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INVESTIGATIONS ON GENETIC VARIABILITY IN A MOUSE ADVANCED INTERCROSS

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“...Great music has the faintest notes, the great form is without shape...”

Chapter 41, Dao De Jing, by Lao Zi
Translated by Arthur Waley

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GENERAL INTRODUCTION

Litter size is one of the best studied economic characteristics for animal breeders and scientists. From ancient times to the present day, the breeding of litter sizes has continuously shaped livestock breeding. Modern research has gradually revealed the complexity of litter size genetics. Its complexity results mainly from the fact that it is a combination of traits and mechanisms that are also influenced by all these components, e.g. ovulation, fertilization, embryo development, maternal physiology and fetal survival before birth and the interactions between them. This leads to a low heredity of litter size, which has been proven to be the main cause of unfavorable low response in artificial selection for litter size. For this reason, it is desirable to thoroughly investigate the relevant hidden genes and mechanisms and to improve selection techniques.

In the past decades animal scientists made abundant efforts both on the genetic and the breeding aspects of the trait. For identifying relevant genes SNP arrays and whole genome sequencing were applied in QTL-mapping in recent decades. For modifying selection approaches, multi-step genomic selection was implemented. Eventually, the cooperation of both directions of research provided more power to livestock breeding. A detailed introduction is given in the following.

The breeding of domesticated animals typically has been connected to their husbandry conditions from ancient time. The breeding objectives in the early husbandry phase were more likely visible traits, e.g., body weight, body size or litter size. This kind of the breeding, together with the effect of geographical isolation, has been lasting for tens of centuries till nowadays, and consequently formed many local breeds all over the world. Nowadays the performance of different local breeds is highly diverse for most domesticated animals in terms of focal traits like body weight and litter size. The pig, sheep and mouse are discussed in detail as examples for the diversity of litter size.

Pig

Pig (*Sus scrofa*) is one of the most important livestock in animal husbandry and is broadly bred mainly for meat in the world. The total stock of pig all over the world has reached 755 million in 2018 (Livestock and Poultry: World Markets and Trade, published by the United

States Department of Agriculture in 2018, <https://www.fas.usda.gov/data/livestock-and-poultry-world-markets-and-trade>). The domestication of the pig was initially started in a large region of the Near East and East Asia approximately between 8,500 and 8,000 cal BC (Larson *et al.* 2005, 2007, 2010; Krause-Kyora *et al.* 2013; Ai *et al.* 2015). The breeding approaches were mating with local wild boar and naive artificial selection. The average number of piglets born alive per litter in selected EU countries distributes from 11.2 in Ireland to 12.7 in Denmark (Lawlor and Lynch 2007). The litter size is considerably diverse among local breeds in the world. The Danish pig breed, Landrace, has around 14.2 piglets per litter in average in the first parturition and about 10.3 weaned piglets per litter after first parturition. The corresponding numbers for the purebred Duroc are 9.8 and 7.3, respectively (Rootwelt *et al.* 2012). In contrast, the Chinese prolific breed, Taihu, is increasingly drawing people's eyes due to its remarkably high litter size. The breed Taihu is a Chinese regional breed which is widely distributed in the region of southern China. It has multiple local strains, e.g., Meishan, Fengjing, Erhualian, which have slightly different morphologic characteristics say coat color. The most focused traits of the Taihu breed is its high prolificacy. Most Taihu pig strains reach sexual maturity early up to 2.5 months, and can normally produce two litters per year. The litter size achieves 14.3-17.5 piglets per litter for the first parturition and 13.3-15.2 weaned piglets per litter (Cheng *et al.*, 1985).

