes as the landlocked alewife populations.

The experimental systems discussed here reveal the difficulties and limitations in performing these types of studies in natural populations, where interactions may be masked, amplified or just be difficult to disentangle from a range of other ecosystem processes (see also Strauss, 2014). In addition, the above-described cascading effects across different trophic levels will need several generations despite rapid evolution. At the same time, these studies also demonstrate the pressing need to understand these often simultaneous and intertwined ecological and evolutionary dynamics. For example, primary production in lakes is of high interest, i.e., its importance for carbon sequestration, inland fisheries, community shifts to favor harmful algal blooms, and the sustainability of drinking water reservoirs.

SO WHAT?

We propose that to fully understand eco-evolutionary dynamics and feedbacks in communities, it is essential to quantify the ecological and evolutionary dynamics, including the heritability of the traits involved and their effects on species interactions, e.g., level of defense, competitive ability or susceptibility, and resistance. Most studies, so far, have focused on single or two-species systems and typically one evolving trait. Studies including more interacting species (Ellner and Becks, 2011) or evolving traits (Burton et al., 2010) illustrate that the potential for eco-evolutionary feedbacks does not diminish when including more complexity (Urban et al., 2008; Tirok and Gaedke, 2010; Tirok et al., 2011). There is also a need to identify traits that are most likely to evolve rapidly and affect ecological interactions (Geber and Griffen, 2003; Thompson, 2009). In addition, little is known about how the rate of evolutionary change depends on the strength of selection and how selection strength alters the potential and shape of eco-evolutionary dynamics. This might be particularly interesting for the conditions leading to a feedback. With increasing number and types of interactions, the direction and strength of selection will change and the resulting dynamics

as diversification and speciation. Studies demonstrating rapid evolution are mainly from populations exposed to strong and steady directional selection, e.g., after introducing or removing a predator (e.g., Olsen et al., 2004; Araki and Schmid, 2010), or from systems with fluctuating selection, where species interactions frequently alter the direction of selection (e.g., Duffy and Sivars-Becker, 2007; Palkovacs et al., 2009; Becks et al., 2010, 2012; Turcotte et al., 2011). Spatial dynamics including range expansion and invasion, as well as local adaptation of communities, have also proven to have a large potential for eco-evolutionary dynamics and feedbacks (Reznick et al., 2001; Hanski and Saccheri, 2006; Kerr et al., 2006; Dlugosch and Parker, 2008; Bassar et al., 2010; Kelehear et al., 2012).

Another conclusion that we can draw is that, depending on the assumptions and conditions, every outcome for community dynamics and the direction of adaptive evolution seems to be possible. Integrating multiple interactions into eco-evolutionary feedbacks can result in multiple possible outcomes. This poses a huge challenge for the experimentalist and thus, likely requires an approach with a strong theoretical background. Some theory-based studies predict that rapid evolution does not always play a role (Jones and Gomulkiewicz, 2012) or only plays a role under certain assumptions (e.g., in the form of a trade-off curve Jones and Ellner, 2007; Jones et al., 2009). Therefore, more modeling and theory-driven studies will be necessary to help identify those conditions under which rapid evolution drives further ecological and evolutionary change, in addition to identifying which interactions, besides consumer-resource, are most relevant. These types of studies, including experimental ones, will also be important for better understanding how indirect species interactions facilitate or inhibit cascading effects of eco-evolutionary dynamics and feedbacks. Currently, there is a poor understanding of the consequences of eco-evolutionary dynamics for indirect ecological effects, such as apparent competition or trophic cascades (but see Bassar et al., 2010; Walsh et al., 2012). It is also important to know whether eco-evolutionary feedbacks in nature withhold in the presence of external ecological pressures. Additional experimental studies analysing eco-evolutionary feedbacks and eco-evolutionary dynamics, in general, are needed to critically assess their role in maintaining diversity within and among populations. However, a key requirement for this is that we achieve a better mechanistic understanding of the effects of evolution on ecology. It is, essential for predicting how ecological and evolutionary properties, e.g., stability and dynamics of populations and communities, as well as intra-and interspecific diversity, are maintained. A strong understanding of these processes in nature is imperative since eco-evolutionary feedbacks may be of essence to the maintenance of biodiversity and the potential for further adaptive change.

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CHAPTER TWO
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LETTER

Eco-evolutionary dynamics in a coevolving host–virus system

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Abstract

Eco-evolutionary dynamics have been shown to be important for understanding population and community stability and their adaptive potential. However, coevolution in the framework of eco-evolutionary theory has not been addressed directly. Combining experiments with an algal host and its viral parasite, and mathematical model analyses we show eco-evolutionary dynamics in antagonistic coevolving populations. The interaction between antagonists initially resulted in arms race dynamics (ARD) with selective sweeps, causing oscillating host–virus population dynamics. However, ARD ended and populations stabilised after the evolution of a general resistant host, whereas a trade-off between host resistance and growth then maintained host diversity over time (trade-off driven dynamics). Most importantly, our study shows that the interaction between ecology and evolution had important consequences for the predictability of the mode and tempo of adaptive change and for the stability and adaptive potential of populations.

Keywords

Algae-virus, arms race, coevolution, eco-evolutionary dynamics, fluctuating selection, host–virus, infectivity, resistance, trade-off.

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INTRODUCTION

Theoretical and empirical studies have shown that adaptive variation in ecological relevant traits can lead to evolutionary changes sufficiently rapid to alter the temporal dynamics of populations which in return can alter the evolutionary dynamics (Thompson 1998; Yoshida *et al.* 2003; Duffy & Sivars-Becker 2007; Post & Palkovacs 2009; Ellner *et al.* 2011; Becks *et al.* 2012; Hiltunen *et al.* 2015). The simultaneous changes in ecological and evolutionary properties (eco-evolutionary dynamics) have important consequences for population and community dynamics, ecosystem structure and functioning, and the generation and maintenance of genetic variation and stability (Pelletier *et al.* 2009; Becks *et al.* 2010; Schoener 2011; Koch *et al.* 2014). Despite the large interest in eco-evolutionary dynamics, the entanglement of ecology and evolution has not explicitly been tested with antagonistic coevolving populations, although theoretical predictions and indirect empirical evidence exists (Thompson 1998, 2005; Bohannan & Lenski 2000; Pelletier *et al.* 2009; Hiltunen & Becks 2014).

Antagonistic coevolution has been shown to drive trait and genetic diversity within host and parasite populations (Brockhurst *et al.* 2004, 2014; Best *et al.* 2009; Koskella & Brockhurst 2014). As reciprocal evolutionary changes in antagonistic coevolving populations can be relatively fast and change the ecological interactions simultaneously (Thompson 1998; Hiltunen & Becks 2014), antagonistic coevolution can generate continuous interactions between ecological and evolutionary processes, indicating an important role for eco-evolutionary dynamics. Theoretical models suggest that coevolution needs to be studied in the context of ecology to fully understand whether and how diversity is generated and maintained (Best *et al.* 2009, 2010; Boots *et al.* 2009, 2014). Although the theoretical predictions on coevolutionary dynamics have been tested in several systems [e.g. see examples in Brockhurst & Koskella (2013)], an interaction with ecological dynamics has typically not been shown beyond changes in species interaction strength. There are only a few empirical tests for how the interaction between ecology and evolution can affect coevolution and trait diversity over time, and how these changes in return affect the ecological dynamics. Previous studies discussed for example how smaller population sizes lower the supply of mutations or strength of selection and thus alter coevolution of bacteria and phage (Gómez & Buckling 2011; Friman & Buckling 2013). Considering the short generation times of only a few hours of these organisms and their strong species interactions, sampling intervals spanning several days reduces, however, the power to link the ecological and evolutionary changes. As an example, hosts and their consumer populations can decrease to very low population sizes and increase again within just a few hours. Bottlenecks and their impact on the coevolutionary dynamics might be missed or largely underestimated when sampling with too small time intervals.

Generally, two distinct patterns of host–parasite coevolution are commonly observed during experimental evolution with microbes called arms race dynamics (ARD) or fluctuating selection dynamics (FSD) (Gandon *et al.* 2008; Hall *et al.* 2011; Betts *et al.* 2014; Brockhurst *et al.* 2014; Buckling and Rainey 2002). When hosts (or virus) evolve, increasingly broader resistance (infectivity) ranges over time, coevolutionary dynamics are characterised by an arms race between host and virus (ARD) resulting from directional selection imposed by each antagonist. In contrast, different host (virus) genotypes can alternate in frequency over time, tracking the rarest (most common) genotype of the antagonist. In this case, there is no directional change in resistance (infectivity) range as evolution is driven by frequency dependent selection (fluctuating selection dynamics: FSD). These coevolutionary dynamics

– pure ARD and FSD – can be seen as two extremes of a continuum (Gandon *et al.* 2008; Hall *et al.* 2011). It is not expected that coevolution is consistently driven by one type of dynamics only. For example, the change from one coevolutionary dynamic to another has been observed in prokaryotic experimental systems with multiple coevolutionary cycles and was typically attributed to increasing fitness costs associated with ARD (Brockhurst *et al.* 2004; Hall *et al.* 2011; Koskella & Brockhurst 2014) or to resource availability (Gómez & Buckling 2011; Lopez Pascua *et al.* 2014). Thus, it is likely that the type of coevolutionary dynamics is context dependent. As a consequence, any ecological property or process – such as changes in population size – will be important in these coevolving systems (Brockhurst *et al.* 2004, 2006; Lopez-Pascua & Buckling 2008; Koskella & Brockhurst 2014), as they can alter, for example, associated fitness costs or strength of infection, making it necessary to study antagonistic coevolution in the context of ecology.

From previous observations and theoretical work, there are at least four clear predictions on how coevolution and populations dynamics are linked in antagonistic coevolving species: (1) rapid changes in population sizes of host and parasite are a function of exploitation efficiency of the parasite, that is, the evolution of host resistance and parasite infectivity, (2) density changes affect the rate of infections, which in turn is an important component for the strength of selection, and that these links between ecology and evolution are altered over time by (3) associated fitness costs of resistance and infectivity and (4) population densities, as they determine the supply of new adaptive mutations within populations and affect genetic drift. There is empirical evidence for some of these predictions (e.g. Lenski & Levin 1985; Poullain *et al.* 2008; Gómez & Buckling 2011; Hall *et al.* 2011; Friman & Buckling 2013), but these predictions have not been tested comprehensively within one study, only supported indirectly and as outlined above, not on a sufficient timescale.

In order to establish a comprehensive understanding of how ecological and evolutionary processes together determine the trajectories and outcome of antagonistic coevolving species, we established a novel experimental eukaryotic host–virus system. We used a host–virus system with the asexual reproducing alga *Chlorella variabilis* and a lytic dsDNA virus of the *phycodnaviridae* family (Chlorovirus strain PBCV-1) in continuous cultures. Three replicated continuous cultures (chemostats) of isogenic algae were inoculated with an isogenic strain of the virus, whereas three chemostats remained without virus and served as controls. Algal and virus densities were assessed daily over a period of 3 months and additional time-shift experiments (Gaba & Ebert 2009) allowed us to follow coevolutionary changes in algal host and virus. Individual growth rate assessments of all algal hosts used for the time-shift experiment provided insights into fitness related costs associated with the evolutionary changes. We further explored the ecological and evolutionary dynamics and the underlying mechanisms comparing results from a mathematical model and the chemostat experiments.

Overall, our results show the tight link between coevolutionary changes and ecological population dynamics confirming the outlined predictions. Our study is the first to comprehensively demonstrate how the increase of resistance range coincides with an increase in growth costs, how ARD switch to trade-off driven dynamics (TDD) due to evolutionary constraints in the virus, how the types of coevolution corresponded to different population dynamics, and how the costly resistance of the host stabilised host and virus population dynamics while less resistant and general resistant hosts cycled over time.

MATERIALS AND METHODS

Chemostat experiments

Experiments were performed in continuous flow-through systems (chemostats) with a modified version of bold's basal medium. One isolated algal clone was used to start all six chemostats in order to minimise the initial genetic variability. Three out of six chemostats were inoculated with purified and concentrated virus at day 12. Virus (Brussaard 2004) and alga densities were counted daily. Samples of virus and alga populations were stored every second day by plating algae on BBM agar plates and storing virus at 4 °C (Van Etten *et al.* 1983) after filtering (0.45 μm cellulose syringe filter; Supporting Information).

Time-shift experiments

To examine the evolution of resistance and infectivity of algae and virus, eleven time-points (Grey vertical lines: Fig. 1) per chemostat were selected. For each time-point, ten individual host clones were randomly isolated from the agar plates and re-grown in batch cultures (11 time-points × 10 clones per time-point = total of 110 clones per replicated chemostat). Each host clone was exposed to each virus population separately; to the virus population from the same time-point from which the host clones were isolated, to each virus population from time-points from their relative past and to each virus population from time-points from their relative future (110 host clones × 11 time-points = 1210 combinations per chemostat). All algal clones were individually assayed as resistant or susceptible to a particular virus population by comparing growth rates of alga clones exposed to virus, to growth rates of the same alga clone without the virus. For each alga-virus combination, algae and virus were diluted to equal densities resulting in MOI of 0.01 particles/algal cell. Four technical replicates per combination were incubated in 96 well plates and maintained in continuous light. Growth rates were calculated based on ODs (Tecan, Infinite M200PRO, 680 Männedorf, Switzerland) measured at 0 hours and after 72 h. To assess whether the algal clones were resistant or susceptible to a particular virus population, we compared the mean growth rate plus 2 standard deviations of the four technical replicates to the mean growth rate minus 2 standard deviations of the control (growth without virus).

Data analysis

All data analyses were performed in Rstudio (Rstudio 2014) and R (RCoreTeam 2014) using the lme4 (Bates *et al.* 2014)

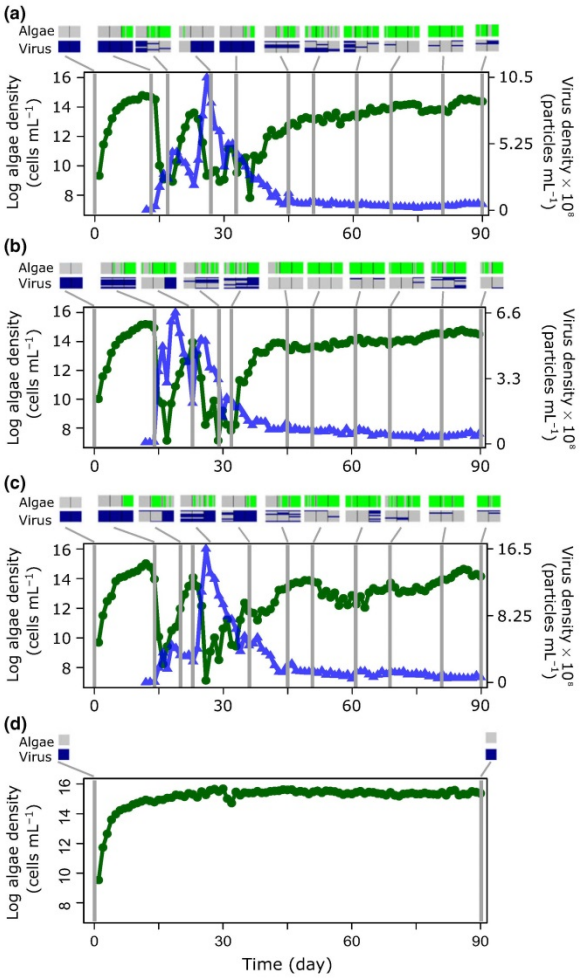

Figure 1 Coevolutionary and population dynamics of algae-virus (a–c) and algae chemostats (d). Green (dots): algal densities (natural logarithm); blue (triangles): virus densities. Grey vertical lines indicate days of time-shift experiments. Colour coded squares above grey lines show alga evolution and virus evolution. The algae squares represent susceptibility assays of algae from one time-point in the past (first square), contemporary time-point (second square) and one time-point in the future (third square) to the contemporary virus population. Similarly, virus squares represent infectivity essays of virus from one time-point in the past (first square), the contemporary time-point (second square) and one time-point in the future (third square) to contemporary algae. Algae: grey = susceptible to virus; green = resistant to virus. Virus: grey = unable to infect algae; blue = able to infect algae. Ten algal clones per time-point were tested against the whole virus population per time-point.

and multcomp (Hothorn *et al.* 2008) packages. Densities of host populations (last day) were compared between alga-virus and control chemostats using student's t test (unpaired and equal variance) after confirming equal variances between samples (F test to compare variances: $F_{2,2} = 5.852$, $P = 0.29$). Host resistance ranges were calculated for each individual host clone by calculating to how many virus populations (from their relative past, present and future) a host clone was resistant. As each host clone was exposed separately to each virus population used for the time-shift experiment (11 in total), the maximum resistance range is 11 (general resistant host). Thus, a general resistant host is resistant to all virus populations (from all time-points) from their chemostat. Virus infectivity ranges were calculated as how many host clones out of 110 clones (10 clones per time-point × 11 time-points per replicate) could be infected by a particular virus population. Average values were normalised to maximum infectivity.

We divided resistance and infectivity data from the time shift experiment into two periods: until a general resistant host was first observed (Fig. 1a: days 13–45, Fig. 1b: 14–32, Fig. 1c: 14–51; ARD in Fig. S1) and all later time-points (Fig. 1a: days: 51–90, Fig. 1b: 45–90, Fig. 1c: 61–90; TDD, see below; Fig. S1). We calculated for every host clone the proportion of virus populations from their relative past, present and future (virus time-shift) the clone was resistant to. The virus populations used to calculate these proportions were restricted to the same period from which the host was isolated (ARD or TDD period; Fig. S1). If coevolution was driven by ARD, we expected that hosts are highly resistant to all virus populations from their relative past (within the period from which the host was isolated; Fig. S1), and not resistant to all virus populations from their relative future (within the period from which the host was isolated; Fig. S1). As such, virus time-shift should be significant for host resistance and resistance should be significantly different between future (low resistance) and past (high resistance) virus time-shifts. To test this, we used a generalised linear model (GLM, quasi-binomial errors) with resistance proportions per algal clone (as response) across virus time-shift and compared this model to a null-model. We performed the same analysis to test whether host time-shift was significant for virus infectivity. Resistance between virus time-shift was further compared using multiple comparisons of means with Tukey contrasts. Looking for selective sweeps, hosts were assigned to distinct resistance types based on unique resistance-profiles (Fig. S1) during the ARD period with one time-point extra (to be able to track sweep of general resistant hosts) and time-point zero left out (as the host and virus were not yet exposed in the chemostats to each other at time zero). Rates of coevolution were calculated from slopes for the proportion of hosts resistant to virus from one time point in the past, contemporary and in the future (Brockhurst *et al.* 2003). We used mixed effect models with MOI (proxy for force of infection) and type of dynamic (ARD or TDD) as fixed, and replicate as random effect to test for a correlation between rates of coevolution and infection strength.

Host per capita growth rates were obtained from growth rates of individual host clones growing without the virus. We used linear mixed models (LMM) to test for a correlation between per capita growth rates and host resistance range (resistance range as fixed effect and replicated chemostat as random effect). We tested for a correlation between host population growth rates (obtained from population dynamics) and proportion of resistant host clones (resistant to contemporary virus) using LMM (proportion of resistant host clones as fixed effect and replicated chemostat as random effect). A

selection coefficient was estimated for each time-point used in the time-shift experiment by: $s_p = 1 - [\text{growth of algae in the presence of the contemporary virus/growth of algae}]$.

Mathematical model

We modeled both the population dynamics and coevolution of algae and virus with a fully dynamical eco-evolutionary model using a modified gene-for-gene infection mechanism (Forde *et al.* 2008). The modified gene-for-gene interaction implies that virus type P_i could infect host type B_j if and only if $i \geq j$. We assumed N host types and $N-1$ virus types, implying that host type B_N is generally resistant (Fig. S2). We modeled the coevolutionary interactions of algae $B = (B_1, \ldots, B_N)$ and virus $P = (P_1, \ldots, P_{N\text{-}1})$ in a chemostat environment with continuous inflow of resources and outflow. Host resistance was costly, i.e. host growth rate declined with increasing resistance range. Host and virus evolved by mutations that altered resistance range and host range. We assumed that evolution progressed step-wise, i.e. B_i could mutate into B_{i+1} or reverse to B_{i-1}. For model detailed description, see Supporting Information.

RESULTS

Population dynamics

We observed two distinct patterns in the population dynamics of host and virus; host and virus populations oscillated for the first ~ 45 days, followed by a more stable period with slowly increasing host populations and low virus densities (Figs 1 and 2). In the experiments, cycle amplitudes decreased very rapidly during the first period and the second host maximum (~ day 32) was not observed in all replicates (Fig. 1). Model results also showed oscillations initially but oscillations were not damped as in the experiments (Fig. 2). The control chemostats with only algae showed stable densities (without oscillations) after initial increase to high densities (Fig. 1d). However, host densities in the algae-virus chemostats during the stable period were well below the stable algal densities observed in control chemostats (Fig. 1, Fig. S3; independent t test: $t = 4.95$, d.f. = 4, $P = 0.0078$).

Coevolutionary dynamics

Using time-shift experiments, we tested whether and when hosts evolved resistance to the virus, and whether and when the virus evolved counter adaptations in return. An infection matrix summarising the time-shift data (Fig. S1) shows that susceptible host clones were replaced by resistant host clones at later time-points when tested against the same virus population (black arrows, Fig. S1). Similarly virus populations that could not infect the host were replaced at later time-points by virus populations that were able to infect previously resistant hosts (red arrows, Fig. S1). Thus, we found that algae and virus populations coevolved rapidly and observed 2–3 cycles of hosts evolving resistance (black arrows, Fig. S1), and 1–2 cycles of virus evolving counter-adaptations to infect previously resistant hosts (red arrows, Fig. S1). As experiments were started isogenically, resistance and infectivity evolved *de novo*. Coevolution resulted in an initial rapid increase in virus infectivity and host resistance ranges (Fig. 3, Fig. S4). Host resistance range reached its maximum when a general resistant host evolved around days 32–51 (Fig. 3, arrows). Generalist hosts could not be infected by any virus population from any time-point, suggesting that the virus was evolutionary constrained and unable to overcome the general resistance mechanism of the host (virus infectivity did not reach its maximum in any replicate; Fig. S4). Importantly, this constraint was not related to low encounter rates, as MOI values (multiplicity of infection) remained high (Fig. S5). Algae isolated from the end point of control chemostats did not evolve any resistance against the ancestral virus (Fig. 1d), confirming that evolution of resistance resulted solely from the algae-virus interactions.

The initial coevolutionary dynamics were consistent with ARD, i.e., until the time-point when the generalist resistant host evolved. All host clones (for ARD-period; Fig. S1) from past time-points relative to the virus were highly susceptible to that virus population (Fig. 4a, Figs S6a, S7), but all host clones from future time-points relative to virus were highly resistant to that virus population. A generalised linear model showed that host time-point (past, contemporary, future) was significant for host resistance during the ARD period (GLM, $F_{2,36}$=25.649, $P = 1.19e^{-7}$) and resistance was significantly different between past, contemporary and future time-points (Tukey mcp; past-future: $P < 0.001$, contemporary-future: $P = 0.003$, past-contemporary: $P = 0.0014$). Likewise, all virus populations (for ARD-period) from past time-points relative to the host population had low infection success, but virus populations from future time-points were highly infective (Fig. 4c, Figs S6b, S8; GLM, $F_{2,36} = 17.238$, $P = 5.61e^{-6}$, Tukey mcp; past-future: $P < 0.001$, contemporary-future: $P = 0.017$, past-contemporary: $P = 0.13$). These patterns were consistent with ARD, where hosts (virus) evolve greater resistance (infectivity). Moreover, hosts that acquired resistance to a particular virus type stayed resistant to all previous viruses, whereas all virus populations were able to infect previous susceptible algal types (Fig. S1). Thus, ARD resulted from directional selection for increasing host resistance and virus infectivity range. ARD with directional selection typically result in selective sweeps (Brockhurst *et al.* 2014). Our data do indeed show consecutive appearance of distinct resistant host types followed by rapid increases to high frequency or temporal fixation (Fig. 5).