A large amount of studies have been conducted for revealing mechanisms connected to fertility and litter size in pigs. The roles of numerous genes have already been identified. All these genes form a complex gene regulation network and serve the functions in the whole reproducing process, e.g. in oogenesis, transportation of oocyte, fertilization, implantation and embryogenesis. For example, the lack of DICER1 (Endoribonuclease Dicer or helicase with RNase motif) enzyme resulted in the termination of meiosis (Stowe *et al.* 2012). Without the protein of gene *HSF1* (Heat shock factor 1) the fertilized embryo stopped the differentiation before 2-cell stage (Kohata *et al.* 2013). Gene *UCHL1* (Ubiquitin C-terminal hydrolase L1) played a vital role in blocking polyspermy (Yi *et al.* 2007). The lack of AGO2 (Argonaute 2) caused the failure of embryogenesis at first cleavage (Krawczynski *et al.* 2015). DNA methylation and demethylation also play important roles during oogenesis and embryogenesis. The participation of DNMT1 and DNMT3A was necessary for embryo evolving to 8-cell stage (Huan *et al.* 2015). The successful embryogenesis also required the participation of proteins like BCLXL (B-cell lymphoma extra-large), HDAC1 (Histone deacetylase 1) SOX2 (SRY-box containing gene 2), NANOG (Nanog homeobox), OCT4 (Octamer-binding protein 4) (Kirchhof *et al.* 2000; du Puy *et al.* 2011; You *et al.* 2012; Zhou *et al.* 2014).

Progesterone and estrogen are two of the most crucial hormones in the whole reproductive procedure. Previous studies have demonstrated the importance of the participation of these two hormones on the embryo development. Progesterone is necessary for the development of embryos (reviewed by Pope and First 1985). Extra supplementation of progesterone after mating increased the number of viable fetuses (Ashworth 1991). A specific allele of the estrogen receptor (*ER*) locus was identified to be associated with increased litter size (Rothschild *et al.* 1996). Abnormal ratio of the levels of estrogen and progesterone was suspected to have correlation with early embryonic mortality in gilts (Archibong *et al.* 1987). A polymorphism in *ER* gene, as the responder of the signal transported by estrogen, was associated with litter size and can explain around 0.45 to 0.75 piglets for litter size as additive effect (Rothschild *et al.* 1996; Short *et al.* 1997).

Except the polymorphism of the *ER* gene a large amount of polymorphisms of other reproduction-related genes have been identified to account for the changes of litter size. A polymorphism within gene *COX-2* (Cyclooxygenase-2) was detected to contribute an additive effect of 0.3 piglets for litter size (Sironen *et al.* 2010). An SNP located at the 3'-untranslated region of the third exon of the porcine *LIF* gene (leukaemia inhibitory factor) was detected to have an additive effect and significant positive dominance effect for litter size in two German pig lines (Spötter *et al.* 2009). The *IGF* family is a well-known gene family that is related with reproductive traits, especially litter size in pig through affecting follicular growth or ovarian function (Cox 1997). Previous studies have indicated that the *IGF* family members *IGF1*, *IGF2* and gene *IGFBP-1* (IGF-binding protein-1) were the important participants of the implantation process and many other critical cell responses, e.g., mitosis, differentiation, and angiogenesis (Spicer and Echterkamp 1995). A study reported an SNP located in gene *IGF2* and an SNP located in gene *IGFBP3* were associated with number of piglets born dead (Rempel *et al.* 2010). In human a mutation in gene *IGF-IR* (insulin-like growth factor I receptor) was verified to result in the intrauterine (and postnatal) growth retardation (Abuzzahab *et al.* 2003).

Quantitative genetic studies help people to understand the genetics of reproductive traits as a whole instead of single gene identifications. In pig the observed heritability of litter size is low, and also varies in different populations. Lamberson *et al.* reported the heritability of 0.15 for litter size in a selected pig population in the US (Lamberson *et al.* 1991). In local Canadian Yorkshire and Landrace swine the low heritability for litter size, between 0.06 and 0.15, was estimated (Roehe and Kennedy 1995). The heritability of up to 0.16 was estimated

in a swine line selected for litter size (Holl and Robison 2003). Johnson *et al.* reported the heritability of 10 to 15% for litter size in a litter size selected pig line, and observed the phenotypic standard deviations of 2.5 to 3 pigs (Johnson *et al.* 1999). The similar phenotypic standard deviation (3.2 pigs) was also reported in an F₂ pig population (Cassady *et al.* 2001).