The coevolutionary dynamics changed after a general resistant host emerged; ARD stopped and host (virus) time-point was not significant for resistance (infectivity) (Fig. 4b,d, Figs. S7, S8, GLM, $F_{2,36} = 1.82$, $P = 0.18$; $F_{2,36} = 1.12$, $P = 0.89$). Furthermore, we found that the generalist host did not go to fixation but that less resistant host types (resistant to different virus types and to a different number of virus types) coexisted with the generalist (Fig. 3). A significant cost was associated with host resistance in terms of reduced per capita growth rates (Fig. 6a, Fig. S9). Growth rates decreased with increasing host resistance range (LME: $\chi^2 = 93.90$, d.f. = 1, $P < 2.2e^{-16}$), showing that costs accumulated with evolving resistance to increasingly more virus types. Consequently, the general resistant host had the lowest per capita growth rate.

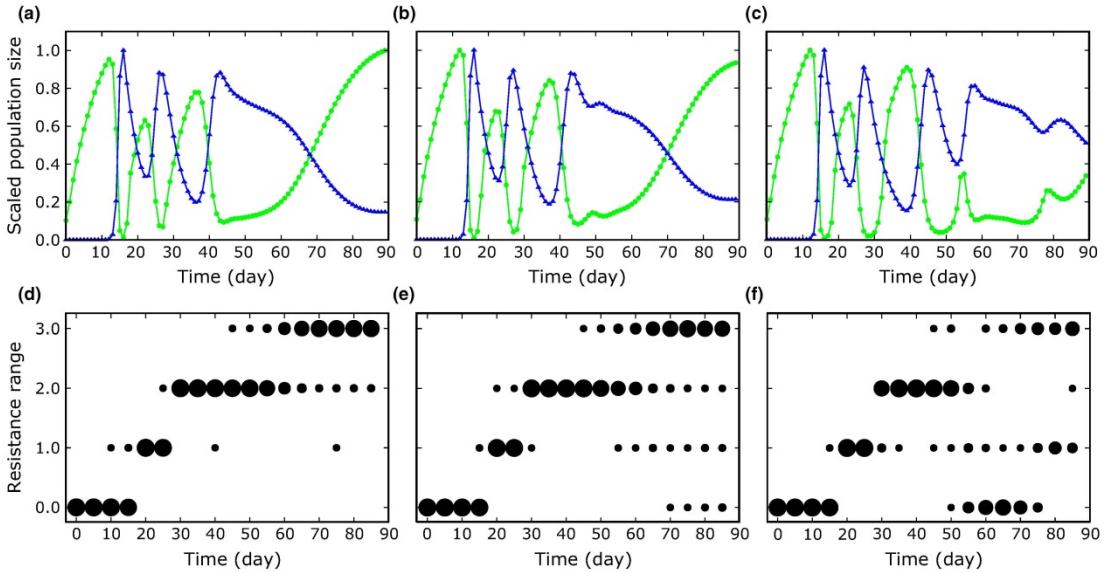

Figure 2 Population dynamics (a–c) and host resistance range (d–f) resulting from models with different host trade-off values. (a–c) population densities are scaled to maximum density of algae or virus. Green (dots): algal densities; blue (triangles): virus densities. (d–f) Host resistance range was calculated as the number of virus types to which an algal clone is resistant. Size of the dots correspond to the number of host clones (1–10) with a certain resistance range (10 random clones per time-point were sampled from the populations). (a, d) Model results without trade-off; (b, e) model results with experimentally observed trade-off; (c, f) model results for strong trade-off (see Material and Methods and Supporting Information).

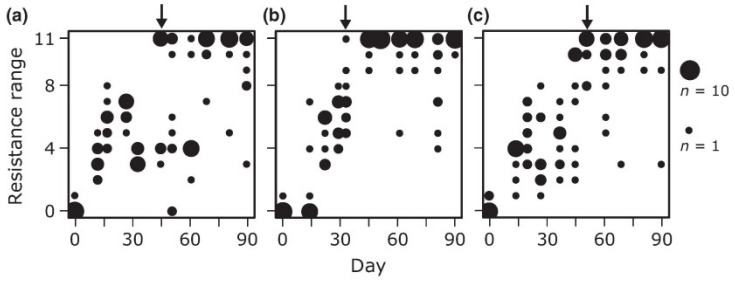

Figure 3 Evolution of host resistance range in chemostat experiments. Resistance range calculated as number of virus populations (from all time-points) to which an algal clone is resistant. Host resistance range increases over time from no resistance (0) to a general resistant type (=11; resistant to all virus populations; first occurrence of general resistant host type is indicated by arrows on top). (a–c) Replicates corresponding to Fig. 1a–c. Size of the dots correspondent to number of host clones (1–10). Every replicate (a–c) shows the 11 time-points from which hosts were isolated.

Model results and trade-off

To better understand the shift in ecological and evolutionary dynamics and the underlying mechanisms we used a mathematical model of a host-virus chemostat system. Specifically, we tested for the role of the resistance-growth trade-off and followed population and evolutionary dynamics assuming three different scenarios with different trade-off strengths (SI). Overall, we found two distinct periods over time similar to our experimental data. Before the generalist evolved, host-virus populations cycled and evolution was characterised by ARD; after the generalists' emergence, population dynamics became more stable (host increasing, virus decreasing to low densities) and evolutionary dynamics changed, depending on the strength of the trade-off considered. When there was no trade-off (Fig. 2a, d, Fig. S10a,b), the general resistant type almost reached fixation and only one other host type was maintained (when using similar sampling as in experiments, Fig. 2d), but only due to mutations. Assuming the trade-off we observed in the experiments, the general resistant type dominated (Fig. 2b,e, Fig. S10c,d) but several different other host types coexisted. Increasing the costs of resistance further led to even higher levels of diversity maintained (Fig. 2c,f, Figs S10e,f, S11). Overall, the presence of the generalist stabilised host-virus population dynamics as observed in the experi-

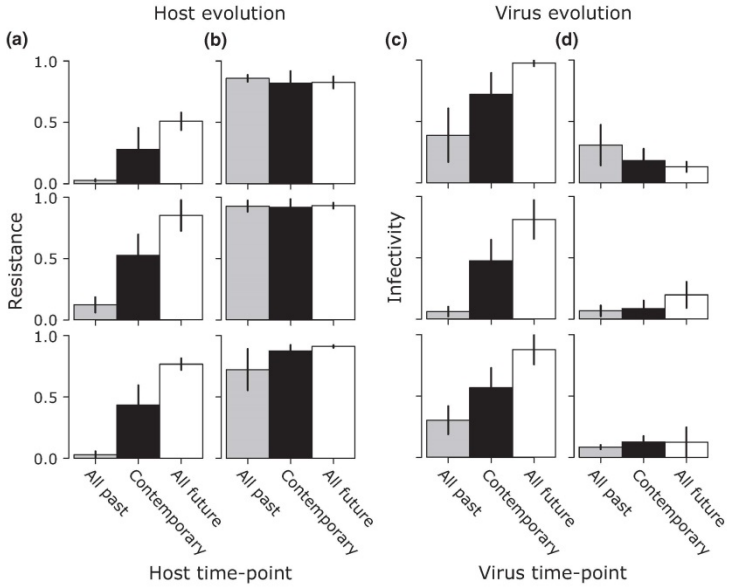

Figure 4 Coevolutionary dynamics in algal-virus populations in chemostats. (a) Average resistance of host clones (fraction of host clones that were resistant ± SE) exposed to virus populations during ARD-period and (b) TDD-period. (c) Average infectivity of virus populations (fraction of host clones that could be infected ± SE) during ARD-period and (d) TDD-period. (a, b) Contemporary are host and virus combinations from the same time-point, all past are combinations of hosts from all previous time-points with the virus populations and all future are combinations of hosts from all further time-points with the virus populations. (c, d) Contemporary are virus and host combinations from the same time-point, all past are combinations of virus populations from all previous time-points with the host, all future are combinations of virus populations from further time-points with the host. (a, c) Data show clear arms race dynamics as time-point is significant for host resistance and virus infectivity.

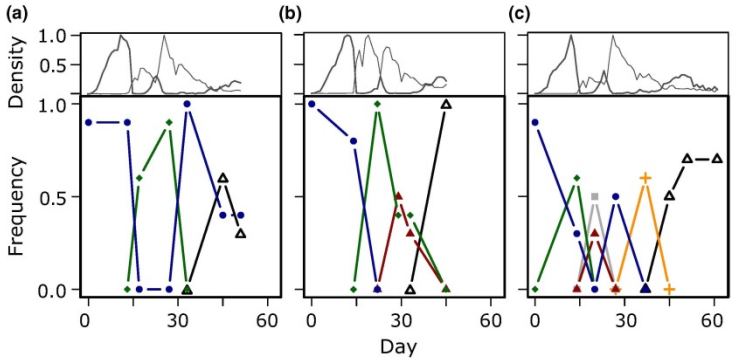

Figure 5 Frequencies of resistance host types over time from chemostat experiments. Frequencies of distinct resistant host types during ARD (until generalist) are shown in different colours and symbols. Blue (dots) are initial not-resistant hosts. Only distinct resistant types with frequencies higher than 0.2 are shown. Scaled population dynamics (density) of host (bold grey line) and virus (thin grey line) are shown above each frequency plot as densities scaled to the maximum population size. (a–c) correspond to Fig. 1.

ments, but the rate at which host increased while virus population size decreased depended on the diversity of host types. Finally, we simulated the dynamics with the experimentally observed trade-off for 360 days (Fig. S12). Here, diversity was maintained while the general resistant host remained at high frequencies and frequencies of host types with lower resistant ranges changed over time while population densities showed only small fluctuations.

Eco-evolutionary dynamics

Overall, host population growth was positively correlated with the fraction of resistant host clones in the population (LME: $\chi^2 = 31.88$, d.f. = 1, $P < 0.001$; Fig. S13). Furthermore, changes in host susceptibility and virus infectivity correlated with distinct changes in population sizes (Fig. 1); host populations decreased when they were susceptible to the contempo-

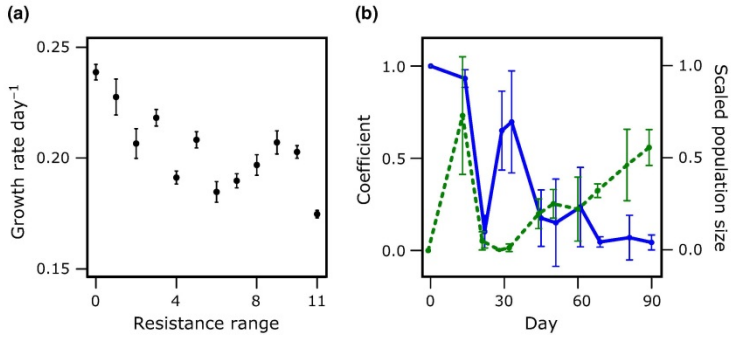

Figure 6 Evolution of trade-off and eco-evolutionary dynamics in chemostats. (a) Trade-off between host resistance range and average per capita growth rate (± SEM). (b) Changes in selection coefficient (blue solid line, ± SD) and algal census population size (green dotted line, ± SD). For all $n = 3$.

rary virus population (e.g. Fig. 1c, day 14) and increased when hosts were resistant to the contemporary virus (e.g. Fig. 1c, day 20). Thus, the reciprocal antagonistic changes through *de novo* evolution of resistance and infectivity constantly changed the ecological effect of the two antagonists. Rates of coevolution were significant different for the ARD and TDD period (LME: type of dynamic: $\chi^2 = 11.85$, d.f. = 2, $P = 0.003$; type of dynamic × MOI: $\chi^2 = 5.38$, d.f. = 1, $P = 0.02$), but we did not observe a correlation between force of infection and rates of coevolution (LME: MOI: $\chi^2 = 5.39$, d.f. = 2, $P = 0.068$, Fig. S14). We further observed that selection and *census* population size N of the host varied over time (Fig. 6b). In particular, the population size of the host was reduced to very low numbers (~ 1000 cells mL^{-1}) during ARD, but was large (increasing) when ARD ended. Selection by the virus cycled during the ARD period, but was low during the TDD period. Thus, there were time-points when s_p and N were small, time-points when s_p and N were high and time-points with one high, the other low.

In a model without evolution the virus rapidly decreased host densities (until the end of the simulated time, Fig. S15) and the lack of further population growth indicated that populations were unable to recover without evolutionary change and underlines the important link between evolution and ecology.

DISCUSSION

We experimentally studied eco-evolutionary dynamics in coevolving host-virus systems and combined our analysis with a corresponding mathematical model. Host and virus densities showed damped oscillations for the first half of the experiment and stabilised hereafter with host densities remaining well below densities observed in control chemostats. Algae and virus coevolved through ARD initially and we observed selective sweeps of new resistant host types. ARD ended with the asymmetrical evolution of a general resistant host, which did not go to fixation due to a trade-off between host-resistance and growth. We thus refer to these dynamics as TDD. Interestingly, the frequencies of the more susceptible types changed over time.

Besides the maintenance of diversity, theory and empirical studies suggest that trade-offs are important for the type of antagonistic coevolution (Sasaki 2000; Hall *et al.* 2011; Lopez Pascua *et al.* 2014). A trade-off can limit the evolution of an ever-increasing host (virus) resistance (infectivity) range as costs accumulate with increasing amounts of resistance (infectivity) alleles. The accumulating cost of resistance would restrain the evolution of a general resistant (infective) host (virus) as the trade-off weakens their response to directional selection leading eventually to a shift from ARD to FSD. In our study, the trade-off had no direct consequences for the evolutionary outcome during ARD. The trade-off did not limit the host's ability to respond to directional selection as we observed the evolution of a general resistant host in all replicate chemostats. Model analysis confirmed that ARD ended only after the evolution of a general resistant host. Furthermore, the model showed that evolutionary and population dynamics during ARD assuming no trade-off (Fig. 2a,d) were almost identical to model results with the experimental trade-off, indicating that the trade-off was indeed less important here.

Our study shows strong links between ecology and evolution. These eco-evolutionary dynamics are evident from several observations. First, we observed that host population growth depended on the fraction of resistant hosts in the population. Second, the appearance of newly resistant host types was clearly reflected in the population dynamics of both alga and virus (Fig. 5). The evolution of new host (or virus) types affected the ecological interaction strength between the antagonists, and lead to changes in population dynamics (one antagonist increased in density, the other one decreased). The changes in population densities then altered directional selection strength, resulting in further evolutionary change, and so on. As a result, the population dynamics of hosts and virus showed damped oscillations during ARD (sustained oscillations in the model). This interaction between ecology and evolution continued until a general resistant host appeared and ARD ended. A third link between ecology and evolution was observed when the population dynamics stabilised during TDD. Here, virus densities decreased to low values, while host densities increased. From this point on, host populations had high resistance on average (Fig. 4b) as the general resistant host reached high frequencies and no further population cycles were observed in the experiments. Thus, the evolution

of a general resistant host stabilised population dynamics. Here, the trade-off became important for the maintenance of (host) diversity. Directional selection for resistance weakened (due to low virus densities) and higher host densities strengthened intraspecific competition between faster growing but more susceptible and general resistant hosts. Model analysis showed indeed that the amount of diversity maintained depended on the strength of the trade-off (Fig. 2, Figs. S11, S12). The trade-off had further consequences, as lower per capita growth rates of resistant host cells (which dominated the host population) resulted in lower host population densities (during TDD) compared to control chemostats. Thus, population size changes can immediately alter interspecific and intraspecific interaction strength, whereas population size depends on the coevolutionary state or history and changes within a few generations.

The eco-evolutionary dynamics had considerable further consequences for our understanding of the dynamics of two coevolving antagonists. Although the population and evolutionary dynamics were relatively similar between experimental replicates, differences in *census* population size (ecology) and selection (evolution) over time (Fig. 6b) likely played a considerable role for the timing and emergence of novel adaptive mutations. For example, the generalist host appeared first at different time-points (Fig. 3: day 33, 45, 51) and we observed differences in appearance and increase in distinct resistant host types between the replicated chemostats (Fig. 5). These observations indicate that changes in population size and selection can weaken or strengthen stochastic effects during reciprocal adaptations as predicted by theory (Gokhale *et al.* 2013). Although we find that coevolution during the first half of the experiment was driven by ARD, average resistance of hosts from further time-points (relative to virus population) was not complete in all replicates (Fig. 4a). This observation resulted from a less resistant host type that re-emerged in two out of three replicates (Fig. 5a,c), which is not predicted under pure ARD. During these periods, host densities were very low, potentially resulting in slower emergence of novel mutations. Together with the random loss of genotypes (drift) and the lack of novel mutations, ARD temporary softened (i.e., moved towards FSD like dynamics). As no host type was resistant to the virus at that time, the ancestral not-resistant host type invaded the host population again. Interestingly, this non-resistant host type had the highest growth rate and thus was able to out-compete hosts with higher resistance ranges. These results indicate that, as soon as hosts could not respond to directional selection imposed by the virus, the trade-off determined the dominating host type. However, when new adaptive mutations emerged, coevolution could again continue through ARD. Indeed, we found that MOI (as proxy for force of infection and selection) was not significantly correlated with the rate of coevolution, confirming that coevolution depended not only on infection strength but on the supply of mutations, drift and the trade-off as well.

Our study also synthesises several previous results and predictions into a coherent picture. Similar to studies with prokaryotic systems, we found a shift from ARD to FSD (Gómez & Buckling 2011; Hall *et al.* 2011), although the underlying mechanism was different here (TDD) and previous studies did not link evolutionary dynamics to detailed temporal changes in population sizes. Furthermore, other studies discovered asymmetrical coevolution between host and virus, which can impede extensive coevolution (Lenski & Levin 1985), but can lead nonetheless to multiple rounds of coevolution (Poullain *et al.* 2008; Hall *et al.* 2011) as in our study. Lenski & Levin (1985) also showed the stabilisation of population dynamics during FSD and suggest the maintenance of host diversity through a trade-off as we have found here. A stabilising effect of rapid evolution has also been demonstrated in studies on eco-evolutionary dynamics (Becks *et al.* 2010) but they did not consider coevolution between consumer and resource population. Overall our detailed analysis goes beyond previous studies by showing that the dynamic effect of selection and population size is an inherent part of eco-evolutionary dynamics, with important implications for the evolutionary dynamics.

CONCLUSION

Viruses have been shown to play an important role in termination of algal blooms (Fuhrman 1999; Brussaard *et al.* 2005), affect nutrient and energy cycling (Suttle *et al.* 1990; Suttle 2007; Haaber & Middelboe 2009) and plankton community structure (Suttle 2007; Short 2012). Our experiment showed rapid recovery of algal populations through the evolution of general resistance, finally reducing the effect of virus on algal mortality.

We showed here the important entanglement of ecology and evolution in antagonistic coevolving. Coevolution affected population density and both densities and evolution then in return, resulted in further evolutionary changes, and so on. Although the fitness-associated costs of resistance did not alter coevolution itself as the switch from ARD to TDD resulted from an evolutionary constraint in the virus, they determined the host population sizes and maintenance of variation during TDD. Our data indicate that low population densities affected coevolutionary dynamics through mutation supply and/or drift as we observed softening of the ARD. Overall, the outcome and trajectory of coevolution with subsequent effects on the ecological dynamics and community structure were determined by many factors, which are intertwined and operate on same timescales (Fig. S16). As such, the eco-evolutionary dynamics have important consequences for stability of populations and genetic diversity of populations, as well as how selection and demography affect evolutionary trajectories.

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AUTHORSHIP

J.F and L.B. conceived and designed the study, J.F. performed experiments, M.S. developed and analysed the model,

J.F., M.S. and L.B. analysed the results and wrote the paper.

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Eco-evolutionary dynamics in a coevolving host-virus system

Jens Frickel, Michael Sieber and Lutz Becks

Material and Methods

Chemostat experiments. Experiments were performed in sterile continuous flow-through microcosm systems (chemostats) and consisted of a 500 ml glass bottle containing 400 ml modified BBM (Bold's basal medium; nitrate was replaced by equal moles of ammonium chloride). Sterile air and BBM were continuously supplied (dilution rate D =0.1d^{-1}). The chemostats were mixed by stirring and maintained at 20°C under continuous light. Samples for assessing population densities were taken daily using standard sterile methods. Algal densities were counted using a hemacytometer and virus particles were counted using flow cytometry (FACS Calibur, Becton Dickinson, San Jose, California) after SYBR Green I staining following Brussaard *et al*. (2004). Algae-virus chemostats ran for 90 days, algae control chemostats for 90, 75 and 40 days (Fig. S3). Representative samples of virus and alga populations were stored every second day by plating algae on BBM agar plates and storing virus at 4°C (Vanetten *et al.* 1983) after filtering (0,45 um reg. cellulose syringe filter).

Mathematical model. We assumed a modified gene-for-gene type of interaction between virus and algae with N algal types and N-1 virus types (Fig. S2). The modified gene-for-gene interaction implies that viral type P_i could infect host type B_j if and only if $i \geq j$ (Fig. S2) making host type B_N general resistant to all virus types. We modeled the

coevolutionary interactions in a chemostat environment with continuous inflow of resource S , algae B= ($B_1, \ldots, B_N$) and viruses P= ($P_1, \ldots, P_{N-1}$) by the following set of equations:

$$\frac{dS}{dt} = D(S_0 - S) - c \sum_{i=1}^{N} g_i\,(S)\, B_i$$
$$\frac{dB}{dt} = M_p\,(g(S) * B) - (\emptyset\, A\, P) * B - DB$$
$$\frac{dP}{dt} = M_p\,\beta\left(\emptyset\, A^T\, B\right) * P - (\emptyset\, A\, B) * P - D\, P$$

Here, * denotes component wise multiplication and superscript T the transposed matrix. The chemostat environment is characterized by the dilution rate D and inflow resource concentration S_0. Resource consumption and algal growth rate was given by a type-specific monod term of the form:

$$g_i(S) = \frac{a_i\, S}{H + S}$$

with resource conversion efficiency c. Viral adsorption rate is denoted by $\emptyset$ and β new virus particles are released upon lysis of an infected algal host cell. Note, that these parameters are the same for all virus types, which only differ in their respective host ranges. The infection matrix A describes host-virus interaction and mutations from one type into another are incorporated via the mutation matrices M_B for the hosts and M_P for the virus respectively. See below for the concrete definitions of these matrices. Becoming more resistant was costly for the host, i.e. host growth rate declined with increasing resistance range. As suggested by the experimental data, we assumed a linear trade-off between resistance range and growth rate, defined by:

$$a_i = \frac{a_N - a_1}{N - 1}\,(i - 1) + a_1, \qquad i = 1, \ldots, N$$

where a_1 is the growth rate of the ancestral type and a_N the growth rate of the universally resistant type B_N. With this definition, the difference a_N - a_1 between the growth rates of the two types at the opposite ends of the resistance range determines the slope of the linear trade-off. Note, that if $a_N = a_1$ there is no cost of resistance and all types have the same ancestral growth rate, i.e. $a_i = a_1$ for all i. If $a_N = a_1$, then all types have the same ancestral growth rate and the greater the difference, the greater the cost for becoming more resistant. The three trade-off scenarios used to explore the data were set as: no trade-off ($a_N = a_1 = 0.25$); experimentally observed trade-off ($a_N = 0.15$); strong trade-off ($a_N = 0.05$). We assumed no costs for larger host ranges in the virus. Hosts and viruses evolved by mutations that altered resistance range and host range, respectively. We assumed that evolution progresses step-wise, i.e. B_i mutates into B_{i+1} or via reversals to B_{i-1}. The virus evolved in the same way. From now on we assumed $N = 4$, which was enough to reproduce the experimental data and in particular the number of rounds of reciprocal coevolution, but small enough to be easily handled computationally. The host ranges of the virus types were described by the 4 X 3 matrix:

$$A = \begin{pmatrix} 1 & 1 & 1 \\ 0 & 1 & 1 \\ 0 & 0 & 1 \\ 0 & 0 & 0 \end{pmatrix}$$

Here, each row determines the sensitivity of the four host types to each of the three virus types (columns), with 1 denoting that the host type can be infected by the respective virus

type and 0 that it is resistant to a virus type. Note, that the last row corresponds to the completely resistant type B_4.

The matrices describing evolution of the host with mutation rate ε were given by:

$$M_B = \begin{pmatrix} (1-\varepsilon) & \varepsilon/2 & 0 & 0 \\ \varepsilon & (1-\varepsilon) & \varepsilon/2 & 0 \\ 0 & \varepsilon/2 & (1-\varepsilon) & \varepsilon \\ 0 & 0 & \varepsilon/2 & (1-\varepsilon) \end{pmatrix}$$

and similar for the virus:

$$M_P = \begin{pmatrix} (1-\varepsilon) & \varepsilon/2 & 0 \\ \varepsilon & (1-\varepsilon) & \varepsilon \\ 0 & \varepsilon/2 & (1-\varepsilon) \end{pmatrix}$$

To ensure consistence of the mutation matrices at the extremal types B_1,B_4 and P_1,P_3, we assume that it is more likely to mutate from those types into intermediate types than vice versa. This represents a conservative choice as we are especially interested in the dynamics of the extremal types and this ensures that those types are not overrepresented in the mutation matrix. However, we note that choosing other reasonable boundary conditions does not change the dynamics or conclusions of the model.

Table S1. Parameter values used for the mathematical model.

Parameter	Biological meaning	Value
D	Chemostat dilution rate	0.1 (d^{-1})
S_0	Inflow resource concentration	30 (µg mL^{-1})
a_1	Algae maximum growth rate of ancestor B_1	0.25 (d^{-1})
H	Algae Half-saturation constant	1 (µg mL^{-1})
c	Algae conversion efficiency	$2.3 . 10^{-5}$
ø	Virus adsorption rate	$7.5 . 10^{-8}$ (d^{-1})
β	Virus burst size	100
ε	Mutation rates	10^{-3}

Figures

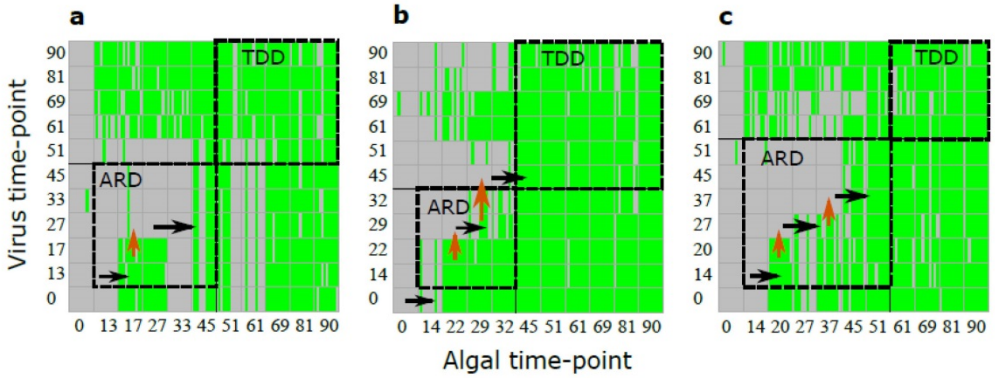

Figure S1. Infection matrix time-shift experiment. Resistance (green) and susceptibility (grey) of algal host clones (10 clones per time point) to virus populations over all time points per chemostat. Black arrows indicate evolution of host resistance to virus (a: 2; b, c: 3 evolutionary cycles) and orange arrows indicate evolution of infectivity of virus to host (a: 1; b,c: 2 evolutionary cycles). Black dashed lines indicate areas of ARD or TDD used in data analysis of coevolutionary dynamics.

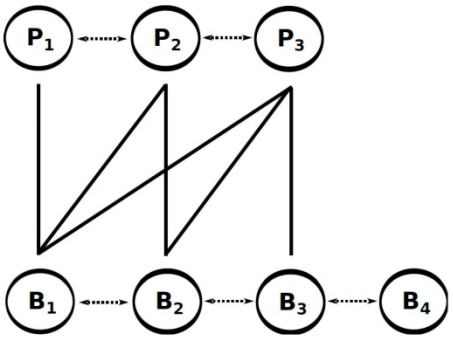

Figure S2. Host-virus interactions model. P (1-3) different virus types. B (1-4) different algae types. Host ranges of the virus types is depicted by solid lines. The B_4 algal type cannot be infected by any virus type and is general resistant. The dotted lines between host and virus types indicates possible mutation pathways.

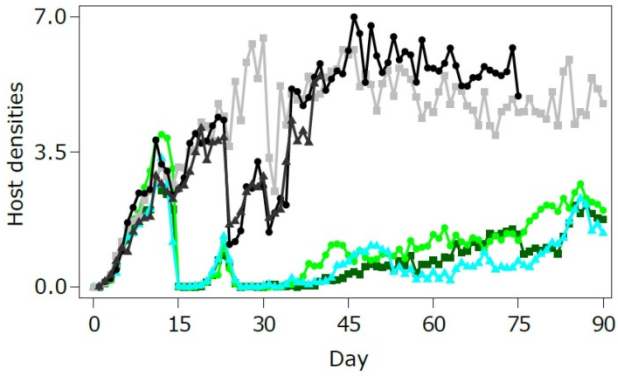

Figure S3. Population dynamics algae-virus and control chemostats. Population densities are in million cells per ml. Grey tint colours are control chemostats, green tint colours are algae-virus chemostats. Two control chemostats were stopped earlier due to technical difficulties.

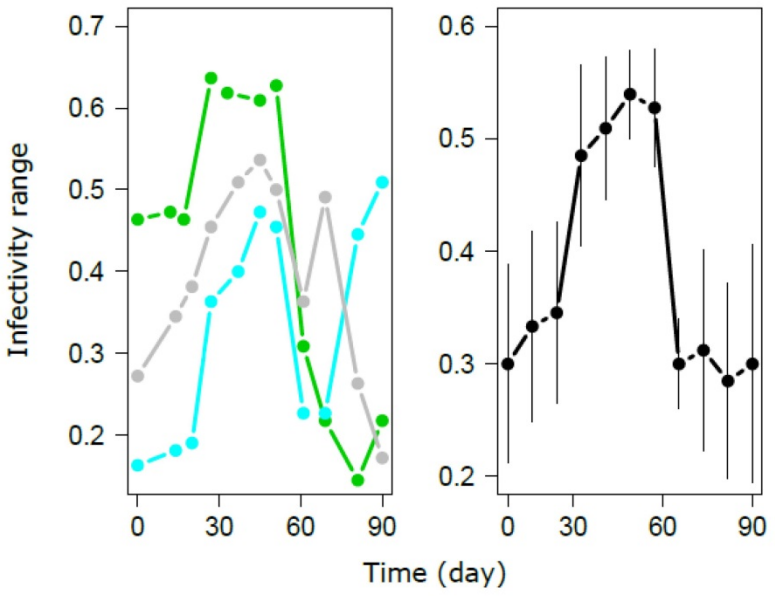

Figure S4. Evolution of virus infectivity in chemostat experiments. Infectivity range calculated as number of algal host clones (from all time-points; 110 per replicate. Infecitvity range is scaled to 1= maximum of 110) that could be infected by the virus population from each time-point. a) Shows virus infectivity over time per chemostat, b) shows average infectivity per time-point (± s.e.m.)

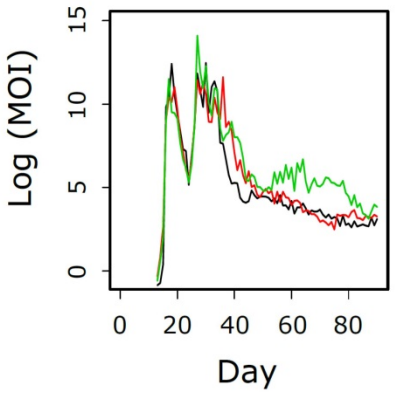

Figure S5. Multiplicity of infection (MOI). Multiplicity of infection, calculated as amount of virus particles per algal host cell, over time. Black: chemostat Fig. 1a; red: Fig. 1b; green: Fig. 1c. MOI values were natural-log transformed.

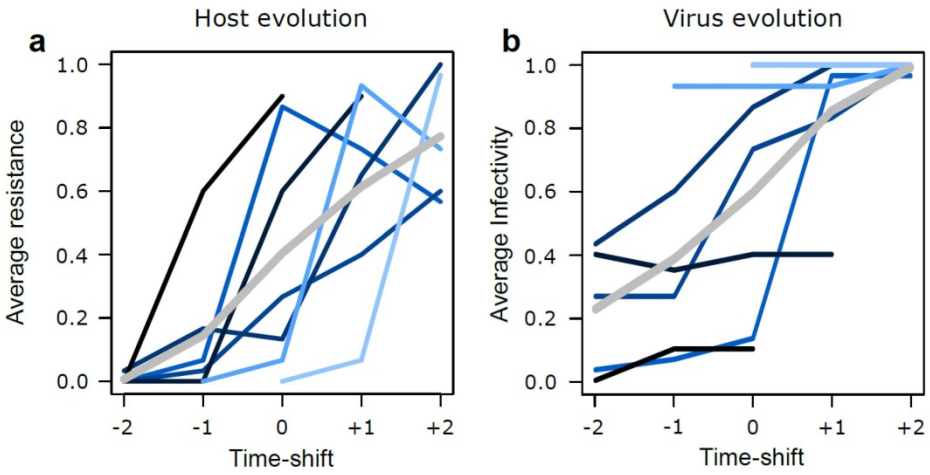

Figure S6. Evolutionary dynamics of host and virus in chemostat experiments. a) Host evolution shows directional selection for increasing host resistance. Contemporary hosts (0), hosts from one or two time-points in the future (+1,+2) and past (-1,-2) were exposed to virus populations. Ten host clones were used for each time-point (per chemostat). Blue shadings (light to dark) indicate the time-point of virus populations (from start to end of ARD-period). Average resistance of three replicate chemsotat is shown per time-shift. Grey is the overall average resistance per time-point. b) Virus evolution shows directional selection for increasing infectivity. Contemporary virus (0), virus populations from one or two time-points in the future (+1,+2) and past (-1,-2) were exposed to host clones (10 host clones per time-point). Blue shadings (light to dark) indicate host clones used from start to end of the ARD-period. Average infectivity of three replicate chemostats is shown per time-shift. Grey is the overall average infectivity per time-point.

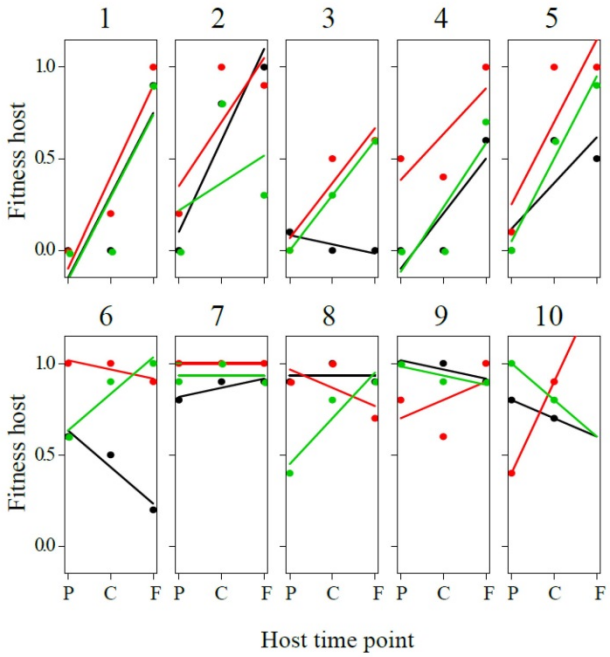

Figure S7. Evolutionary dynamics of host in chemostat experiments. Each panel represents one time point (1 = second time point used for time shift experiment) and dots show fraction of resistant host clones within the host population (10 individual host clones per time point). Hosts from only one pervious time point (P), the contemporary time point (C) and only one time point further (F) were exposed to the contemporary virus population (black: chemostat Fig. 1a; red: 1b; green: 1c). Fitted lines are derived from linear regressions.

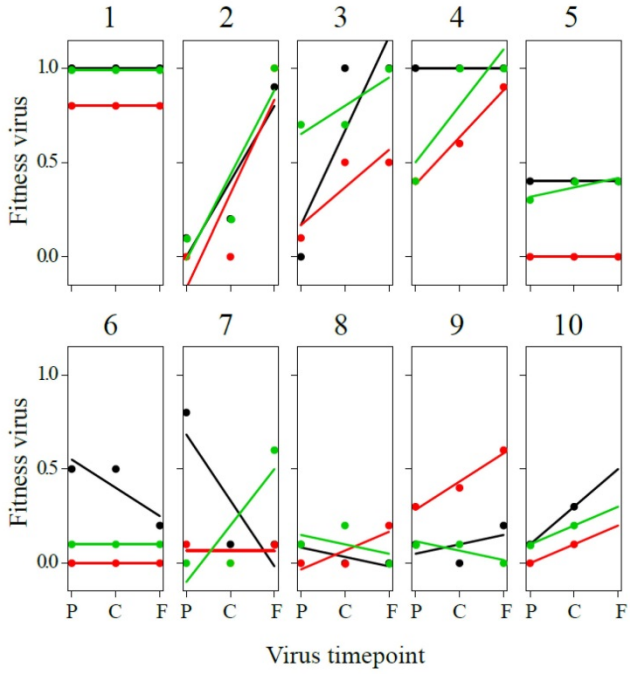

Figure S8.Evolutionary dynamics of virus in chemostat experiments. Each panel represents one time point (1 = second time point used for time shift experiment) and dots show fraction of susceptible host clones (as a measure for virus fitness, n=10). Virus populations from only one pervious time point (P), the contemporary time point (C) and only one time point further (F) were exposed to the contemporary algal population (black: chemostat Fig. 1a; red: 1b: red; green: 1c). Fitted lines are derived from linear models.

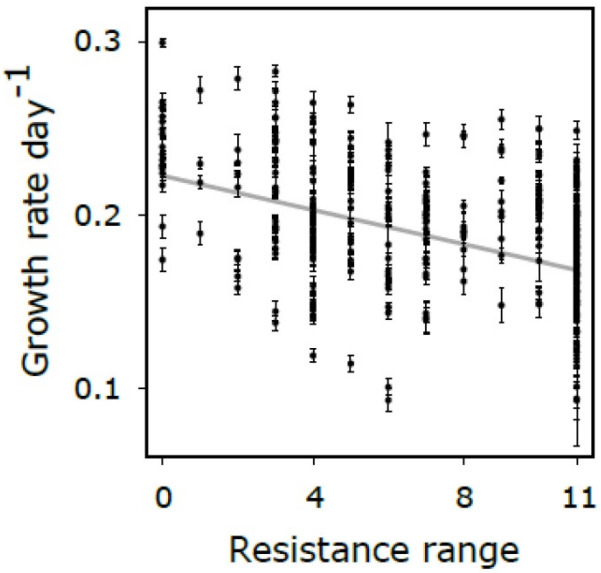

Figure S9. Trade-off between host resistance range and per capita growth rate of individual clones in chemostat experiments. Average growths per day of 4 technical replicates are shown (± s.e.m). Fitted line derived from a significant linear model for growth rates with resistance range as fixed effect and chemostat as random effect (LMM, X^2=93.902, df=1, n=3, p<2.2e-16).

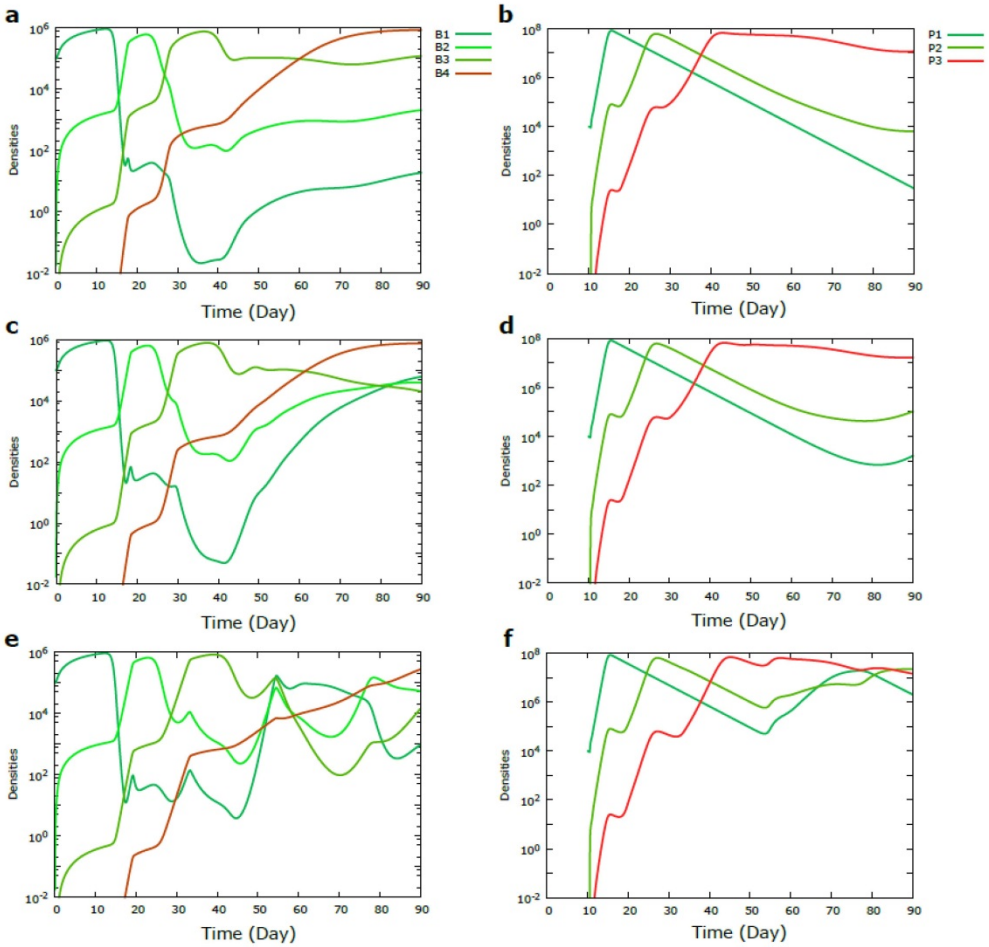

Figure S10. Densities of host and virus types resulting from models with different host trade-off values. a,c) and e) densities of different algal types from not-resistant (B_1) to general resistant type (B_4). b,d) and f) densities of different virus types from P_1-P_3 with respectively increasing host ranges. a,b) model results without trade-off; c,d) model results with experimental observed trade-off, e,f) model results for strong trade-off.

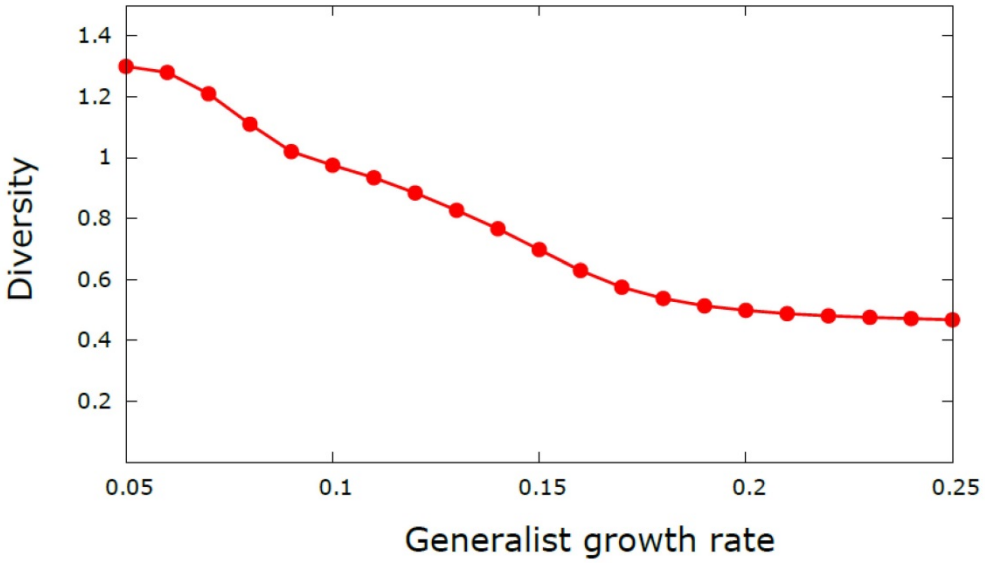

Figure S11. Host diversity resulting from models with different trade-off values.

Average host diversity (Shannon index) was calculated from day 45 to day 90. Growth rate of general resistant host indicates the strength of the trade-off.

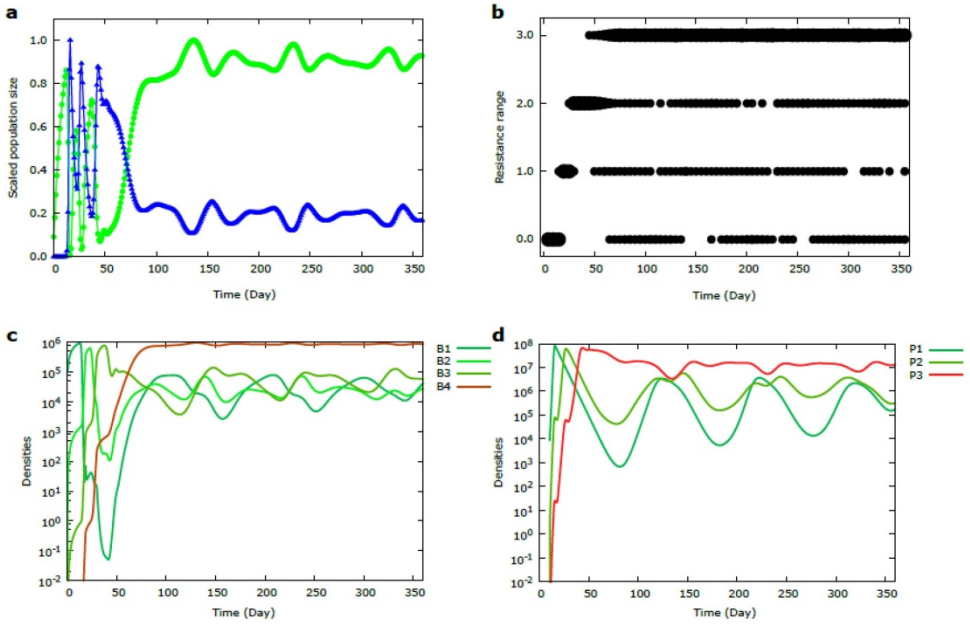

Figure S12. Population and evolutionary dynamics modeled with experimentally observed trade-off value over one year. a) scaled population dynamics of algae (green, dots) and virus (blue tirangles). b) evolution of host resistance range (number of virus types to which an algal clone is resistant). Size of the dots correspondent to number of host clones (1-10). d) densities of different algal types from not-resistant (B_1) to general resistant type (B_4). d) densities of different virus types from P_1-P_3 with increasing host ranges (respectively).

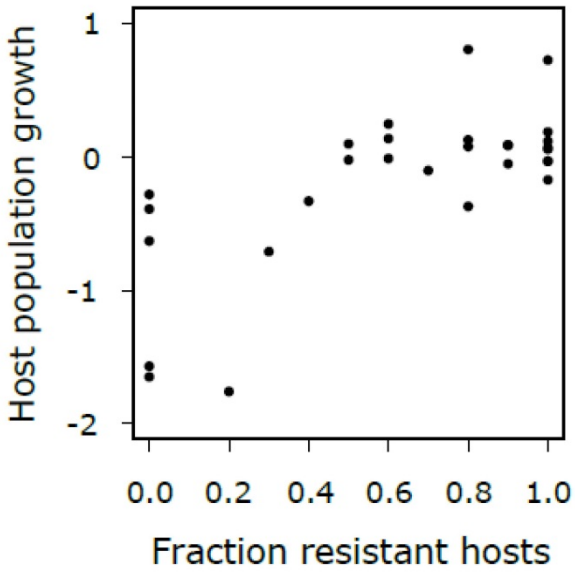

Figure S13. Host population growth and fraction of resistant hosts in population. Population growth rates were averaged over 3 days (Fig. 1) around the days used for the time shift experiment and correlated with the fraction of resistant hosts for the same time point (based on 10 individual host clones, n=3).