Although litter size exhibited low heritability, people still were attracted to identify its potential QTL. Buske *et al.* reviewed the achievements of QTL studies before 2006, which focused on the fecundity in sows (Buske *et al.* 2006). A large number of QTL analyses identified many QTLs for reproductive traits, which were linked to a variety of candidate genes, e.g., different allele types of gene *ER* (Rothschild *et al.* 1996; Depuydt *et al.* 1999; van Rens *et al.* 2000), *RYR1* (Matousek *et al.* 2003), *FSHb* (Li *et al.* 1998), *RBP4* (Rothschild *et al.* 2000), and so on. In recent years the whole-genome association study were carried out for detecting the candidate genes for reproductive traits and became another power tool. For example, 5 QTLs were identified for litter size at birth, e.g. *SCO1*, cytochrome c oxidase assembly protein, *SEMA5A*, semaphorin 5A, and *ABHD5*, abhydrolase domain containing 5 (Metodiev *et al.* 2018). It is still promising to explore more candidate genes for reproductive traits despite of their low heritability.

Sheep

Sheep (*Ovis aries*) and goat (*Capra hircus*), which can provide meat, milk and wool, are the livestock of substantial economic importance. The live animal production has reached 1,172 million for sheep and 1,005 for goat in 2013 (FAO 2015). It was reported that the ancient ancestors of modern sheep and goat were domesticated around 10.5 thousand years ago in the Middle-East region (Naderi *et al.* 2008; Alberto *et al.* 2018). From then on the domestic sheep and goat have been spread into the world ultimately. In contrast to the establishment of local breeds of other livestock the local breeds of sheep and goat were not bred by mating with local wild relatives, more likely they were bred independently within the population (Fernández *et al.* 2006; Alberto *et al.* 2018). Thus the gene flow and genomic changes for domestic sheep and goat are less complex. The diversity of litter size among local sheep and goat breeds are not large as they typically are uniparous. Davis *et al.* reported that the mean litter size varies between 3.4 (± 0.2) for the Lleyn breed (sampled in Wales) and 1.5 (± 0.2) for Mountain Sheep (white, sampled in Germany) and the mean number of lambings can vary between 6.3 (± 0.3) for the breed D'Man in Morocco and 2.3 (± 0.4) for Blueface Leicester (Davis *et al.* 2006). As sheep and goats are uniparous species the twinning rate (proportion of twin births) in a population are also commonly used to specify the fecundity in a population.

It should be noted that the lactating goat (nanny) and sheep (ewe) each have two glands, each drained by a single teat with a single streak canal. Because of these morphological restrictions and since small ruminants often are kept under harsh conditions with only sparse supply of nutrients it may be difficult for a ewe to raise twins or even triplets. Breeders therefore do not consider an increased twinning rate is as desirable under all conditions (Crawford 2001).