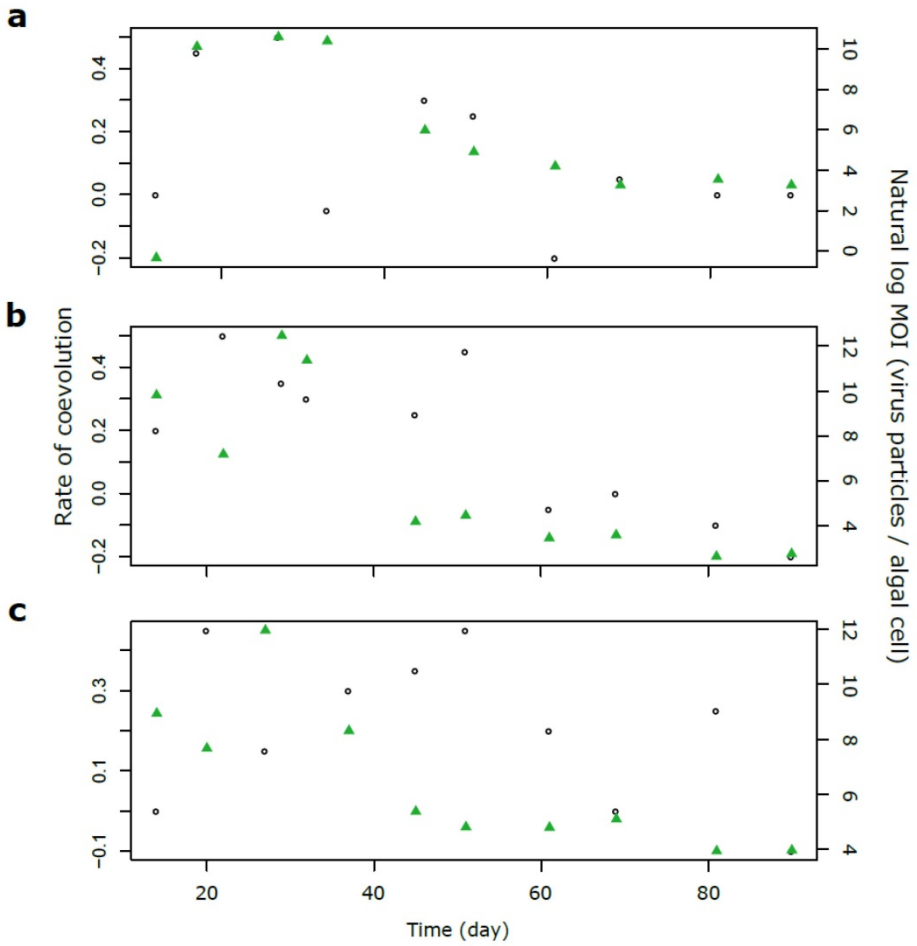

Figure S14. Multiplicity of infection and rates of coevolution. MOI values were log natural transformed (green triangles) and calculated for every time-point used in the time-shift experiments. Rates of evolution (black open circles) are represented as the slopes estimated between the proportion of hosts resistant one time-point in the past, the contemporary time-point and one time-point in the future. a-c correspond to a-c in figure 1.

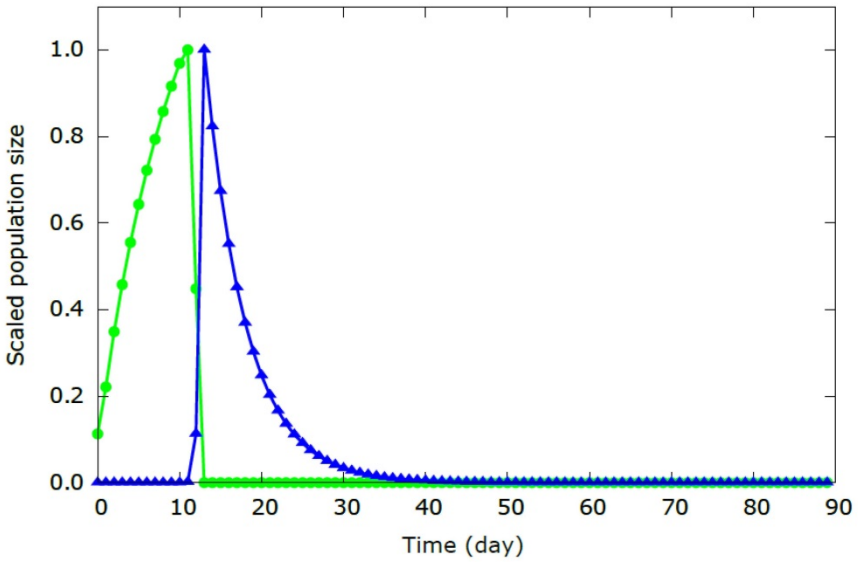

Figure S15. Population dynamics resulting from a model without evolution.

Population densities were scaled to maximum densities of virus or host. Green (dots): algal densities; blue (triangles): virus densities.

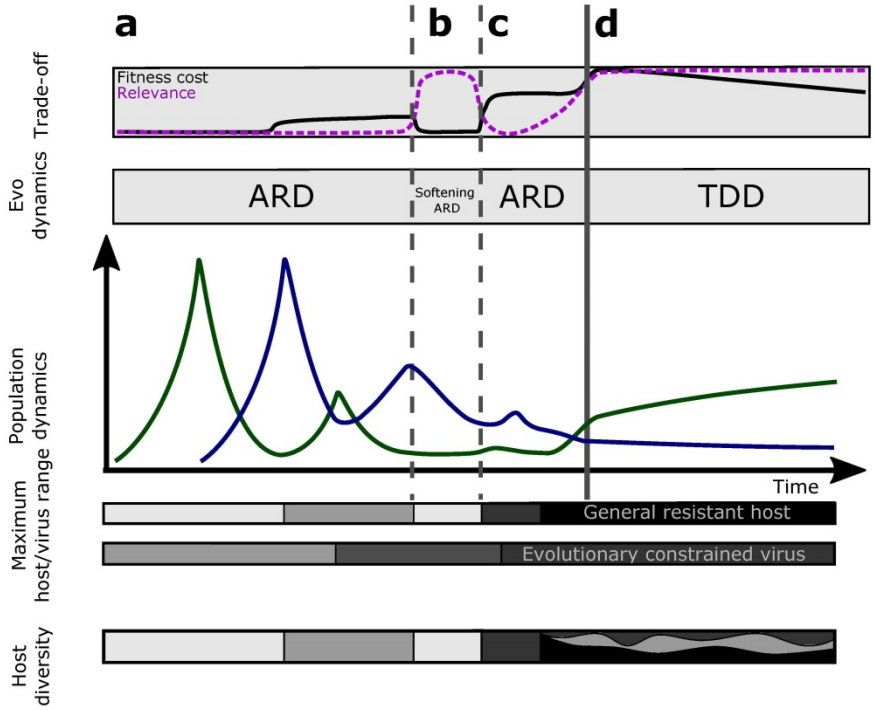

Figure S16. Conceptual figure summarizing the ecological and evolutionary dynamics. Figure summarizes important concepts concerning antagonistic coevolution in an eco-evolutionary context as explained in the discussion. Periods a-c represent ARD phase and period d represents TDD phase. Trade-off box represents fitness cost (black; associated with evolution of resistance) and relevance of this trade-off (purple dotted line). Evo-dynamics box corresponds to the type of coevolution. Population dynamics of host (green) and virus (blue). The maximum host/virus range represents the maximum resistance range of host clones present in the population at that time, and maximum infectivity range of virus clones present in the population. Colours (light to dark) represent different types (from low range to high range). Final maximum host range is a general resistant host and final maximum virus clone (evolutionary constrained) is unable to infect this general resistant host. Host diversity box represents how many different host types are present during that time. Colours correspond to different host types represented in maximum host range box.

CHAPTER THREE

Manuscript

The ghost of predation and coevolution past for eco-evolutionary dynamics

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Abstract:

Continuous feedbacks between ecology and evolution significantly affect populations and communities (eco-evolutionary dynamics). Although species interactions within food webs are characterized both by direct and indirect effects, eco-evolutionary dynamics were so far mainly assessed with direct species interactions. Moreover, most studies that did include potential indirect effects focused on short time-scales of only a few generations, potentially missing delayed and multigenerational consequences. Thus, the significance of indirect effects for eco-evolutionary dynamics is largely unclear. We test here for both indirect and direct ecological and evolutionary mechanisms driving eco-evolutionary dynamics by comparing two species systems (host-virus and predator prey) without indirect effects with a more complex system (host-virus plus predator) with indirect effects. We show that direct and indirect effects of predation and coevolution (between host and virus) have cascading and transgenerational consequences that were necessary for the mechanisms driving diversity, community structure and the coexistence of species.

One Sentence Summary: Direct and indirect effects of predation and coevolution have cascading and delayed consequences for eco-evolutionary dynamics

Main Text

The entanglement of ecology and evolution has been emphasized and shown in many different study systems (*1-6*). Studies on eco-evolutionary dynamics and feedbacks used mainly small food webs with two interacting species (see *7*) or focuses on short time scales (few generations; *8, 9, 10*). Natural food webs are, however, more complex, with a large number of interacting species over longer time scales. Thus by design, those studies exclude two important aspects of natural food webs.

First, complex food webs with more than two interacting species can result in indirect effects of one species on other members of the community (*11-14*). Density-mediated indirect effects occur when the dynamics of one or two organisms are driven by a change in the density of other organisms that are not directly interacting (*15*). At the same time, greater complexity increases the possibilities for rapid adaptive evolutionary responses within populations due to more interacting species (e.g. multiple pairwise evolution and diffuse evolution; *16, 17*). Likewise, rapid evolution can potentially result in cascading and indirect effects on other members of the community when a species‘ change in phenotype in response to the presence of a second species alters the interactions with other community members (i.e. trait-mediated indirect effects *15, 18*). Within the context of eco-evolutionary dynamics, such indirect effects can be key processes as both density- and trait-mediated indirect effects can result in cascading effects across trophic levels (*8, 13, 15, 18-20*) and thus affect important feedbacks between ecology and evolution. Second, studying eco-evolutionary dynamics on short time scales (few generations) does not allow for delayed (transgenerational) feedbacks between ecology and evolution. Such delayed effects occur when the effect of a particular ecological (e.g. the presence of a species) or evolutionary process (e.g. selection pressure) continues for many generations even after the initial causal process has stopped (e.g. predator removal; *21*).

Thus, in natural communities, with complex food webs and where species interact over many generations, indirect effects and delayed feedbacks should be important for eco-evolutionary dynamics. Ultimately, they will not only explain the mechanisms driving community dynamics and structure (*22*), but also help predicting future biodiversity changes - which determine the evolutionary potential of populations.

In an integrative and controlled experimental study, lasting for ~ 100 host generations, we tested for the impact of direct and indirect effects on eco-evolutionary dynamics with increasing food web complexity. Specifically we asked whether and how indirect effects

control feedbacks between ecology and evolution, and whether these effects are important for coexistence of multiple species (community structure). Furthermore, we investigated both immediate and delayed effects over multiple generations of species interactions. We manipulated food web composition in a factorial design: predator-prey and host-virus communities (two species, thus no indirect effects) and communities where predator and virus competed for the same resource (prey=host) with possible indirect effects through the shared resource of the virus and predator. For each experimental community, three replicated continuous cultures (chemostats) were started from the same isogenic clone of the asexually reproducing algae *Chlorella variabilis* (Materials and methods are available as supplementary materials at the Science website: SM). After an initial growth period of the algae, i) an asexually reproducing rotifer clone (*Brachionus calyciflorus*) was added as a predator ('predator-prey system'; Fig. S1), or ii) an isogenic strain of a double stranded DNA virus (Chlorovirus PBCV-1) was added as viral parasite ('host-virus system'; Fig. 1A), or iii) both the rotifer and virus were added as competing consumers ('complex system'; Fig. 1B). We followed population and coevolutionary dynamics for 90 days (~ 100 prey/host generations). Coevolution between host and virus was measured by time-shift experiments. In these we expose the host (isolated in regular time intervals during the experiments) to virus populations isolated from their relative past and future. This enabled us to estimate when hosts evolved resistance to a particular virus population, and when virus evolved to infect previously resistant hosts again (*23*). We individually exposed 10 random host clones isolated from 11 time-points throughout the experiments to virus populations isolated from these 11 time-points. From these data we estimated host-resistance range (the number of virus populations a host clone was resistant to; SM) and changes in average resistance and infectivity over time (Fig 2A, S3).

When exploring the population dynamics over time, we observed a one-quarter-phase lag between predator and prey population densities in the predator-prey system (Fig. S1B), and we did not observe evolutionary changes within the algal prey population (SM). As the population oscillations followed the classical ecological theory of predator-prey dynamics, we concluded that the dynamics in this predator-prey system were mainly driven by ecology (*24*), i.e. the direct interaction between predator and prey without evolutionary change. In contrast, the host-virus dynamics in the host-virus system were driven by both ecological and evolutionary changes. The initial damped population oscillations (Fig. 1A, ~day 12 - 45) were driven by rapid coevolution between host and virus (discussed in *25*) and the population

oscillations stabilized after several rounds of coevolution through the evolution of a general resistant host which could not be infected by any virus coming from past, contemporary or future time points. After the evolution of the general resistant host, a trade-off between host resistance and per capita growth rate maintained trait variation in the host population (i.e. host resistance; Fig. S2). The host population then consisted mostly out of general resistant hosts, which coexisted with less resistant hosts (Fig. 2), enabling the persistence of the virus despite the high level of average resistance in the host population (see *25*).

In the complex system, where both consumers were added initially, the predator population went extinct after one initial cycle in all replicates (~day 16, Fig. 1B). The following host-virus dynamics were relatively similar to the two species system, with i) initial population oscillations followed by stabilization and ii) two rounds of coevolution and the evolution of general resistant host. When the population dynamics stabilized, we tested whether the predator would go extinct again or could coexist with the virus and algae. To do so, we added the predator again to the complex system (day 57, Fig. 1B) and all three species could now coexist. Thus, the host-virus and the complex system showed changes in both ecology and evolution over time.

Ecological and evolutionary processes can, however, simultaneously drive the dynamics of communities at the same time (eco-evolutionary dynamics). We therefore applied the 'Geber method' (*26*), to quantify to what extent ecological changes (=host densities) or coevolutionary changes (=evolution of host resistance and virus infectivity) contributed to virus population growth rates. We found indeed, that both ecology and evolution affected virus population growth rates (Fig. 3) on same the timescale (simultaneously) and to a similar extend as demonstrated by their lack of statistically significant differences (Fig. 3, Table S1; ANOVA comparing ecological and evolutionary contributions: $F_{1,96}$=0.56, p=0.46). This is not only in agreement with eco-evolutionary theory, but also represents a significant characteristic of eco-evolutionary dynamics. Interestingly, the extent to which ecology and evolution affected virus growth rates changed over time (Fig. 3, table S1; ANOVA contribution * time: $F_{1,96}$=31.14, p=2.24*10^{-7}) with both having strongest effects early on in the experiments, indicating that eco-evolutionary dynamics might be strongest within the first few generations of novel species interactions (e.g. invasion, colonization, experimental manipulation). Notably, this observation suggests that estimating eco-evolutionary dynamics from short-term experiments with only a few generations might lead to an overestimation of the role of evolution. These eco-evolutionary dynamics differed, however, significantly when

comparing the complex with the host-virus system (Table S1; ANOVA: system $F_{1,96}$=15.43, p=1.62*10^{-4}: system * time: $F_{1,96}$=11.58, p=9.76*10^{-4}).

These differences in eco-evolutionary dynamics arose from several direct and indirect effects caused by the additional consumer in the complex system. Although the predators went extinct after one cycle they affected, as delayed effects, the ecology (i.e. densities) of algae and virus until after their extinction. We found that the algal densities in the complex system were reduced to much lower densities (below detection limit) compared to those of the host-virus system (~1,250 cells/ml), which is a direct effect of the past presence of predators. Furthermore, even though the predator was extinct, the subsequent virus densities reached significantly lower densities in the complex system compared to the host-virus system (t-test: comparing maximum virus densities before algae populations growth >0 after virus addition: t=7.75, df=2, p=0.016). This is clear evidence for an indirect density mediated effect of the predator through a reduction in the resource for the virus.

Besides direct and indirect effects on ecology, the initial presence of predators also affected the coevolutionary dynamics between algae and virus in the complex system. We found that alga populations in the complex system recovered significantly slower (t-test: number of days till algae populations growth >0 after virus addition: t=4.16, df=4, p=0.014). As algal population recovery was only possible due to the evolution of host resistance, we used time-shift experiments to follow the evolution of host resistance range. This allowed us to investigate how the initial presence of the predator altered coevolution between host and virus by comparing the evolution of host-resistance range in the complex system with that of the host-virus system. Host-resistance range increased in the host-virus and complex system (Linear model: test host resistance range over time: $F_{1,642}$=484.1843, p<2.2*10^{-16}, Fig. 2A) due to coevolution (Fig. S3). This increase, however, was significantly different and delayed in the complex system (Linear model: test for host resistance increase between host-virus and complex system: $F_{3,642}$=22.0594, p=1.321*10^{-13}, Fig. 2A). It is therefore evident that the sole past presence of the predator ('predation-past') affected the evolutionary dynamics between host and virus indirectly by delaying them in two ways: first 'predation-past' reduced the host population size and delayed the emergence of potential new adaptive mutations for resistance against the virus, and second it decreased the population densities of the virus. As both host and virus densities were decreased, encounter rates between the antagonists were lower, weakening selection for resistance. Both slower emergence of mutations and weakened

selection resulted in slower coevolution (Fig. 2A). Thus 'predation-past' caused an indirect eco-evolutionary feedback by altering future coevolutionary and population dynamics.

When the predator was added again to the complex system (day 57, Fig. 1B), all species coexisted until the end of the experiments as a result of a trait-mediated indirect effect. During the period when the predator was extinct, a general resistant host evolved and decreased the effect of the virus on the host population size. When re-introducing the predator to system the second time, the host population could support both consumers in the system, because rotifers could now overcome periods with low amounts of algae by consuming resistant hosts, which were inaccessible for the virus. Hence previous coevolution ('coevolution-past') indirectly affected community structure of the system, which could now sustain a new species (predator).

In agreement with the host-virus system, a trade-off between host resistance and per capita growth rates evolved in the complex system. This trade-off evolved similarly as in the host-virus system as it was not significantly different between the two systems (Fig. S2, Linear model: $F_{1,346}$=3.56, p=0.06). Here again, the trade-off maintained diversity in the host population (general resistant hosts coexisted with less resistant hosts), but only until the point when the predator was added again to the complex system. From this point, host diversity was significantly reduced compared to the host-virus system (Fig. 2B, t-test compare diversity between host-virus and complex system for period after adding the predator: t=3.76, df=21.83, p=0.001) due to a trait-mediated indirect effect. To be specific, we found that non-resistant hosts grew mainly as single cells, but with increasing resistance ranges, hosts grew in increasingly larger colonies ($F_{1,68}$=51.5, p<0.001; Fig.S4). As rotifers are filter feeders and ingestion rates depend on the particle size, we tested the efficiency by which the filter-feeding predator consumed general resistant cells (large colonies) and non-resistant host cells (single cells). The rotifers consumed general resistant cells at significantly lower rates than non-resistant host cells ($F_{1,33}$=45.07, p<0.001;Fig. S5). Thus, general resistant hosts were simultaneously less vulnerable to predation and the predator selected for the same algal phenotypes as the virus, reducing thereby diversity of host-resistance types.

Although multiple consumers could coexist due to higher alga densities, it remained unclear how they coexisted while selecting for the same algal phenotype (both predator and virus selecting for the large colony forming general resistant host). We tested how virus and predator densities cycled relative to algal densities (host/prey) and average colony size by inspecting the time series data when all three species coexisted, and by using wavelet-

coherence analysis (SM). Due to the trade-off, less resistant algae (small colonies or single cells) were able to outcompete general resistant algae (large colonies) when algal densities were high (Fig. 1B). Predators were then able to increase in densities (rotifer-algae cycled with a quarter-phase lag; Fig. 1B) by consuming mainly smaller colonial hosts (less resistant) and with less efficiency larger colonial hosts, which lead to an increase in average host colony size (algae colony size cycled three quarter after algal maximum; Fig. 1B). When rotifer densities decreased, the trade-off between the different algal types enabled the smaller colonial, faster growing and less resistant hosts to outcompete bigger colonial hosts again, resulting in almost in-phase cycles of virus and host densities (Fig. 1B). After virus densities decreased again, algal densities increased and a new cycle started.

Our results confirm that eco-evolutionary dynamics can result in cascading and delayed effects within food webs (*9, 27, 28*) and that selection of parasitism and predation together shape evolutionary and ecological responses (*29-32*) which are intertwined on one timescale. Evidently, the eco-evolutionary dynamics were different when more species interacted. Most importantly, our study clearly shows how direct and indirect effects of predation, 'predation-past' and 'coevolution-past' have cascading and transgenerational delayed consequences for eco-evolutionary dynamics, which are crucial to understand the mechanisms driving community structure and diversity.

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Figures

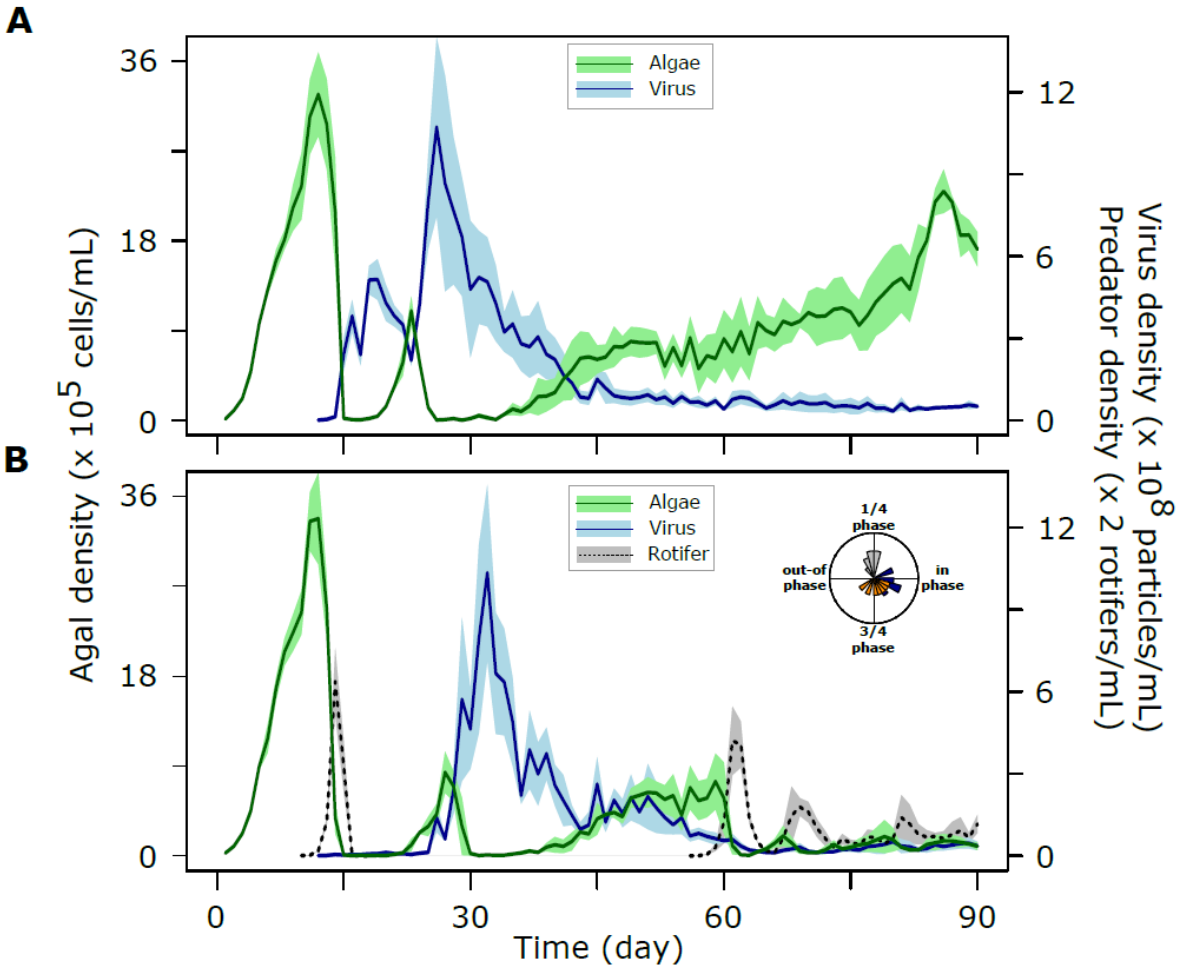

Fig. 1. Population dynamics of host-virus system (A) and complex system with host-virus-predator system (B). Population densities are shown as daily averages (n=3). Colored areas around lines show standard error (of the three replicates) around average. **A)** Population dynamics of host-virus system, with initial damped oscillations of algae and virus, and stabilization around day 45 with algae increasing to high densities and virus decreasing to low densities. **B)** Population dynamics of complex system with algae as resource for two consumers (virus and predator). Predator showed one initial cycle (day 15) and got extinct hereafter. The remaining host-virus dynamics oscillated, followed by stabilization day ~45. The predator was added again (day 57) and both predator and virus coexisted with the algae and show cycling population densities. Phase-shift insert shows how virus densities (blue), predator densities (grey) and average colony size of algae (orange) cycled relatively to algae (in phase = maximum algal density within one cycle). Virus and algae cycled almost in phase, whereas rotifers cycled with a quarter-phase lag after algal maximum. High predator and virus densities resulted then in an increase of average colony size of algae with three-quarter phase lag after algae.

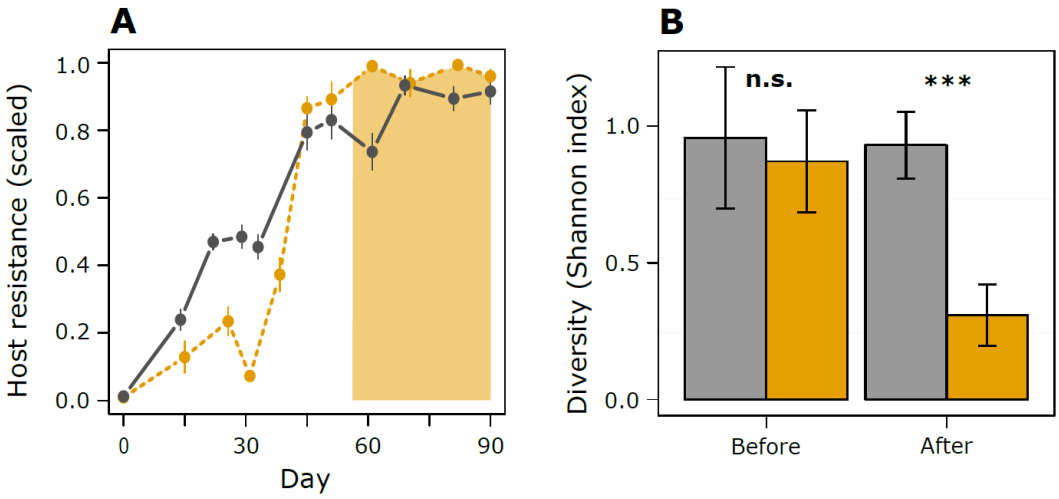

Fig. 2. Evolution of host resistance range (A) and host diversity (B) in host-virus system (grey) and complex system (orange, dashed line). A) Average host resistance range is the number of virus populations to which an algal clone is resistant (maximum = 11, normalized to 1; each data-point is average of 10 clones for each replicates) increased over time from susceptible (0 = non-resistant) to maximum (1 = general resistant host = resistant to all virus populations) but did not reach 1 as general resistant hosts coexisted together with less resistant host clones. Host resistance range increased significantly different between two systems. **B)** Average diversity of host resistance ranges was calculated for each replicate and for each time-point after the evolution of general resistant hosts (10 host-clones per time-point). Host diversity was high in both the host-virus (grey) and complex system (orange) period before the predator was added to the complex system ('Before' = time-point when generalist detected – day 57), but significantly reduced after the predator was added to the complex system again ('After': day 57 – day 90, correspond to orange shaded area in (A); ***: $p<0.001$, n=3, error bars: s.e.m).

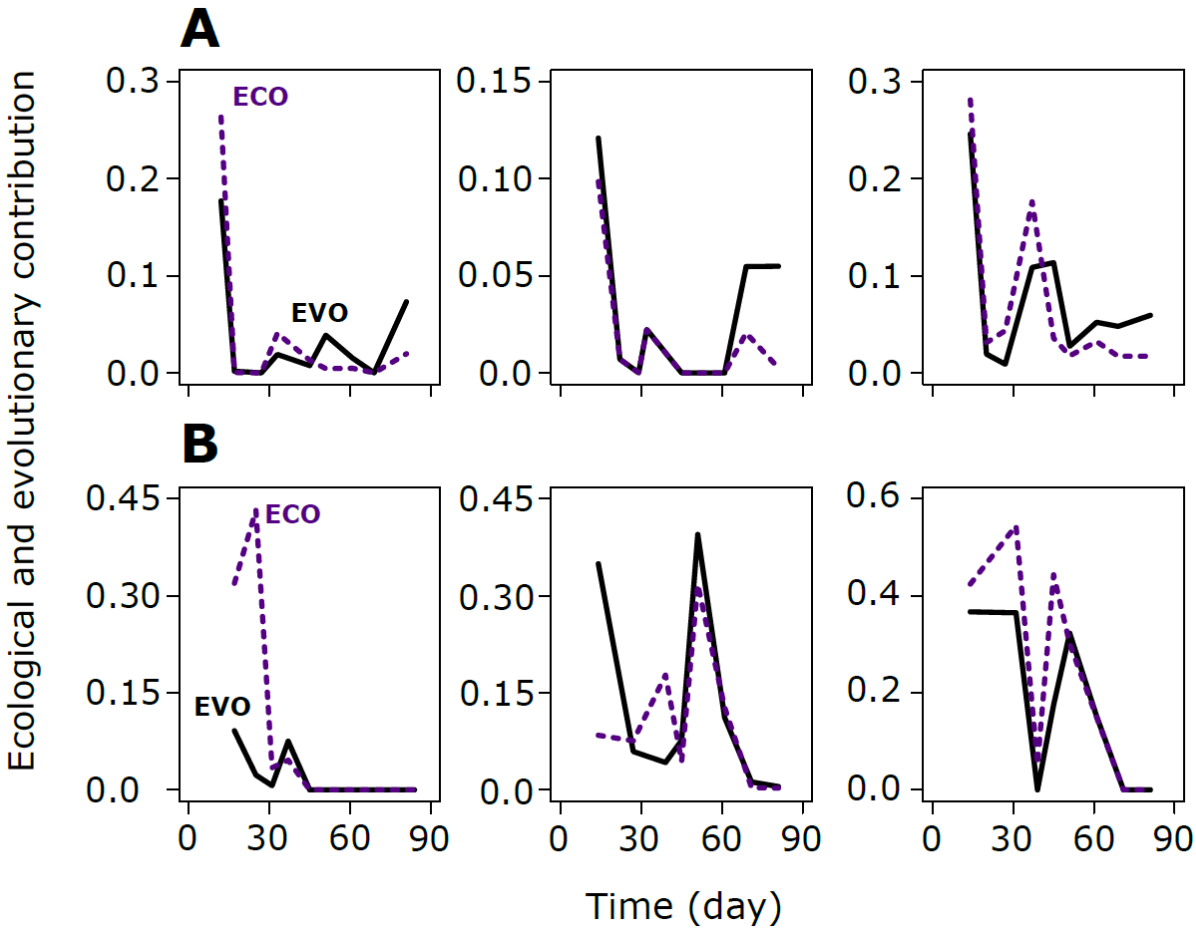

Fig. 3. Eco-evolutionary dynamics in host-virus (A) and complex system (B). The effects of ecology (change in host densities; purple dotted line) and coevolution (change in fraction of resistant hosts in the population) on virus population growth were disentangled using the Geber method as a measure of eco-evolutionary dynamics. The extent to which ecology and coevolution contributed to virus population growth rates was not significantly different in all replicates of the host-virus (A) and the complex system (B). (**A)** Both ecology and evolution contributed to a greater extend at the start of the experiments, but were significantly lower towards the end. **B)** The effects of ecology and evolution were greater at the start of the experiments and became significantly smaller towards the end.

Supplementary Materials:

Materials and Methods:

Chemostat cultures. Continuous flow-through experimental systems consisted of 500 ml glass bottles containing 400 ml of sterile Bold's basal medium where nitrate was replaced by ammonium chloride. Sterile air and medium were supplied continuously at a rate of 10% per day. The cultures were maintained at 20°C with continuous light and were mixed by stirring. One isogenic clone of *Chlorella variabilis* (strain NC64A) was used to start all chemostat cultures. For each experimental system, the isogenic consumers (predator, virus or both) were added at day 12 in 3 replicated chemostat culutures (per experimental system). Purified and concentrated virus was used to inoculate the chemostats. Predators were added from a stock culture containing asexual rotifers (*Brachionus calyciflorus*) with *Chlorella variablilis* as resource. The rotifers were cleaned from algae before adding to the chemostats by filtering and starving overnight.

Population dynamics. Samples for assessing population densities were taken daily using standard sterile methods. Algal and rotifer densities were enumerated in life samples (*4, 25*). Samples for assessing virus densities were filtered through a 0.45 µm cellulose syringe filter, the filtrate fixed with 1:100 gluteraldehyde and stored at -80°C after freezing in liquid nitrogen. Virus densities were counted later by flow cytometry following Brussaard (*25, 33*).

Time-shift experiments. Time-shift experiments were performed as described in Frickel and Becks (*25*). Briefly, during experiments algal and virus samples were stored (algae: agar plates, virus: at 4°C after filtering through 0.45 µm cellulose filter). From each chemostat, eleven time-points were used to perform time-shift experiments. Per time-point, 10 random algal clones were picked from the agar plates and cultured in batch culture. Each algal clone was diluted to equal densities and challenged to the virus population (virus densities diluted to a MOI of 0.01 particles/algal cell, 4 technical replicates per combination) from each time-point separately (11 time-points X 10 algal clones per time-point X 11 virus populations = 1210 combinations per chemostat) in 96 well plates. Growth rates of algae exposed to the virus were calculated based on OD measurements after 0h and 72h. To assess whether the algal clones were resistant or susceptible to a particular virus population, we compared the mean growth rate plus 2 standard deviations of four technical replicates to the mean growth rate minus 2 standard deviations of the control (host clone growth rates without virus). If the virus treatment value was smaller than the control, the algal clone was considered susceptible

to this particular virus population. If the virus treatment value was greater or equal than the control, these algae were considered resistant to this particular virus population.

Statistical analysis. Data analysis was performed in Rstudio (0.98.1091) (*34*) and R (*35*) using the lme4 package (*36*). Algal population recovery was compared between systems by assessing the amount of days until first positive growth of algae after virus addition and performing student t-test after verifying equality of variances ($F_{2,2}$=0.75, p=0.86). Maximum virus densities during this period was compared between algae-virus and complex system after testing equality of variances ($F_{2,2}$=7.74, p=0.0029) and performing a student t-test corrected for unequal variances. Host resistance range was calculated as to how many virus populations (0 to 11) the host was resistant to and was calculated for each host clone used in the time-shift experiment (10 host clones per time-point). The average host resistance range (of 10 clones and 3 replicates) was then normalized to a maximum of 1 (1 = all host clones are resistant to all 11 virus populations; general resistant host). A linear model was used to investigate host resistance range evolution over time in the host-virus and complex system. Host resistance range was used as response, with 3 polynomial terms fitted for time (continuous) and experimental system as a factor (host-virus or complex system).

Previous work showed a trade-off between host resistance range and growth rates in the host-virus system (*25*). We tested for a similar trade-off between growth and host resistance in the complex system and looked for significant differences in trade-off between the two systems. The analysis was limited to hosts from time-points before the predator was added for a second time in the complex system. We used a linear model with host growth rate as a response and tested for a correlation with host resistance range (continuous variable) and tested for a different trade-off between experimental systems (factor: host-virus or complex system).

Shannon index was calculated as a measure of diversity for the same time-points used in the time-shift experiment (based on the host resistance range of ten host clones per time-point). Diversity (after the evolution of a general resistant host) between the two systems was compared before and after the predator was added a second time after testing and verifying equality of variance (equality of variance: before predator; $F_{5,5}$=1.92, p=0.49, after predator; $F_{11,11}$=1.19, p=0.78).

Average colony size of each host clone was assessed by counting average colony size (number of cells per colony) of host clones used in the time-shift experiments until the

general resistant host evolved (in one host-virus chemostat replicate, 10 host-clones per time-point). We tested for a correlation between host resistance and average colony size using a linear model. To test for morphological differences of algae in the predator-prey system, the average colony size of algae was calculated from daily population-density counts. Daily average colony size of algae from the predator-prey system was not different from daily average colony size of algae growing in identical chemostats but without any consumers (t-test unequal variance: t=1.39 df=314.2 p=0.16; data not shown), indicating that the predator-prey interactions did not result in morphological change of algae.

We used wavelet coherence analysis (*37*) to determine phase shifts of algae, virus and rotifer populations as well as mean colony size within one chemostat using the MATLAB wavelet coherence package (Wavelet software was provided by C. Torrence and G. Compo, and is available at URL: http://atoc.colorado.edu/research/wavelets/)). This method allows measuring the local correlation between two non-stationary time series over a specific period. The value of wavelet coherence falls into the range of 0 (no phase coupling between the two time series) and 1 (perfect phase coupling) and from these analyses we extracted the dominant phase shifts. We used this method to detect significant phase shifts between the algae and rotifers in the predator-prey system (days 9-90) and between algae, rotifers, virus and mean clump size in the algae-virus system (days 57-90). We extracted all phase angles located within significant regions of the cross-wavelet spectra but outside the cone of influence (see *37*).

Rotifer ingestion rates. To test the efficiency by which rotifers consumed the general resistant and non-resistant host cells, the amount of cells consumed by predators was assessed over six concentrations of algal cells (1.3 – 3.8 $*10^6$ cells/ml). For each concentration, five rotifers were added to one mL of algae in 24 well plates (three replicates per algal concentrations) and three replicates of the same concentration served as control (no rotifers added). We then calculated the amount of cells consumed after 24h by comparing algal densities of controls with algal densities in the rotifer containing wells. For all tests, algae were diluted in BBM (without ammonium chloride) to minimize algal growth over 24h. A linear model was used to test differences in amount of algae consumed over the different concentrations between general resistant and non resistant hosts.

Eco-evolutionary dynamics. To test for eco-evolutionary dynamics and the relative importance of ecology and evolution in our experimental systems, we used the "Geber-

method" introduced by (*26*). This allowed us to decompose rates of an ecological response into components driven by simultaneous evolutionary change and ecological factors. As a response, we used virus population growth rate calculated from three days around each time-point (time-points were the same as those used in the time-shift experiment). The ecological component affecting virus growth rates was host population density. As evolutionary component, we looked at host resistance when exposed to the contemporary virus population, which reflects both evolution of host resistance and virus infectivity (= coevolution). Host resistance was estimated for 10 host clones per time-point, and the fraction (0-1) of resistant hosts was used as evolutionary component. Ecological and evolutionary contributions were then calculated for each time-point (excluding time-point 0, as hosts and virus were not exposed to each other yet). To further test for differences in ecological and evolutionary components ("contributor" = ecology or evolution), differences over time ("time") and differences between the two experimental systems ("system" = host-virus or complex system,), we used a linear model with absolute values of ecological and evolutionary components as a response, contributor and system as factors and time as a continuous variable (LM: absolute values of ecological and evolutionary contribution to virus population growth ~ contributor x system x time; Table S1).

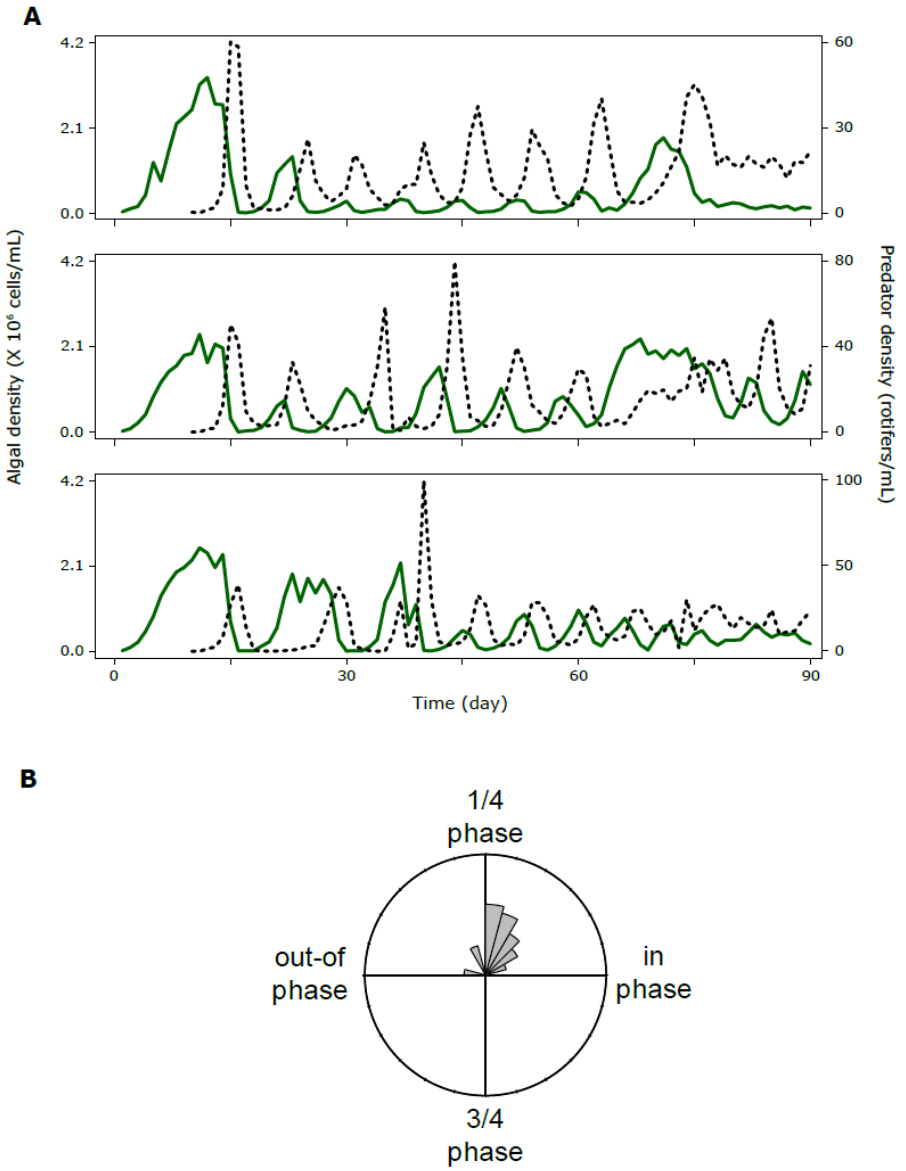

Fig. S1. Population dynamics (a) and phase-shift analysis (b) of predator-prey system. **A)** Daily algal (green full line) and predator densities (black dotted line) in 3 replicate chemostat cultures. **B)** Phase-shift analysis show that rotifers cycle predominately with one quarter lag after algal local maxima.

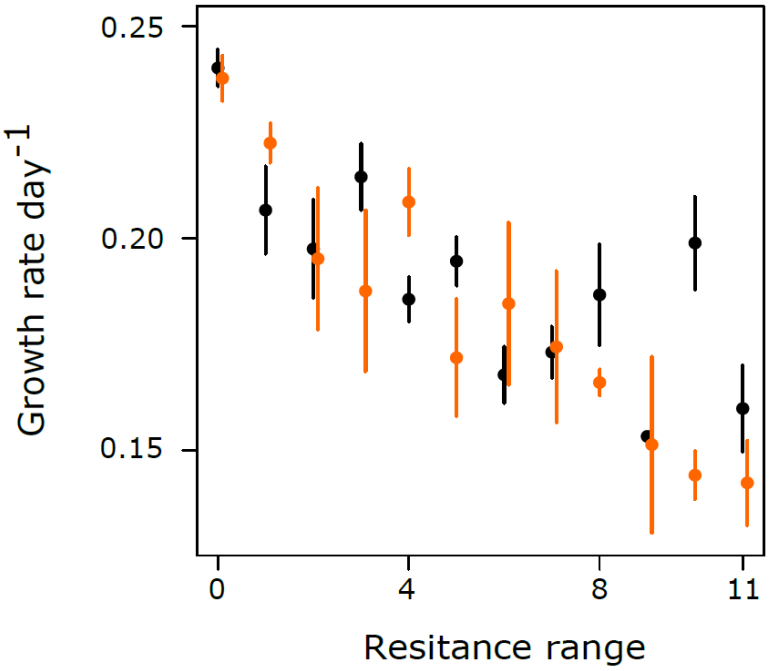

Fig. S2. Trade-off between host resistance and growth. Host per capita growth rate (± se.) decreased with increasing host resistance range (number of virus populations a host is resistant to, maximum is 11). The trade-off observed in the host-virus (black) and complex system (orange) was similar (not significant different). Only hosts from time-points before the predator was added for a second time in the complex system were used (time-points were used for host-virus and complex system).

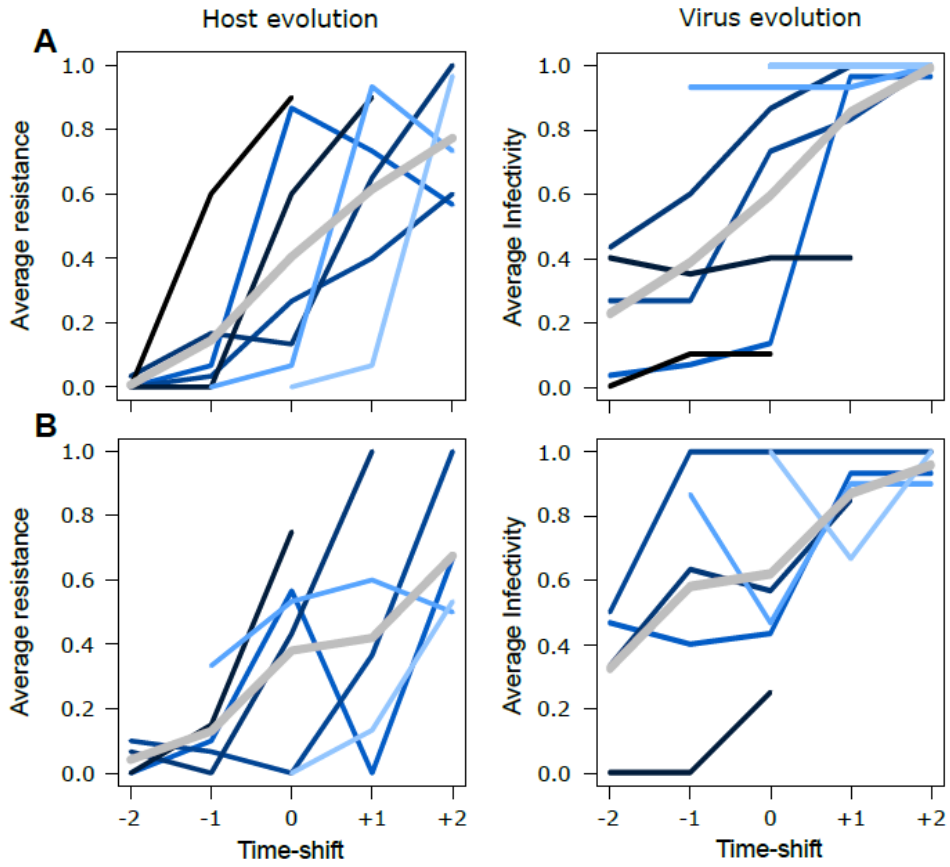

Fig. S3. Coevolutionary dynamics of host and virus in host-virus system (A) and in the complex system (B). A) Host evolution shows directional selection for increasing host resistance. Contemporary hosts (0), hosts from one or two time-points in the future (+1,+2) and past (-1,-2) were exposed to contemporary virus populations. Ten host clones were used for each time-point (per chemostat). Blue shadings (light to dark) indicate the time-point of virus populations (from start of experiments until the time-point when a first general resistant host appeared). Average resistance of three replicate chemsotat is shown per time-shift. Grey is the overall average resistance per time-point. Virus evolution shows directional selection for increasing infectivity. Contemporary virus (0), virus populations from one or two time-points in the future (+1,+2) and past (-1,-2) were exposed to host clones (10 host clones per time-point). Blue shadings (light to dark) indicate host clones used from start until first general resistant host was observed. Average infectivity of three replicate chemostats is shown per time-shift. Grey is the overall average infectivity per time-point and show directional selection for increasing host resistance and virus infectivity over time, comfirming that host and virus were coevolving through arms-race dynamics. **B)** Same analysis was performed with hosts and viruses coming from the complex system and show directional selection for increasing host resistance and virus infectivity, confirming host and virus coevolved through arm-race dynamics.