Interestingly in sheep numerous twinning-related genes and their mutations/alleles were identified and proved to have direct and significant effects in the underlying genetic mechanisms. The most well-known fecundity related gene in sheep, bone morphogenetic protein receptor type 1B, (*BMPR1B*, also known as the booroola gene with its mutation/allele symbol *FecB*) was found in the Australian sheep breed Booroola Merino (Wilson *et al.* 2001; Mulsant *et al.* 2001; Souza *et al.* 2001). Ewes that are homozygous *FecB^B/FecB^B*, heterozygous *FecB^B/FecB⁺*, and noncarriers *FecB⁺/FecB⁺* of the *FecB^B* Booroola mutation can be segregated on the basis of ovulation rate recordings of five or more, three to four, and one to two, respectively (Davis *et al.* 1982; Mulsant *et al.* 2001). The bone morphogenetic protein 15 (*BMP15*) and allele types *FecX^I* and *FecX^H* (Galloway *et al.* 2000), *FecX^B* and *FecX^G* in the Belclare and Cambridge sheep breeds (Hanrahan *et al.* 2004), *FecG^r* and *FecX^O* in French Grivette and the Polish Olkuska sheep populations (Demars *et al.* 2013) were identified to be associated with the twinning rate of ewes. Moreover, it was reported that the oocyte-derived growth factors growth differentiation factor 9 (*GDF9*), played an important role in mammalian fertility and was proved to affect the occurrence of births with multiple lambs in the Belclare and Cambridge sheep breeds (Hanrahan *et al.* 2004), in the Icelandic Thoka sheep (Nicol *et al.* 2009), in the Brazilian Santa Inês sheep (Silva *et al.* 2011) and in the Norwegian White Sheep (NWS) breed (Våge *et al.* 2013). The causative alleles were denoted *FecG^H* (Hanrahan *et al.* 2004), *FecG^T* (Nicol *et al.* 2009), *FecG^E* (Silva *et al.* 2011) and *FecG^F* (Våge *et al.* 2013). Notably most mutations related to twinning rate in sheep are thought to be breed-specific, in other words, may not be detected across breeds. Allele type *FecB^B* (one allele type of booroola gene), however, was found in several breeds, e.g. Booroola Merino (Mulsant *et al.* 2001), Small-tailed Han and Hu (Chu *et al.* 2011; Cao *et al.* 2016). More alleles may, however, be detected to be present in other breeds too, once a wider range of populations have been investigated. The effects of causative genes in sheep and goat are rather intense. The average difference of litter size between two homozygous allele types (say, *FecB^B/FecB^B* and *FecB⁺/FecB⁺*) have been reported to be up to two lambs per litter - one lamb per litter comparing to three lambs per litter (Davis *et al.* 2006).

Mouse

The mouse model is one of the most important and also widely applied mammalian models. A variety of mouse models have been developed to meet diversified purposes of life scientists, e.g. analyzing gene functions (e.g. Zhu *et al.* 2010), biological pathways (e.g. Greten *et al.* 2004) or disease mechanisms (e.g. Mann *et al.* 2018) through genetically modified mice or discovering candidate genes for important traits as e.g. high litter size in relevant selection lines (Holt *et al.* 2004; Langhammer *et al.* 2014).

Mouse model has many benefits compared to other common model organisms. Mouse is a rodent mammal, so any biological discoveries revealed in mouse also have reference value, not only for livestock, but also for humans, especially in terms of complex biological processes like mitosis and hormone regulation. Because of its small body size and the short lifespan the mouse is usually easier, faster and cheaper to maintain and next generations can easily be generated. The mouse and humans have high similarities in terms of anatomy, physiology and genetics. The mouse reference genome has high quality due to a large amount of gene annotations and sequencing. With the abundance of the gene ontology annotation project across different species (<http://www.geneontology.org/>) the candidate genes for reproductive traits detected from mouse lines can provide new sight for other species, e.g., pig or human.

In animal breeding the mouse model has frequently been applied in order to understand the inheritance of economically important traits and how results from genetics can be applied in breeding programs. Frequently studied traits were growth and reproductive traits. With regard to the latter up to date (Oct 04, 2018) the Mammalian Phenotype Ontology Annotation database (<http://www.informatics.jax.org/mp/annotations/MP:0005389>) has recorded 16,904 annotations of mouse lines/strains with abnormal reproductive phenotypes constructed by knock-out, transgene or other gene-editing treatments. Among them there were 579 annotations for female infertility, 1,079 annotations for male infertility, 38 annotations for decreased ovulation rate, 25 annotations for increased litter size and 472 annotations for decreased litter size.

A large number of genes were connected with the mechanisms of reproductive traits, most prominently litter size. For example, a lin-28 homolog A (*Lin28a*) transgenic mouse line showed significantly larger first litter size than a control line ($p < 0.001$, Zhu *et al.* 2010). A disruption of membrane cofactor protein (Mcp or Cd46), which was found on the inner

acrosomal membrane of sperm, increased the average number of new born pups for Cd46^{-/-} males compared to wild-type males ($p < 0.02$, Inoue *et al.* 2003). A p55 knockout (p55^{-/-}) mouse model exhibited consistently small litter size (Quinn *et al.* 2009).