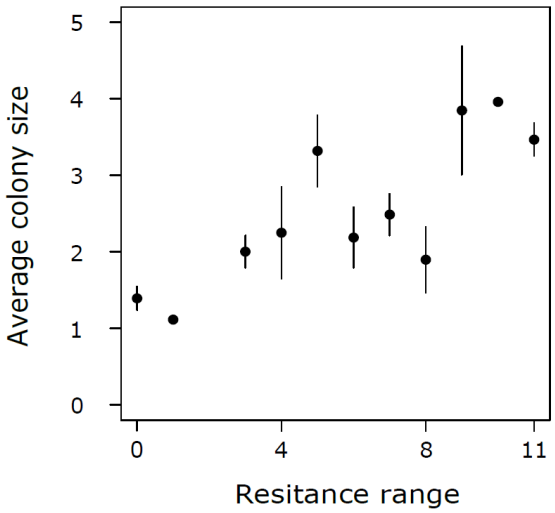

Fig. S4. Host colony morphology. Average host colony size (number of algal cells per colony ± s.e.m.) increased with increasing host resistance.

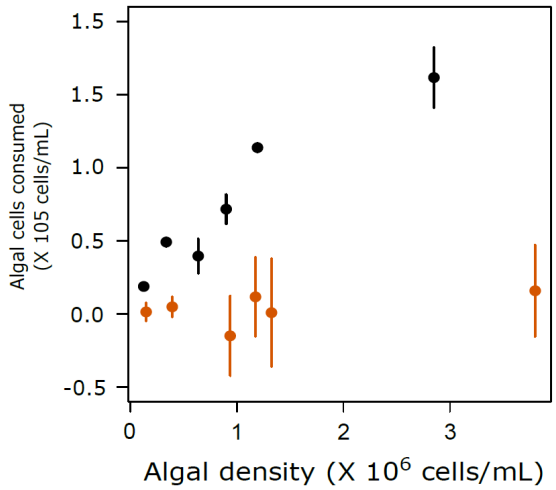

Fig. S5. Predation efficiency on general resistant and not-resistant algae. Average number of algal cells (± s.e.m., N=3) consumed by rotifers in 24h. The predator consumed not-resistant algae (black) more efficient then general resistant algae (orange). The number of consumed not-resistant cells increased with increasing algal densities, but almost no general resistant cells were consumed over all concentrations of algae.

Table S1. Anova table eco-evolutionary dynamics. All interactions for the linear model to analyze eco-evolutionary dynamics over time with increasing complexity are shown. The linear model contained absolute values of ecological and evolutionary contribution to virus population growth as a response with contributor (ecology or evolution) and system (host-virus or complex system) as factors and time as a continuous variable

Single terms	**F value**	**DF**	**P**
Contributor	0.56	1, 96	0.46
System	15.43	1, 96	1.62×10^{-04}
Time	31.14	1, 96	2.21×10^{-07}
Interaction terms			
Contributor X System	1.039	1, 96	0.31
Contributor X Time	2.30	1, 96	0.13
System X Time	11.57	1, 96	9.76×10^{-04}
Contributor X Time X System	0.28	1, 96	0.6

References (33-37)

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CHAPTER FOUR

Manuscript

Coevolution of an algal host with a dsDNA virus drives phenotypic parallelism and the parallel evolution of a large genomic duplication.

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Abstract

Different lineages of the same species that independently evolve similar adaptations in identical environments (parallel evolution) denote a certain level of repeatability of evolution. Parallel evolution is most frequently found on the phenotypic level, but is less frequently observed looking at the underlying genetic level (mutations). Using experimental evolution with many replicate populations provides good methods to replay evolution and test for repeatability (parallelism) of evolution. In particular, experimental antagonistic coevolution provides evidence for both high levels of parallelism and divergence which differ on phenotypic and genotypic level, impeding generalizations about repeatability of evolution. However, both evolutionary dynamics and important feedbacks between ecology and evolution (e.g. population size, bottlenecks and selective sweeps) will affect evolutionary trajectories both on individuals' phenotype and genotype. Thus, a detailed understanding of the population evolutionary and ecological history is needed in order to explain the processes that drive parallel or divergent evolution on different levels of biological organization. Here, we found high levels of phenotypic parallelism (host resistance) between replicate experimental coevolving populations of an asexual eukaryotic host (alga) and a large dsDNA virus. Yet, when examining at the genotypic level, variants (point mutations and small indels) did not show any parallelism in the host populations across replicates. However, all host populations evolved a duplication in a large genomic region, reflecting the parallelism found at the phenotypic level (host resistance). Our results indicate that coevolution drove further genetic divergence (based on variants) between coevolving populations both due to demographic effects and selective sweeps.

Introduction

Throughout evolutionary history, convergent evolution between species is often observed. That is, different species evolved similar phenotypes independently to adapt to similar environments, either through identical genetic changes or with a different underlying genetic basis (1). Convergent evolution through identical genetic changes indicates a certain level of repeatability of evolutionary trajectories and might seem counterintuitive in regard to the randomness at which genetic changes (mutations) occur. To specifically test for the repeatability of evolution, studies using experimental evolution with many replicates of isogenic and asexually reproducing populations of microbes have proven insightful. When identical populations evolve in the same environment independently of each other, two patterns can be observed. Populations can adapt to the same environment differently, resulting in divergent evolution between the populations (e.g. 2), or they acquire the same adaptive solutions (e.g. 3, 4, 5) . In the latter case, convergence between populations of the same species drives the populations to the same fitness peak (6) and evolution is considered to be parallel . Generally, parallel evolution is most frequently found on the level of individual fitness (or phenotype), but looking at the underlying genetic basis of adaptation evidence for parallelism is rare. Indeed, parallel evolution is less frequently observed looking at functional groups of genes, reduces even further when looking at single genes and is finally almost non-existing at the base-pair level with individual point mutations (7-9). Thus, patterns of parallel (and divergent evolution) differ when looking at different levels of biological organization (10, 11).

Antagonistic coevolution between parasites and hosts is of particular interest concerning parallel and divergent evolution, because parasites are thought to be a strong selective force, antagonistic coevolution can accelerate molecular evolution (12) and so, can potentially result in between population divergence (13) . Experimental coevolution with bacteria and bacteriophages added more insights by using genome analysis of coevolving populations. Besides differences based on levels of biological organization, several lines of evidence indicate that parallelism might be related to genome size and organismal complexity (8). For example, relatively high levels of parallelism were found when looking at identical base-pair changes in bacteriophages that coevolved with a host or adapted to a new host type (12, 14-16). However, (base-pair) parallelism seems far less frequent in the more complex bacterial hosts with larger genomes and many more potential targets of selection (8).

Besides adaptive genetic changes due to antagonistic coevolution, several other population genetics parameters can affect degrees of parallelism and divergence between populations. Population size for example determines mutation supply. Bottlenecks with sudden and dramatic reduction of population size and selective sweeps will affect the divergence of populations over the whole genome due to drift and genetic hitchhiking (17-19). These important ecological and evolutionary dynamics can thus affect divergence or parallelism on all levels of biological organization. Importantly, such feedbacks between ecology (e.g. population size) and evolution (e.g. selective sweeps) are inherent parts of the coevolutionary process (20). Thus, besides estimating degrees of parallel and divergent evolution between populations on different levels of biological organization (phenotype and genotype), additional detailed understanding of the populations evolutionary and ecological history is needed in order to explain the processes that drive parallel and divergent evolution.

In this study, we investigate parallel evolution between populations of asexual eukaryotic algal hosts that coevolved with a dsDNA virus. We study the extent of parallel evolution on the phenotypic (evolution of resistance) and genomic level in the algal host. Furthermore, we combine these findings with additional detailed information about population ecological and evolutionary history (e.g. bottlenecks and selective sweeps) and infer their effects on genome wide divergence and parallelism between populations. In a previous study (20) we followed host and virus population densities in continuous cultures (chemostats) over the course of 90 days (Fig. 1a,b) and showed in combination with time-shift experiments that hosts were coevolving with the virus through arms race dynamics with selective sweeps that clearly affected host densities (bottlenecks and rescue; Fig. 1a). Bottlenecks in host densities occurred when no host was resistant to virus, whereas the host populations recovered through evolutionary rescue (hosts evolved resistance to virus) which coincided with selective sweeps of these newly resistant host types. As all our experiments were started from the same isogenic host and virus (without genetic or phenotypic variation) and consisted of three replicate continuous cultures (‘coevolved’ populations; Fig. 1a), we were able to quantify degrees of phenotypic parallel evolution by comparing host resistance in its local population to host resistance in the two other replicates. Doing this for ten time-points during which host and virus were coevolving (from the start to the end of the experiment) gave us a measure of phenotypic parallelism of host resistance. Furthermore, we isolated ten individual host clones from every replicate at the end of the experiments and obtained their whole genome sequences. We then examined whether the degrees of parallel evolution based on host (resistance) phenotypes could be retrieved on the genomic level. To compare adaptations

purely resulting from adapting to the experimental setup, we used three replicate chemostat cultures with only algae (no virus; 'evolved' populations; Fig. 1b) and ten sequenced alga clones (per replicate) from the last day of these experiments.

Results

Experimental evolution.

Demography. We previously described and discussed the population dynamics of algae and virus in detail (20). In the current study, we focused on the algal (host) populations. In short, we found that the demography of algal populations was very different between the evolving and coevolving populations (Fig. 1a,b). Algal densities in the evolving populations were stable at their carrying capacity throughout the entire experiment (Fig. 1b), whereas we observed at least two bottlenecks in the densities of all coevolving populations before they stabilized and steadily increased (Fig. 1a).

Evolutionary dynamics. Using time-shift experiments, we showed that host and virus were coevolving through arms race dynamics (20). We found multiple cycles of hosts evolving resistance to virus and virus evolving to infect previously resistant hosts again. Algal densities recovered (Fig. 1a) after each bottleneck due to evolutionary rescue through the evolution and selective sweeping of new resistant host types. Thus, there was a clear correlation between the evolution of resistance and demography. Hosts became increasingly more resistant over time and finally became general resistant to all virus types. The population dynamics of coevolving populations stabilized through the evolution of general resistant hosts (resistant to all virus types) and the host population at the end of the experiments (day 90) consisted mostly out of general resistant host clones (Fig. 1c; sympatric host-virus combinations).

We here tested whether the host-virus interactions resulted in parallel evolution (same phenotypes evolving across all three replicates) or locally adapted alga and virus populations, which is indicative for divergent evolution (distinct phenotypes across replicates). We calculated the degree of parallel and divergent evolution for ten time-points (from the start till the end of the experiments) between the three replicate coevolving populations following Buckling and Rainey (21) by comparing resistance of hosts with virus from their own replicate chemostat to their resistance with virus form the other replicates. We found high levels of parallel evolution between replicates over time (on average 87%; Fig. 1d). Host

clones from the last day of the experiments (day 90) showed 81% of parallel evolution. Parallel evolution resulted in general resistant hosts, which were resistant to all virus types from their own replicate (Fig. 1c; Sympatric host-virus combinations), but also to all virus types from the other replicates (Fig. 1c; Allopatric host-virus combinations).

Variant discovery.

To address the same question at the genotypic level, we analyzed and identified positions containing variants (single-nucleotide polymorphism: SNPs and small indels) using whole genome sequences of ten individual isogenic clones coming from the last day of every replicate population ('evolved' = 3 replicates x 10 clones, 'coevolved' = 3 replicates x 10 clones). Ten clones of the isogenic population used to start all replicates ('ancestor' = 10 clones) were used to identify variant positions already present in the ancestor population, and these positions were removed from further analysis (for a detailed description, see Materials and Methods). One of the sequenced clones of the evolved populations was discarded from these and further analysis, due to sequencing errors (Table S1b). In order to identify potential adaptive variants, the data set was filtered for variants that were on high frequencies within any evolved or coevolved population (> 70 % contained the variant; Fig. 2a, Fig. S1). A total of 117 positions contained variants at high frequencies in the evolved or coevolved replicates. The coevolved populations showed 5 times more high frequency variants then the evolved populations (coevolved = 94, evolved = 19, coevolved and evolved = 4) and only 4 positions had variants at high frequencies in both the coevolved and evolved populations (Fig. 2a, Fig. S1). Most variants in the coevolved populations were private to one replicate, whereas most variants of the evolved populations were also present in other replicates of the evolved populations, but at lower frequencies (Fig. 2a, Fig. S1).

We used the same data set to construct a genetic distance tree based on Euclidean distance of the frequencies of the variants within every replicate of evolved and coevolved populations. This data set contained only variants that were at high frequencies within one or more replicates of the coevolved or evolved populations (with the corresponding frequency in the other populations) and thus represents general patterns of parallel or divergent evolution between populations. There was a clear separation of evolved and coevolved populations (Fig. 2b). The evolved populations clustered significantly closer to the ancestor (contained less variants at high frequency) then coevolved populations, which evolved further away from ancestor and evolved populations (average distance ancestor-evolved = 31,045,

ancestor-coevolved = 50,66; Fig. S2a: t.test, t=4.18, df=4, p=0.014). Moreover, greater divergence was observed between the three replicates of the coevolved populations then between the replicate evolved populations (average distance between coevolved = 64.89, between evolved = 24.45 Fig. S2a; t.test, t=7.18, df=4, p=0.0020). This analysis with high frequency variants captured general patterns of parallel and divergent evolution of potential adaptive variants. But likewise, the genetic distances calculated with all variants (including variants that were not on high frequencies) and the genetic distance tree showed a similar pattern (Fig. S2b-d).Thus, the divergence from the ancestor and between populations was a general pattern of sequence divergence over the whole genome.

The impact of all high frequency variants was annotated and divided in 5 classes; high (frameshift, splice donor variant), moderate (missense variant), low (synonymous variant) and non-coding (variant in intron or intergenic region). No annotation was available for eight variant positions (chloroplast) and these were removed from further analysis. There were significantly more variants in the coevolved populations (Fig 3a; generalized linear model: number variants ~ treatment, $T_{23,22}$=2.892, p=0.0085). Overall, different amounts of variants were contained within these 5 classes. Most variants were synonymous and thus had a low impact, or were in introns and intergenic regions (Fig 3a). Most importantly however, the distribution of variants within these classes was significantly different between coevolved and evolved populations (Fig 3b; linear model: proportion ~ impact*treatment: $F_{3,16}$=4.068, p=0.025). For example, non-coding variants contributed to half (Fig. 3b; 49 %) of all variants in all three replicates of the coevolved populations, whereas this was much lower in the evolved populations (Fig. 3b; 18 %). Furthermore, most of the variants in the evolved populations were synonymous substitutions (73%; low impact) but synonymous substitutions only made up a small part of all variants in the coevolved populations (22.7%).

Specifically looking at (high frequency) variants within genes, a total of 35 genes contained one or more high, moderate or low impact variant. When a gene had more than one variant, the highest impact variant was used in further analysis to estimate the impact on the gene. Significantly more genes had variants in the coevolved populations (generalized linear model: number of genes ~ treatment: $T_{17,16}$=2.48, p=0.025) and the proportion of genes with non-synonymous substitutions (Fig. 3c; moderate: 38.8% + high: 26.37%) was significantly higher compared to those in the evolved populations (Fig. 3c; moderate: 16.7% + high 5.6%; linear model: proportion ~ treatment * synonymous or non-synonymous: $F_{1,8}$=5.90, p=0.041). All replicates of the coevolved populations had unique set of genes that had a variant (at high

frequency) and only one gene contained variants in both the coevolved and evolved populations.

Structural variation.

We identified one distinct region in the genomes of the coevolved populations that had a significant increase in copy number compared to the ancestor and evolving populations (Fig. 4c, Fig. S3; from one copy to two copies). Copy number increased in all coevolved populations in a region of ~75 kb (Fig. 4c, Fig. S3e-g; 'common region': Table 1). This region contained 15 genes, and no variants were observed in this segment. However, this duplicated region was not the same size between the three replicates of the coevolved populations (Fig. 4d-f). For one replicate population, this duplication started 59 kb upstream of the common region and contained an extra 15 genes ('unique region: Table 1). The two other replicates had extra 2 kb and 7 kb downstream of the common region (containing 0 and 2 genes respectively, Table 1). One replicate of the coevolved populations showed a less clear signal for increased copy number (not in all clones; Fig. 4d, Fig. S3e), which potentially resulted from an overall low coverage (Table S1a). One out of ten sequenced clones in the second coevolved population did not show this increase in copy number (Fig. S3f), whereas all sequenced clones in the third coevolved population showed copy number increase for this region (Fig. S3g). Thus, overall 23 out of 30 clones showed copy number increase in all the coevolved treatments. We found no evidence for copy number increase in the ten sequences ancestor clones (Fig. 4a, Fig. S3a). We did found evidence for copy number increase in the evolved populations, but only in two out of 30 sequenced genomes (Fig. 4b, Fig. S3b). Furthermore, one of the coevolved populations had one additional large duplicated region (38 kb, 'unique region; Table 1) with copy number increase from one to two copies on a different scaffold.

Functional annotation of genes. We looked for gene orthologues (based on *Arabidopsis thaliana*) and performed GO-enrichment analysis to get further insights into the functions and cellular components of genes that contained high and moderate impact variants (high frequency non-synonymous substitutions; Table S2). Genes from the evolved populations did not significantly enrich a particular cellular function, but were generally involved in metabolic processes. Genes from the coevolved populations were significantly correlated with cellular functions of oxygen-evolving-complex and thylakoid part and also had

molecular functions mostly related with metabolic processes (ammonia metabolism, disulfide oxidoreductase activities and DNA polymerase activity).

Genes contained within the common genomic duplicated region (Table S3) were highly correlated with the plasma membrane and small ribosomal subunit, with molecular functions mostly involved in transport activity (vesicle mediated transport, endo- exocytosis, Golgi-apparatus) and protein kinase activity (signal transduction). Specifically, two genes in this region were related to protein kinase activity and signal transduction with orthologue *Arabidopsis thaliana* genes encoding casein kinase 1 like proteins and VH1-interacting kinase proteins. These types of proteins are known to regulate gene expression by numerous extracellular signals (regulate signal transduction pathways). Moreover, protein kinase gene activity is often associated with pathogen exposure in plants (22-24) and RNA silencing (25). Three other genes within this duplicated region were related to transport and had gene orthologues in *Arabidopsis thaliana* encoding Golgi nucleotide sugar transporters, ABC transmembrane transporters and AP2 adaptor complex. Biosynthesis of polysaccharides or glycoproteins (and subsequent transport to the cell wall) is an important defense for plants against pathogens. Furthermore, transport of secondary metabolites with for example ABC transporters or excretion of other substances such as cell wall polysaccharides through vesicle mediated transport (exocytosis, AP2 adaptor complex) have been shown important for the outcome of plant-pathogen interactions (26-28).

Discussion

In our study, we found that antagonistic coevolution between an alga (host) and virus resulted in parallel evolution of resistance between replicated experimental populations. We investigated how parallel evolution correlated with the genomic architecture of coevolved host populations. Although parallel evolution was found on the phenotypic level (evolution of resistance), parallelism did not result from acquiring the same variants (SNPs and small indels). However, the host genomes did show common adaptations by duplication of a large genomic region, which happened repeatedly and independently in all three replicates.

The three replicates of the coevolved populations showed high levels of parallel evolution of resistance, which resulted in general resistant hosts (Fig. 1c, d). Parallel evolution could be inferred from two observations related to host phenotypes. First, we observed high levels of parallel evolution of host resistance over all time-points between the three replicates (Fig. 1d). As we observed multiple coevolutionary cycles between host and virus, high parallelism over all time-points indicated that evolutionary trajectories were similar between the three replicate populations. Moreover, we found that the demography (population dynamics) of coevolving populations was very similar between replicates (Fig. 1a). All three populations showed at least two bottlenecks, with subsequent rescue at more or less similar times. This observation confirmed parallelism of the evolutionary trajectories, but also indicated that the timing at which newly resistant adaptations appeared was similar between the three populations. Second, parallel evolution resulted in general resistant hosts. General resistant hosts from the last day of the experiments were resistant to all virus types (that evolved at any time) from their own coevolving population (Fig. 1c, sympatric), but were also resistant to all virus types from the other coevolving populations (Fig. 1c, allopatric). Thus, parallel evolution drove the coevolved populations to the same fitness peak (general resistant hosts).

It was, however, unlikely that any of the variants we detected were driving evolution of (general) resistance and we did not find any indication of parallel evolution between the replicate coevolved populations. All variants that were on high frequencies in one replicate of the coevolved populations were unique or at lower frequencies in the other replicates (Fig. 2a, Fig. S1). Consequently, all genes that contained variants at high frequencies were unique to one replicate of the coevolving populations. These results indicated that selection for resistance was not targeting variants within the same sites or genes. Moreover, genes that

were affected by non-synonymous variants were mostly involved in metabolic pathways (Table S2), further suggesting that these variants and genes were not adaptive in the context of a coevolutionary arms-race and the evolution of host-resistance. As such, the high level of (phenotypic) parallel evolution could not be retrieved on the genomic level when considering variants.

However, we found a large genomic region (Fig. 3; 75 kb) with copy number increase in all three replicates of the coevolved populations. This region contained 15 genes that significantly related to functions in the plasma membrane (Table S3) and had *Arabidopsis thaliana* orthologues that are known to be important in plant-pathogen interactions (Table S3). Thus, acquiring an extra copy of these genes could be a rapid response in terms of increased gene expression (4) related to pathogen response.

This region was not exactly the same size in all replicate populations (Fig. 4 d-f; Table 1), confirming that the duplication happened in all three coevolved populations independently and was a highly repeatable evolutionary process. Large genomic duplications have been shown to occur relatively frequent in prokaryotic (29-32) and eukaryotic genomes (33-35) in response to limiting resources or as compensation for deleterious mutations and are thought to be a much quicker evolutionary response than for example adaptations through SNPs (29, 34, 36). Moreover, certain regions of genomes are more receptive to such duplications, because they depend on the genetic background in which they occur. Thus, adaptation through large duplications can result in parallelism (30, 33). We indeed found some evidence for copy number increase in the evolved populations (Fig. 4b and Fig. S3b, not in the ancestor population: Fig. 4a and Fig. S3a), but at very low frequency (two out of 30 sequenced genomes). This observation supports the idea that duplications can occur readily in the same region. However, we only detected this at very low frequency in the evolved populations, indicating that the duplication was not adaptive in case of the evolved populations.

In all, the high level of parallel evolution based on host phenotypes was clearly reflected when looking at structural variation. Our results demonstrate that duplication events can occur readily in the same region and lead to parallelism between replicate populations. Importantly, not all host clones from the coevolved populations were general resistant (Fig. 1c). In total, 8 out of 30 sequenced hosts were not general resistant (Fig. 1c, Fig. S3e-g). Six from these hosts did show copy number increase in this region, suggesting that the

duplication indeed increased resistance, but that the duplication alone might not be sufficient for general resistance against all viruses. The evolution of general resistance might require additional adaptions. Nevertheless, the independent evolution of the large duplication in all three replicates together with the functions of genes within this region strongly suggests that this duplication was highly adaptive in terms of resistance.

Adaptation through duplications had further consequences concerning variants in the coevolved populations when comparing with evolved populations. Most variants (at high frequency) in the evolved populations were synonymous substitutions or were in intergenic or intron regions (Fig. 3a,b) and thus, did not result in changes of the amino acid sequences. Consequently, only 4 genes were affected by a variant and these where related to metabolic pathways (Table S2). The small number of genes was to be expected for the evolved populations because these algae were already pre-adapted to their growth medium. Moreover, these populations were at stable and high densities throughout the whole experiment (Fig. 1b), resulting in strong competition between individual algal cells and consequently in effectively purging of deleterious mutations. However, we found 5 times more variants at high frequencies (Fig. 3a) and significantly more variants affecting amino acid sequence in the coevolved populations (Fig. 3a). The distribution of variants within non-coding, high - , low - or moderate - impact classes was significantly different between coevolved and evolved populations. Surprisingly, non-coding variants contributed to half (Fig. 3b; 49 %) of all variants in all three replicates of the coevolved populations, whereas this was much lower in the evolved populations (Fig. 3b; 18 %). As such, we found a more uniform distribution of variants across the genome in the coevolved populations (Fig. 3b). These contrasting observations between evolved and coevolved populations concerning acquired variants resulted from two fundamental differences. We observed at least two bottlenecks in the population densities throughout the experiments, whereas population densities of evolved populations were high and stable (Fig. 1a,b). Furthermore, coevolution resulted in selective sweeps (20), and our analysis indicated a selective sweep of the duplicated region (as this region was the same size within every replicate). Strong genetic drift caused by bottlenecks lead to the fixation of variants randomly distributed across the genome and selective sweeps resulted in variants hitchhiking in the genetic background. Purging selection was outweighed by the adaptive advantage of the duplication and was thus not as effective as in the evolved populations. Moreover, the algal populations were asexual and thus not able to recombine, resulting in strong clonal inference. As a result, these asexual coevolving host populations

acquired more variants, which were at high frequency and had a more uniform distribution across the genome.

These signatures of selective sweeps and drift could further been observed on the genetic distance tree (Fig. 2b, Fig S2b). The evolved populations only diverged little from the ancestor population and divergence was almost entirely driven by variants that were not subjected to purging selection (synonymous substitutions; did not change the amino acid sequences). The divergence between the three replicate evolved populations was a signature of different allele frequencies or, to a lesser extent, a different set of variants between the replicate evolved populations. Differently, the coevolved populations diverged significantly further from the ancestor population, which resulted from more variants that accumulated in their genomes. Furthermore, genome divergence between the three replicate coevolved populations was significantly greater than between the evolved populations (Fig. 2b, Fig. S2b,c). Interestingly, greater divergence between coevolved populations was a direct consequence of strong genetic drift caused by population bottlenecks and hitchhiking of variants due to selective sweeps of i.e. the large duplication. In fact, parallel evolution of this large genomic duplication resulted in further sequence divergence (variants) between these three populations. Thus, looking for patterns of parallel evolution could result in opposite findings depending what level of biological organization is considered and on the type of genomic evolution (variants or structural variation).

In conclusion, we found that parallel evolution of general resistance between coevolved populations could not be retrieved when looking at variants in the host populations. Our results indicated however, that hosts adapted in every replicate independently by copy number variation of the same genomic region. Thus, although parallel evolution is seldom found on the genotypic level of biological organization when looking at variants only, copy number variation could as well be a very common contributor to parallel evolving or coevolving populations as shown in our study. Interestingly, regardless the high degrees of parallelism on the level of resistance phenotypes and duplication, our results indicated that coevolution actually drove further genetic divergence (based on variants) between coevolving populations. Importantly, this divergence was driven both by demographic effects and selective sweeps, which are inherently part of the coevolutionary process.

Materials and Methods

Chemostat experiments.

Continuous flow-through experimental systems (chemostats) consisted of 500 ml glass bottles containing 400 ml of sterile Bold's basal medium where nitrate was replaced by ammonium chloride. Sterile air and medium were supplied continuously at a rate of 10% per day. The cultures were maintained at 20°C with continuous light and were mixed by stirring. One isogenic clone of *Chlorella variabilis* was used to start all chemostat cultures. Purified and concentrated virus was used to inoculate three replicates of the coevolving populations and three replicates of the evolving populations remained virus-free.

Population dynamics.

Samples for assessing population densities were taken daily using standard sterile methods. Algal densities were enumerated by counting algal cells in life samples using a hemacytometer. Samples for assessing virus densities were filtered through a 0.45 μm cellulose syringe filter, the filtrate fixed with 1:100 gluteraldehyde and stored at -80°C after freezing in liquid nitrogen. Daily virus densities were counted later by flow cytometry following Brussaard (37) and Frickel, Sieber and Becks (20).

Host resistance and quantification parallel evolution.

Host resistance range (sympatric host-virus combination: Fig. 1c) of hosts isolated at day 90 from the experiments was calculated as to how many virus populations from their own replicate a particular clone was resistant to. To do so, each host was tested against 11 virus populations separately coming from different time-points from start to end of the experiment. Thus, a maximum resistance of 11 means these alga clones were general resistant (to all virus populations). During the experiments, virus samples were stored (at 4°C after filtering through 0.45 μm cellulose filter) at regular time-intervals from the start of experiments to the end of the experiments (eleven time-points in total = eleven virus populations). Algae from the last day of the experiments were plated on agar plates and 10 random algal clones were picked from these agar plates and cultured in batch culture. Each algal clone was diluted to equal densities and challenged to the virus population (virus densities diluted to a MOI of 0.01 particles/algal cell, 4 technical replicates per combination) from each time-point separately (10 algal clones X 11 virus populations) in 96 well plates. Growth rates of algae

exposed to the virus were calculated based on OD measurements after 0h and 72h. To assess whether the algal clones were resistant or susceptible to a particular virus population, we compared the mean growth rate plus 2 standard deviations of four technical replicates to the mean growth rate minus 2 standard deviations of the control (host clone growth rates without virus). If the virus treatment value was smaller than the control, the algal clone was considered susceptible to this particular virus population. If the virus treatment value was greater than the control, these algae were considered resistant to this particular virus population. Allopatric host resistance range (Fig. 1c) was calculated similarly, but hosts were exposed to 11 virus populations from the different coevolving populations.

Degrees of divergent and parallel evolution between the three replicate coevolved populations was calculated following Buckling and Rainey (21). We calculated degrees of parallel evolution for each time-point from which virus populations were isolated to calculate host resistance range (11 time-points). To do so, algae from each of these time-points were conserved on agar plates. From every time-point, 10 random host clones were selected from the agar plates and grown in batch cultures. Each of these hosts were separately exposed to the virus population isolated from their own chemostat (and from the same time-point from which that particular alga were isolated) and to the virus population isolated from the two other chemostats. Resistance and susceptibility of each algal clone was then assessed similarly as described above, and was used as a binary response variable and virus (from which replicate population isolated) and algal populations (from which replicate population isolated) and their interaction as factors in a generalized linear model. The deviance explained by the main effects (deviance main effects/ (deviance main effects + deviance interaction)) provided an estimate of the degree of parallel evolution, while the interaction provided an estimate of divergent evolution (deviance interaction/ (deviance main effects + deviance interaction)).

Genomic data and analysis.

We obtained whole genome sequence reads by NGS (Illumina Nextseq 500 high throughput sequencing platform) of ten individual isogenic clones coming from the last day (day 90) of every replicate of the coevolved (3 replicates x 10 clones = 30) and evolved (3 replicates x 10 clones = 30) populations and from the (isogenic) ancestor population (10 clones) that was used to start all the replicates. To isolate individual host clones, single colonies were picked from agar plates and grown briefly to sufficient densities in the same growth medium

(modified BBM). Algal cells were concentrated by centrifugation, and potential bacterial cells were removed using a sucrose-density gradient. Algal DNA was extracted using CTAB-DNA extraction method (38).

The whole genome reads were mapped to the reference genome (39) using the bwa-mem (URL: http://bio-bwa.sourceforge.net/) tool with the default parameters and variants were identified using standard GATK pipeline via HaplotypeCaller following the best practice for variant calling (40; https://www.broadinstitute.org/gatk/) . One of the sequenced clones of the evolved populations was discarded from these and further analysis, due to sequencing errors (Table S1b). Variants were called with ploidy set to one (haploid and isogenic algal genomes). We removed all variant positions found in the ancestor population from the data set containing all potential variant positions (from coevolved and evolved populations). The data set was filtered for variants that were on high frequencies within every replicate population (> 70 % contained the variant) and all variants were then annotated using SnpEff (41; http://snpeff.sourceforge.net/) and a modified version of the reference annotation file (39). The genetic distance between all replicate populations (evolved, coevolved and ancestor) was calculated based on the frequency of variants using Euclidean distances and a genetic distance tree was constructed using hierarchical cluster analysis based on the distance matrix and the phylogenetic plot function of the ape package in R (42). We tested for significant differences between the genetic distance of every evolved and coevolved population relative to the ancestor using student t-test after testing and confirming equality of variances ($F_{2,2}$=0.18, p=0.31). We tested for significant differences of the genetic distance between the three coevolved populations and the three evolved populations using student t-test after testing and confirming equality of variances ($F_{2,2}$=0.83, p=0.91).

In order to identify copy number variation we used mrCaNaVaR program in conjucture with the mrFAST alignment tool (43). We only identified large structural variants excluding simple repeats and mobile elements. As such, simple repeats and mobile elements were annotated using Repeatmasker (44; http://www.repeatmasker.org/) and Tandem Repeat Finder (45; https://tandem.bu.edu/trf/trf.html). The reads were then aligned to all possible locations on the reference that they can align within the given edit distance. We estimated the actual copy number for each segment (500 bases) using mrCaNaVaR read depth method after normalizing for GC content and mapping depth within every sample. We used the DeSeq2 R-package (46) to identify regions with significant copy number change comparing general resistant hosts (coevolving populations) with non-resistant hosts (ancestor and evolving

populations). Large duplications were identified by looking for significant copy number increase of 500 bp regions that were within 1000 bp proximity of one another. Finally, two large duplicated genomic regions were found and breakpoints were identified manually with IGV-browser.

Functions of genes were inferred by using the algal functional annotation tool (47; http://pathways.mcdb.ucla.edu/algal/index.html) by looking for *Arabidopsis thaliana* orthologues.

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Figures

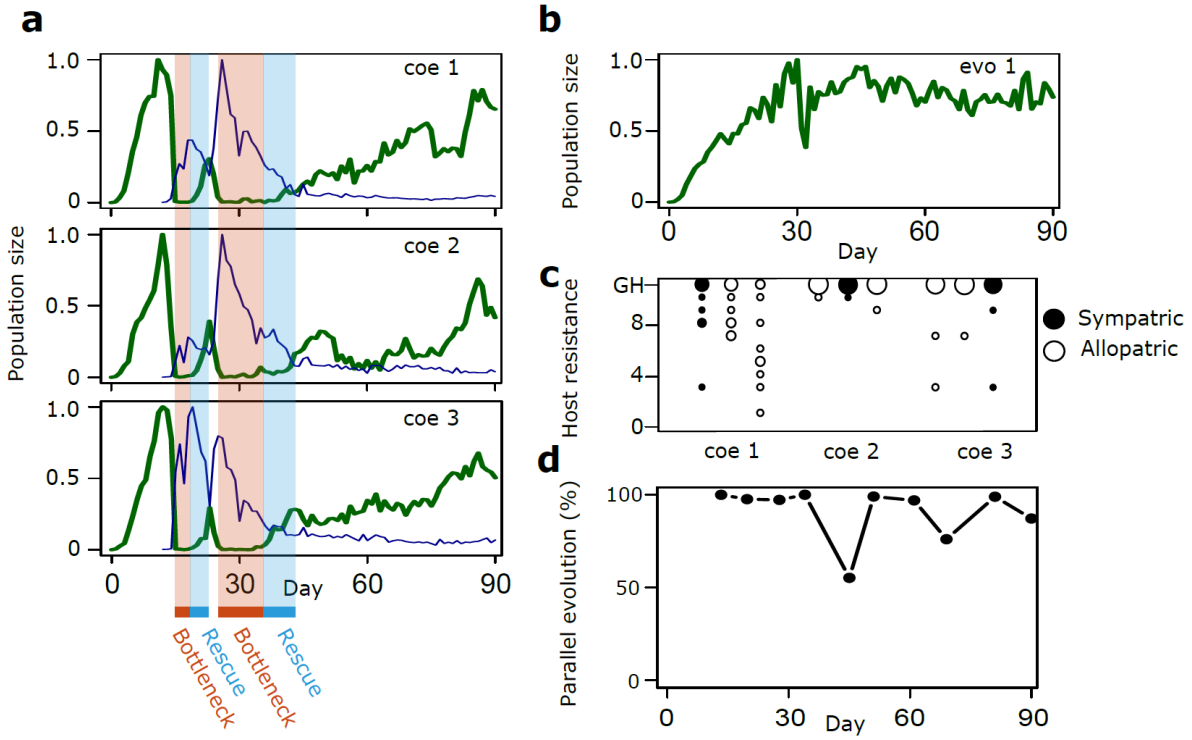

Fig. 1 | Population dynamics and parallel evolution. a) Population dynamics algal host (green) and virus (blue) in three replicate continues cultures over 90 days. Population size of host (green lines) and virus (blue lines) was normalized to maximum values within replicate. Host population dynamics show two bottlenecks (orange-shaded) followed by rescue (blue-shaded) in every replicate. **b)** Population dynamics of algae (green lines) in continues culture without virus over 90 days. Population size of algae was normalized to maximum values within replicate. Population dynamics show an initial increase to high densities followed by stable densities around carrying capacity. Only on out of three replicates is shown. **c)** Host resistance (susceptible = 0 to general resistant host = GH) of 10 algal clones per replicate of the coevolved populations. Algal hosts were isolated from day 90 and for every replicate population, host resistance was tested to all virus types (isolated from 11 time-points, ranging from day 0 to day 90) from their own replicate (sympatric host-virus combination) and to all virus types form the other two replicates (allopatric host-virus combinations). The size of the dots correlates with how many hosts had that particular resistance range. Most algae were general resistant at the last day of the experiments (sympatric host-virus combinations) and hosts that were general resistant were also general resistant when tested against all virus populations of the other replicates (allopatric host-virus combinations) **d)** Degrees of parallel evolution between the three replicates of the coevolving populations. Parallel evolution was calculated for 10 time-points ranging from day 12 to day 90. Hosts of the three replicate evolved highly parallel over time (average = 87%) and hosts from the last day of the experiments showed similar high levels of parallelism (81 %).

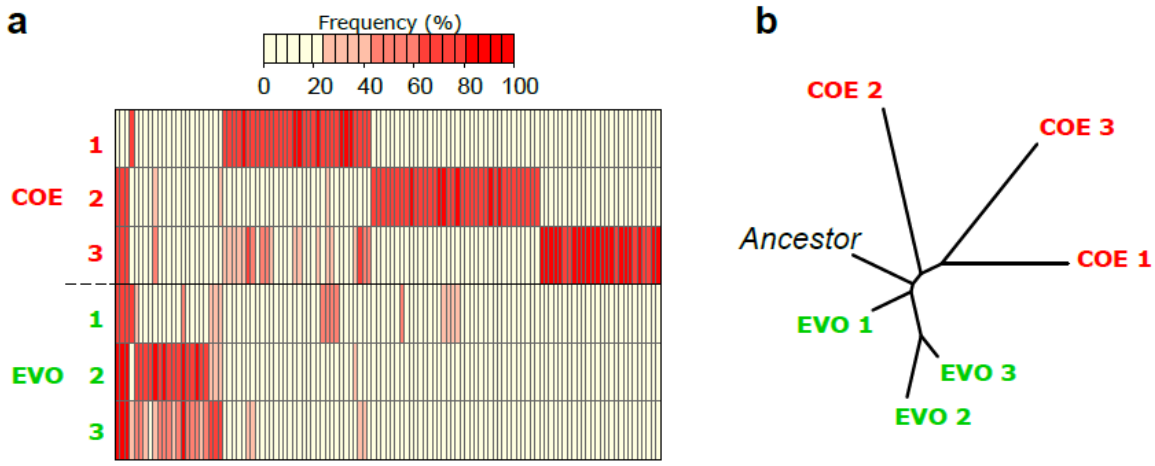

Fig. 2 | High frequency variants and genetic distance tree. a) All variants that were on high frequency in one (or more) replicate of the evolved ('EVO') or coevolved ('COE') populations. Colors represent the frequency of the variants within replicates (n=10 per population) and variants were clustered by occurrence in a population (not arranged according to genome position). Most variants found in the evolved populations were also found in another replicate of the evolved populations and most variants found in the coevolved populations were unique to one population. **b)** Genetic distance (high frequency variants) tree representing genetic difference based on Euclidean distances calculated from the frequency of variants in each population. Evolved populations cluster together close to the ancestor. Coevolved populations diverged further away from ancestor and evolved populations, and show greater divergence between replicate populations than evolved populations.

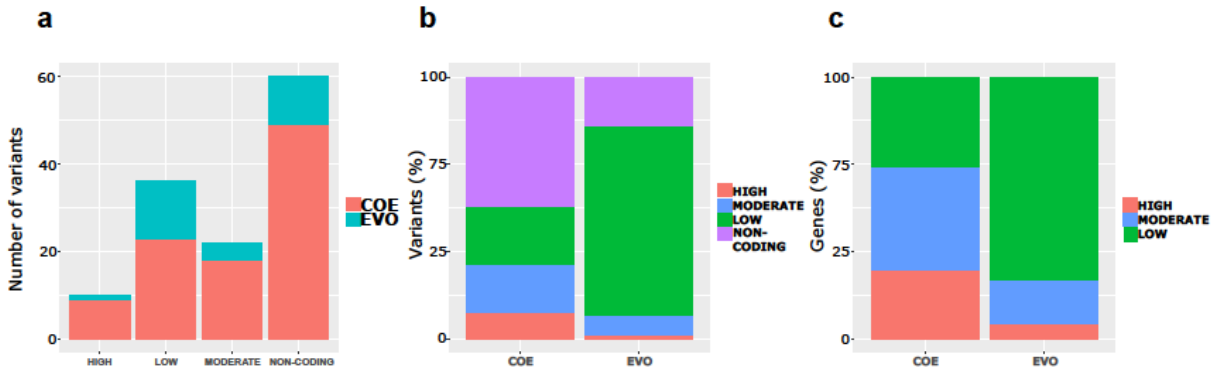

Fig. 3 | Impact per variant and per gene. a) Total amount of (positions containing) variants per impact-class in coevolved and evolved populations. Common variants were included in the total number of variants. Coevolved populations had more variants then evolved populations. Most variants were in non-coding areas or were synonymous (low-impact) **b)** The relative distribution (%) of variants within impact classes in coevolved and evolved populations. The distribution of variants was significantly different between evolved and coevolved populations. **c)** The relative distribution (%) of genes impacted by high, moderate or low variants in coevolved and evolved populations. Most genes in the evolved populations had synonymous variants (no change in amino acid sequence), whereas there were relatively more genes with high and moderate variants (change in amino acid sequence) in the coevolved populations.

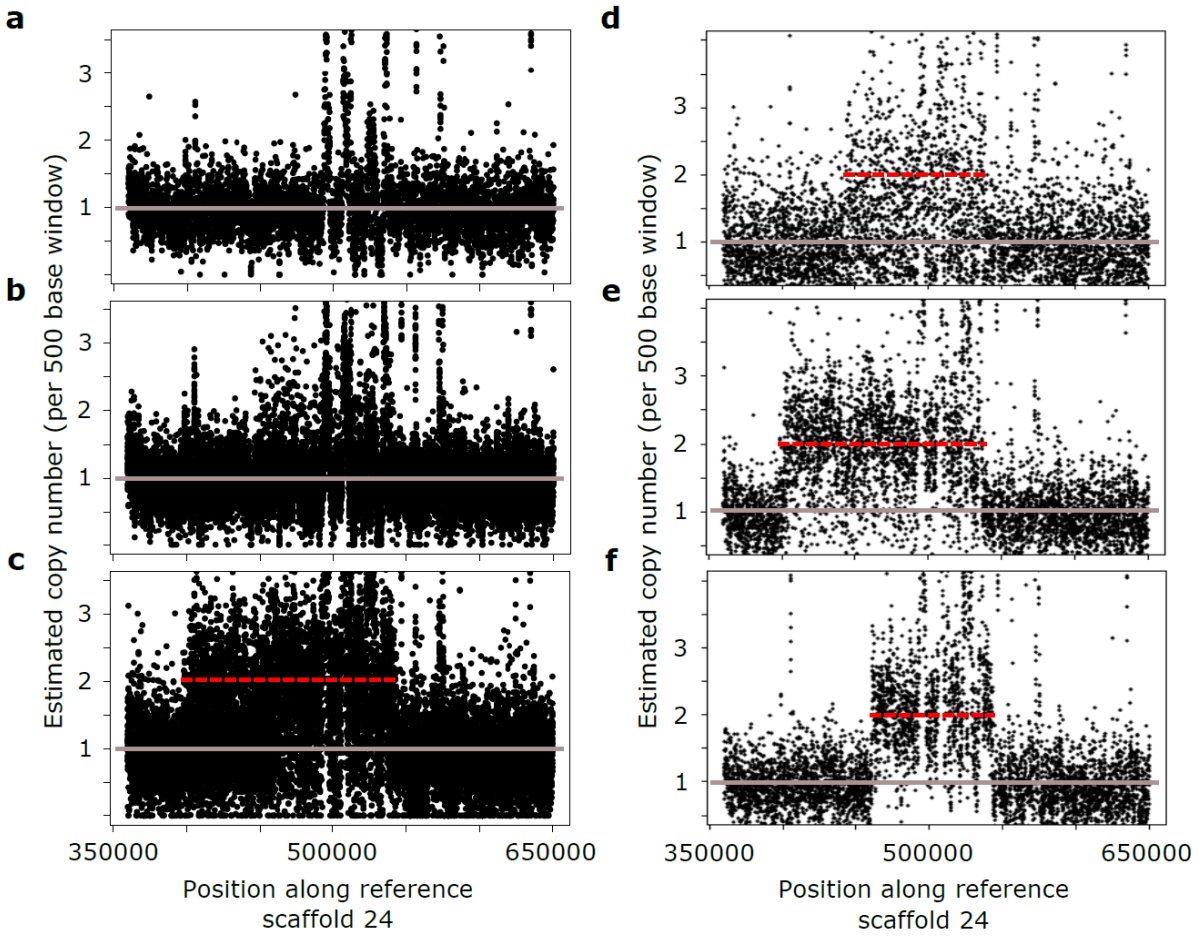

Fig. 4 | Copy number variation. a-f) Estimated copy number for a region from position 350000 to 650000 on scaffold 24. Each dot represents the estimated copy number in a 500 bases window (after correcting for GC content and normalization to average mapping depth). Grey lines indicate 1 copy and dashed red lines indicate regions with copy number increase. **a)** Estimated copy number in the ancestor population (n=10). **b)** Estimated copy number in the evolved populations (n=30). **c)** Estimated copy number in the coevolved populations (n=30). **d-f)** Estimated copy number in the coevolved populations shown for each replicate population separately (for each n=10). The duplication in every replicate coevolved population is different in size.

Tables

Table 1 | Duplication. a) Start and stop position of duplication in the three coevolved replicates. b) Start and stop position and amount of genes contained within the common genomic duplication region (common in all three coevolved populations) and unique region (not common in all coevolved populations).

a.

Scaffold_24				
Replicate	**Start_pos**	**Stop_pos**	**Length**	**Copy_Number**
1	401815	536360	134545	2
2	443410	538526	95116	2
3	461179	543338	82159	2
Scaffold _6				
Replicate	**Start_pos**	**Stop_pos**	**Length**	**Copy_Number**
1	-	-	-	-
2	-	-	-	-
3	1983243	2021686	38443	2

b.

Common region - Scaffold _24				
Replicate	**Start_pos**	**Stop_pos**	**Length**	**Genes**
All	461179	536360	75181	15
Unique region - Scaffold _24				
Replicate	**Start_pos**	**Stop_pos**	**Length**	**Genes**
1	401815	461179	59364	15
2	443410	461179	17769	4
	536360	538526	2166	0
3	536360	543338	6978	2
Unique region - Scaffold _6				
Replicate	**Start_pos**	**Stop_pos**	**Length**	**Genes**
3	1983243	2021686	38443	3

Supplementary information

Figures

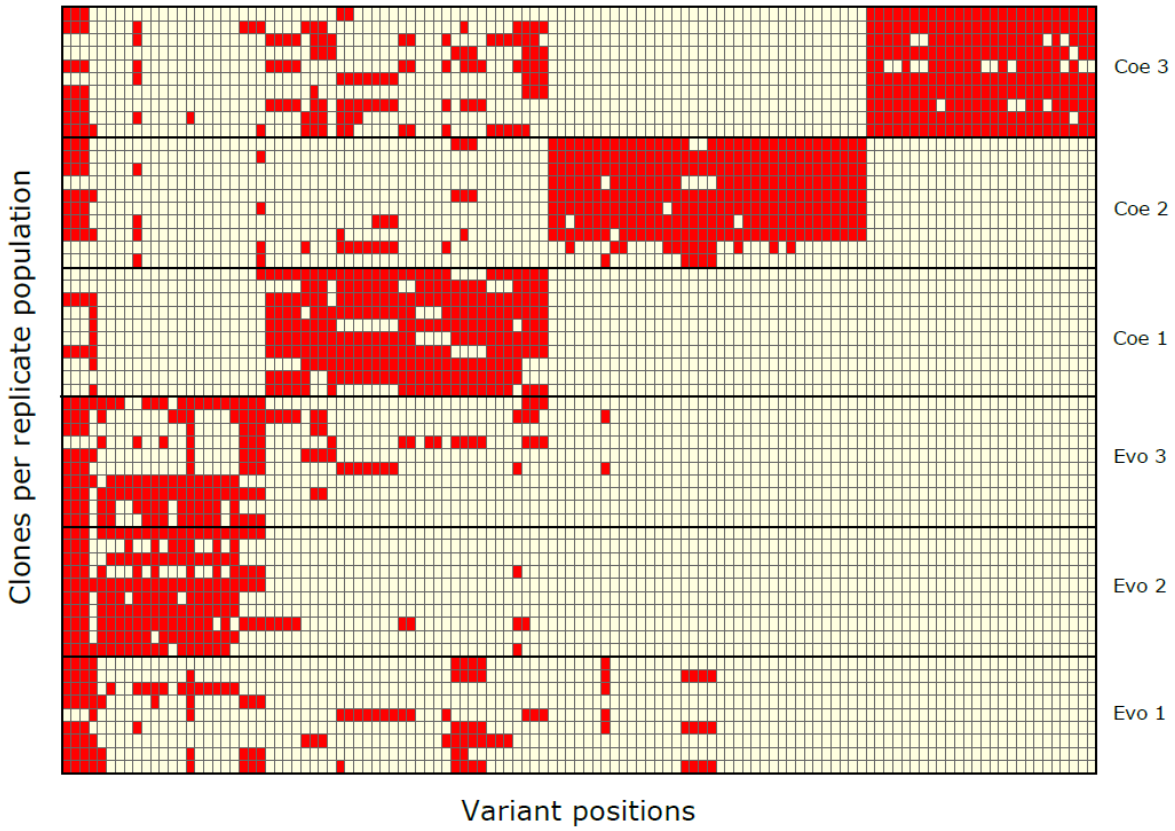

Fig. S1 | High frequency variants per sequenced clone. All variants that were on high frequency in one (or more) replicate of the evolved or coevolved populations. Every column represent a variant position and red colors indicate this variant was present, yellow indicates the variant was absent in that clone. Variants were clustered by occurrence in a population (not arranged according to genome position) Evolved 1 population only contained 9 clones, because one of the 10 sequenced clones was discarded from these and further analysis due to sequencing errors (Table S1b). Most variants found in the evolved populations were also found in another replicate of the evolved populations and most variants found in the coevolved populations were unique to one population.

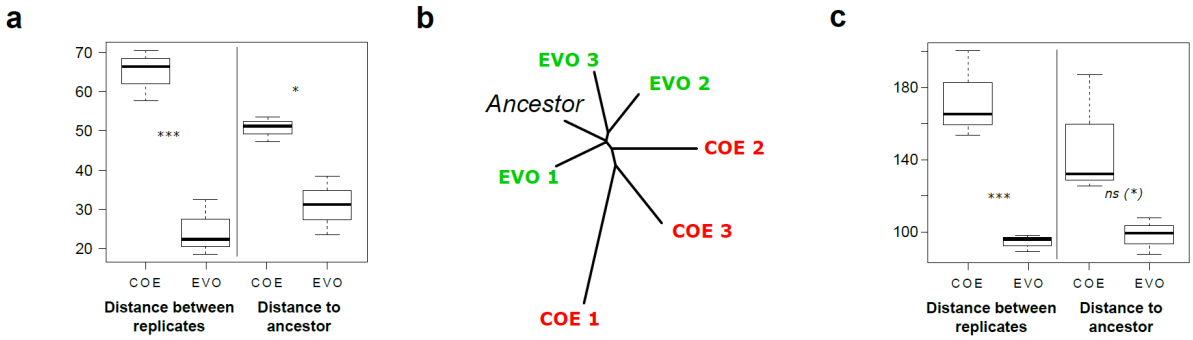

Fig. S2 | Genetic distance. a) The genetic distance based on the high frequency variant data set and calculated as Euclidean distance between replicate coevolved ('COE') populations was significantly greater then between evolved ('EVO') populations (t.test, t=7.18, df=4, p=0.0020). The distance between each population and the ancestor was significantly greater for the coevolved populations (t.test, t=4.18, df=4, p=0.014). **b)** Genetic distance tree based on all variants showed a similar pattern as in Fig. 2b. Evolved populations cluster together close to the ancestor. Coevolved populations diverged further away from ancestor and evolved populations, and show greater divergence between replicate populations than evolved populations. Note that COE 1 population showed a much greater genetic distance. This is most likely due to problematic variant calling because of poor sequence quality (Table S1) resulting in many unreliable low-frequency variants, increasing the distance in this populations. **c)** The genetic distance based on all variants between replicate coevolved ('COE') populations was significantly greater then between evolved ('EVO') populations (t.test, t=5.08, df=4, p=0.0071). The distance between each population and the ancestor was not significantly different due to the inflated distance in COE 1 population most likely as a result of poor sequence quality (*ns*: t.test, t=5.08, df=4, p=0.083). However, when excluding this replicate from analysis, the distance from every population to the ancestor was significantly different (*:t.test, t=5.08, df=4, p=0.025).

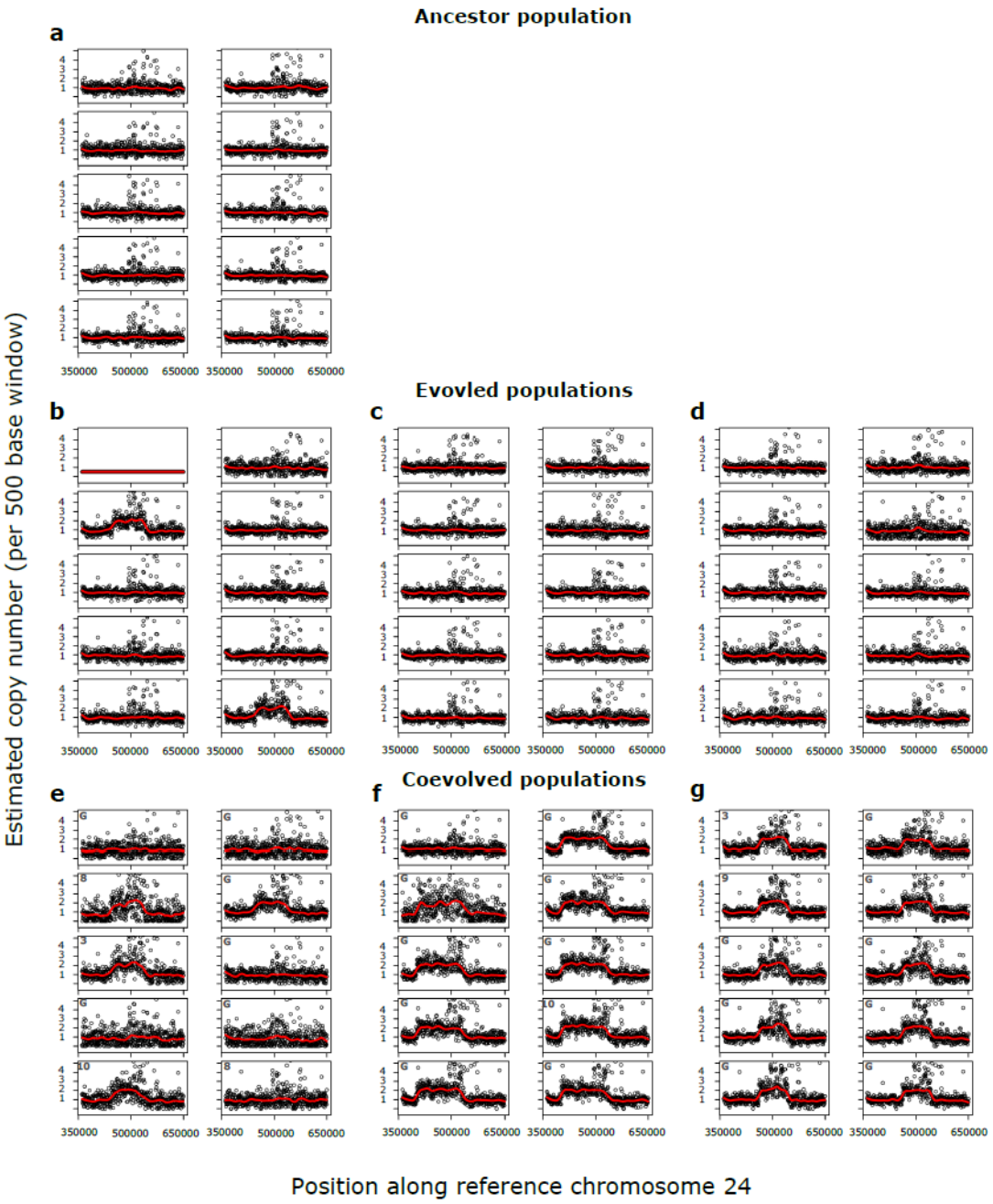

Fig. S3 | Copy number variation. Estimated copy number for all sequenced clones for a region from position 350000 to 650000 on scaffold 24. Each dot represents the estimated copy number in a 500 bases window (after correcting for GC content and normalization to average mapping depth). Red lines are averages inferred by fitting a smooth polynomial regression curve. **a)** Estimated copy number in the ancestor population. **b-d)** Estimated copy number in the evolved populations (b-d correspond to Evo 1 - Evo 3 respectively). **e-f)** Estimated copy number in the coevolved populations (e-f correspond to Coe 1 - Coe2 respectively). Letters and numbers in the left upper corner of every clone represents how resistant this host clones was (G = general resistant or resistant to 11 virus populations, 0-10 = resistant to 0-10 virus populations).

Tables

Table S1 | Summarizing statistics mapping quality. a) Average coverage (read depth) was calculated for scaffold 24 and scaffold 1. Average insert size was estimated for all reads. The percentage of the genome that had no coverage, more than 10 mapped reads and more than 5 mapped reads was calculated per base along the reference genome. b) Summarizing statistics for every sequenced clone of evolved 1 population.

a.

	Average coverage (Scaffold 24)	Average coverage (Scaffold 1)	Average insert size	No coverage (%)	> 10 coverage (%)	> 5 coverage (%)
Ancestor	10.77	10.02	127.28	3.9	40.11	67.67
Coe1	2.96	2.71	31.81	30.94	3.94	15.99
Coe2	9.52	7.43	106.3	8.4	28.11	60.08
Coe3	8.9	7.33	81.85	6.79	26.22	55.92
Evo1	6.55	6.13	136.58	13.94	20.43	51.78
Evo2	9.98	9.57	173.96	3.17	39.87	70.62
Evo3	7.51	7.42	134	7.29	27.99	57.39

b.

	Average coverage (Scaffold 24)	Average insert size	No coverage (%)	> 10 coverage (%)	> 5 coverage (%)
1	0.00	133.33	99.99	0.00	0.00
2	7.89	101.27	13.04	21.32	59.85
3	9.10	129.77	11.49	35.35	71.41
4	3.96	179.20	16.89	5.10	34.77
5	7.84	100.75	12.42	23.85	63.02
6	3.33	113.44	22.05	4.07	25.31
7	10.12	119.45	10.89	42.58	75.49
8	6.13	163.90	13.13	15.25	55.27
9	10.08	109.45	11.05	40.49	74.46
10	7.08	215.23	12.35	16.34	58.18

Table S2 | GO-enrichment with genes containing variants. Genes containing high or moderate impact variants (non-synonymous variants) were used for identifying gene orthologues based on *Arabidopsis thaliana*. Gene orthologues and molecular functions were identified for the evolved and coevolved populations and we looked for particular cellular functions associated with the same set of genes.

Gene Ontology results -- based on Arabidopsis orthology

Molecular Function	**Hits**	**Score**	**Pathway**
Evolved popualtions			
GTP diphosphokinase activity	1	9.88E-04	metabolic pathway
ATP-dependent DNA helicase activity	1	2.47E-03	metabolic pathway
diphosphotransferase activity	1	2.96E-03	metabolic pathway
DNA helicase activity	1	4.93E-03	metabolic pathway
DNA-dependent ATPase activity	1	8.38E-03	metabolic pathway
ATP-dependent helicase activity	1	2.16E-02	metabolic pathway
purine NTP-dependent helicase activity	1	2.16E-02	metabolic pathway
helicase activity	1	3.33E-02	metabolic pathway
ATPase activity, coupled	1	4.73E-02	metabolic pathway
Coevolved populations			
acid-ammonia (or amide) ligase activity	1	5.92E-03	metabolic pathway
ammonia ligase activity	1	5.92E-03	metabolic pathway
glutamate-ammonia ligase activity	1	5.92E-03	metabolic pathway
protein disulfide oxidoreductase activity	1	1.47E-02	metabolic pathway
disulfide oxidoreductase activity	1	2.64E-02	metabolic pathway
aminopeptidase activity	1	3.22E-02	metabolic pathway
DNA-directed DNA polymerase activity	1	4.93E-02	metabolic pathway

Cellular Function	**Hits**	**Score**
Evovled populations		
-	-	-
Coevolved populations		
oxygen evolving complex	1	1.23E-02
thylakoid part	2	4.07E-02

Table S3 | GO-enrichment with genes contained within the common duplicated region of the coevolved populations. The 15 genes contained within the common duplicated genomic region in all coevolved populations were used for identifying gene orthologues based on *Arabidopsis thaliana*. Gene orthologues and molecular functions were identified and we looked for particular cellular functions associated with the same set of genes.

Gene Ontology results -- based on Arabidopsis orthology

Molecular Function	**Hits**	**Score**	**Pathway**
Genes in common duplication Coevolved populations			
phosphatidylinositol-4-phosphate phosphatase activity	1	2.72E-03	transport
phosphatidylinositol-4,5-bisphosphate 5-phosphatase activity	1	5.43E-03	transport
nucleotide-sugar transmembrane transporter activity	1	5.43E-03	transport
phosphatidylinositol bisphosphate phosphatase activity	1	5.43E-03	transport
phosphoinositide 5-phosphatase activity	1	5.43E-03	transport
protein kinase activity	2	1.59E-02	signal transduction
protein serine/threonine/tyrosine kinase activity	1	1.62E-02	signal transduction
inositol or phosphatidylinositol phosphatase activity	1	2.15E-02	transport
transporter activity	3	3.15E-02	transport
phosphotransferase activity, alcohol group as acceptor	2	4.15E-02	transport
carbohydrate transmembrane transporter activity	1	4.53E-02	transport

Cellular Function	**Hits**	**Score**
Genes in common duplication Coevolved populations		
plasma membrane of cell tip	1	2.77E-03
plastid small ribosomal subunit	1	2.77E-03
organellar small ribosomal subunit	1	5.54E-03
plasma membrane	4	5.74E-03

Contributions to the thesis

Chapter one

This chapter was published in the journal Frontiers in Ecology and Evolution (May 2014).

Citation: Koch H, Frickel J, Valiadi M and Becks L (2014) Why rapid, adaptive evolution matters for community dynamics. Front. Ecol. Evol. **2**:17. doi: 10.3389/fevo.2014.00017.

Jens Frickel (JF), Hanna Koch, Martha Valiadi and Lutz Becks (LB) wrote the paper.

Chapter two

This chapter was published in Ecology Letters (February 2016).

Citation: Frickel J, Sieber M and Becks L (2016) Eco-evolutionary dynamics in a coevolving host-virus system. Ecol. Let. 19 (3): In press. doi: 10.1111/ele.12580

JF and LB conceived and designed the study, JF performed experiments, Michael Sieber developed and analysed the model, JF, Michael Sieber and LB analysed the results and wrote the paper.

Chapter three

This chapter will be submitted to Science.

JF and LB conceived and designed the study, JF performed experiments, JF and LB analysed the results and wrote the paper.

Chapter four

This chapter will be submitted to PNAS.

JF, LB and Philine Feulner conceived and designed the study, JF performed experiments. Sven Kuenzel performed sequencing. JF, Emre Krakoc and LB analysed the results. JF and LB wrote the paper.

General conclusion

In this thesis study, I develop a novel model system to study eco-evolutionary dynamics with antagonistic coevolving populations. Overall, the results show how ecological and evolutionary processes are entangled in many complex ways and substantially affect each other. Thereby, my study contributes to the eco-evolutionary dynamics research field because this type of biotic interactions (antagonistic coevolution) was previously not fully integrated within an eco-evolutionary framework. Ecology and evolution are indeed tightly linked in such coevolving populations through changes in population densities and selection imposed by one antagonist on the other. As such, they drive the generation of variation and, depending on the type of coevolution, the maintenance of trait variation within populations. Furthermore, I show that changes in the direction and strength of selection, together with changes in population sizes are inherent parts of eco-evolutionary dynamics. This observation indicates that eco-evolutionary dynamics can introduce a certain level of unpredictability regarding the rate and trajectories of coevolving populations. Although a direct test of this prediction should provide additional evidence, my results show that to fully understand the evolutionary dynamics and trajectories of such populations, a detailed understanding of the demographic history is necessary. These results underline the importance to integrate eco-evolutionary dynamics while studying antagonistic coevolution.

In a more general perspective, an interesting aspect of this study is that I show rapid and extensive (multiple cycles of) coevolution between a eukaryotic algal host and its virus. Increasingly more studies focus on the ecology of alga-virus interactions (e.g. termination of algal blooms), but they typically do not consider rapid antagonistic coevolution. My study shows that both antagonists can potentially evolve rapidly and that algae can evolve general resistance (against at least one strain of the virus). In an ecological context, these findings are important considering how viruses can affect host mortality and thereby influence nutrient and energy cycling as well as plankton community structure (Suttle *et al.* 1990; Fuhrman 1999; Suttle 2007; Short 2012). A detailed field study involving time-series analysis of population densities in combination with evolutionary changes (e.g. time-shift experiments) over time would contribute greatly to this research field.

Fig. 1 summarizes how eco-evolutionary dynamics can operate over different levels of biological organization (see Bailey *et al.* 2009) and how my thesis work relates to them. The work in chapter two for example demonstrates how phenotypes within populations evolve

(e.g. different resistant host and infective virus types) as a result of the interaction between populations within a community, and how such phenotypic variation can influence population dynamics and community stability in return. The species interactions in this study (chapter two) are necessarily all direct interactions, as only two species are interacting. Yet, the results of chapter three show that the effect of eco-evolutionary dynamics does not decrease when considering more complex systems (with more than two species). Although the interaction between ecology and evolution becomes much more complex, operating through direct and indirect cascading and potentially delayed effects, they can even affect community structure and the coexistence of species. It is important to note that this observation also challenges our ability to infer for example previous selection dynamics, or making future predictions regarding biodiversity and community structure. I show for example that predation can have long lasting (transgenerational) indirect effects (ecological and evolutionary) on other members of the community, even when the predator population already went extinct. When sampling a population only at one certain time-point, such effects can thus be missed.

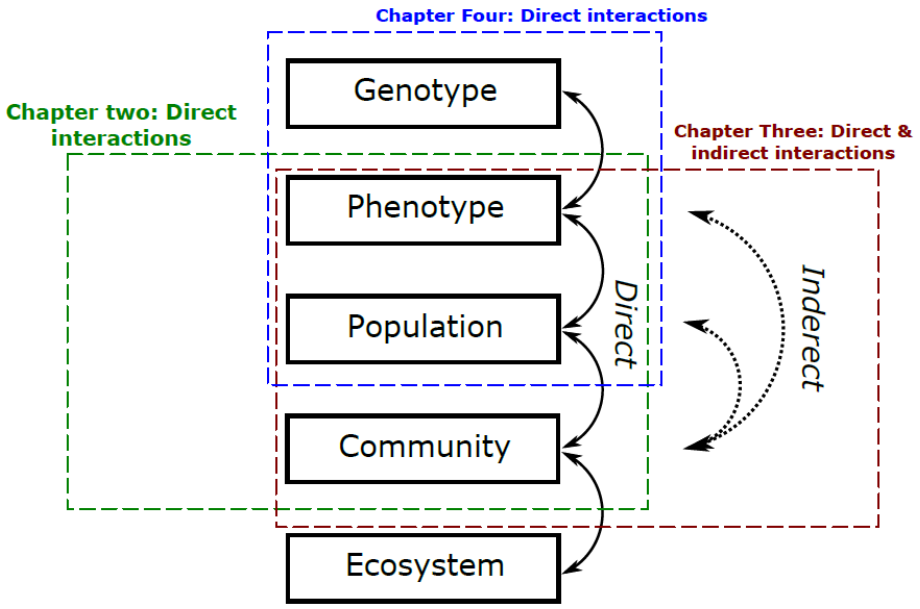

Figure 1 | Conceptual framework of eco-evolutionary dynamics. Interactions between ecology and evolution operate through many levels of biological organization. For example, genetic and phenotypic variation affects population dynamics, community structure and ecosystem functioning. Similarly, community structure can affect phenotypic or genetic variation within populations. Full arrows (direct-interactions) indicate direct effects. Dashed arrows (indirect-interactions) indicate indirect effects, for example when one species is indirectly affected by another through a third species (they are not directly interacting). Boxes in different colors show what level of biological organization is investigated in which chapter of this thesis. Modified from Bailey *et al.* (2009).

Other studies using experiments and mesocosms studied eco-evolutionary dynamics in systems with many species. They showed for example that evolutionary changes can affect other trophic levels in an ecosystem (Post *et al.* 2008; Palkovacs *et al.* 2009; Bassar *et al.* 2010), but these studies were performed over a few generations or by testing the effect of different phenotypes on the ecosystem, thus not allowing for delayed or multigenerational dynamical feedbacks. To really evaluate eco-evolutionary dynamics in more complex natural communities and reach general conclusions about their role and functions in nature, detailed and long term field studies will be necessary. Nevertheless, the study presented in chapter three shows that, even increasing food web complexity form two to three species, eco-evolutionary dynamics operate through direct and indirect interactions (Fig. 1), and are crucial to understand the mechanisms driving community structure and diversity.

Eco-evolutionary theory predicts that variation within phenotypes can affect populations, communities and ecosystems (see Fussmann *et al.* 2007; Bailey *et al.* 2009; Pelletier *et al.* 2009; Schoener 2011; Koch *et al.* 2014). Such phenotypic variation in a heritable trait is determined by the genotype of the organism (Fig. 1). According to this logic, changes in an organisms' genotype can have cascading effects throughout the population, communities and ecosystem if the genotype underlies a phenotypic trait that affects the ecology of species (e.g. growth rates, resistance). In the last chapter of my thesis I show that the reverse is also true. Eco-evolutionary dynamics resulting from host-virus interactions drives changes in population size and changes in the strength of selection. These changes influence the effect of random genetic drift and strong selection results in selective sweeps, which leave clear signatures in the genomes of these organisms in terms of random fixation and hitchhiking of mutations (chapter four). Ultimately, such effects lead to sequence divergence between replicate host populations. Interestingly, divergence between host populations in this study is in contrast with the observation that the replicate populations evolve highly parallel based on host phenotypes. This parallelism can also be observed when looking at structural variation. All host populations have, after coevolving with the virus, a duplication of a large genomic region. Such parallelism indicates that this duplication is highly adaptive in terms of host resistance when coevolving with the virus. However, it is likely that this duplication is not solely driving host resistance in this system (discussed in chapter four). Further analyses are necessary to directly test how this duplication is involved in the evolution of resistance. Sequencing the host populations at regular time-points during coevolution would provide

more insights regarding all small variants or structural variations that are directly involved in coevolution.

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Affidavit

I hereby declare that the this thesis work

- concerning content and design are my own work under guidance of my supervisor. Contributions of other authors are listed in the ‘contributions to the thesis’ section of the thesis;
- has not been submitted elsewhere partially or wholly as part of a doctoral degree and no other materials are published or submitted for publication than indicated in the thesis;
- the work and thesis has been performed and prepared following the Rules of Good Scientific Practice of the German Research Foundation.

Plön, 29.02.2016

Jens Frickel