Selection lines already have been used to identify QTLs in QTL-mapping analyses and genome-wide association studies (GWAS). Several fecundity associated chromosome intervals on MMU1, MMU6, MMU9, MMU13 and MMU17 were identified in a GWAS study by the haplotype association approach of comparing the highly fecund inbred mouse strain QSi5 and other 22 inbred mouse strains (Wei *et al.* 2013), which agreed the QTL regions reported by Liljander *et al.* (Liljander *et al.* 2006). Reproduction-related QTLs were mapped on MMU1, MMU2, MMU8, MMU10 and MMU11 for an F₂ intercross population which crossed from two founder selection lines, the parental high-growth (M16i) and low-body-weight (L6) selection lines (Rocha *et al.* 2004). A QTL (Lfq2) were detected on MMU2 for litter size which accounted for 13.2% phenotypic variance in a crossbred mouse population which were bred from the cross of an litter size selected Quackenbush-Swiss line and the inbred strain C57BL/6 (Kirkpatrick *et al.* 1998). The position of QTL Lfq2 overlapped the position of litter size related QTLs reported in other study (Spearow *et al.* 1999). MMU7 was reported to carry a QTL for litter size, which can account for 7.4% of the phenotypic variance, in an F₂ intercross of the SM/J and LG/J inbred mouse strains which selected for small (SM/J) and large (LG/J) body size (Peripato *et al.* 2004).

In these studies crossing a mouse population which performed high fecundity due to selection with a control population has been proven to be efficient in order to obtain an intercross population that possessed a larger variation of litter size. These intercross populations provided appropriate resources for the QTL-mapping of litter size. Simultaneously these QTL-mapping studies identified a larger number of QTL related to litter size and highlighted several chromosomes, e.g. chromosome 2, which might be important for litter size due to the heavy overlapping of the QTL identified in different studies and also be references for our subsequently fine-mapping.

Litter size

Despite many studies on the genetics of reproductive traits in mice, many questions still remain unanswered.

Litter size can be thought to consist of several component traits, most prominently ovulation rate (Cunningham *et al.* 1979), prenatal survival (Rosendo *et al.* 2007) and uterine capacity

(Clutter *et al.* 1994), which was defined as the maximum number of offspring the dam can produce when ovulation rate, or number of viable embryos is not limiting. An increase of ovulation rate, prenatal survival and uterine capacity seems always to be observed in animal populations directly selected for litter size (Holt *et al.* 2004). Selection for increased ovulation rate, in contrast, may not obtain a correspondingly significant increase of litter size, according to experiments in mice (Clutter *et al.* 1990; Gion *et al.* 1990; Kirby and Nielsen 1993) and pigs (Cunningham *et al.* 1979; Johnson *et al.* 1999). A low fertilization rate or insufficient embryonic survival was assumed to cause this kind of asymmetry (Cunningham *et al.* 1979). Similarly, an increased uterine capacity due to direct selection might also not necessarily result in the expected increased litter size (Clutter *et al.* 1994). Thus in order to obtain the increase of litter size direct selection on it was recommended rather than selecting the components of litter size (Cunningham *et al.* 1979; Clutter *et al.* 1990; Gion *et al.* 1990; Kirby and Nielsen 1993). Further, mechanisms that increase litter size can differ clearly from mechanisms that decrease litter size. An asymmetric response to selection has commonly been observed in two-directional selection experiments (Falconer 1953; Villanueva and Kennedy 1992). Unequal gene frequencies, directional dominance and unsuitable metrics were identified as possible causes to asymmetric selection response (Falconer 1953). Linkage disequilibrium generated by selection was also pointed out to explain asymmetric response, especially in the infinitesimal model (Villanueva and Kennedy 1992). In many studies where a single gene has been modified either only minor increases of litter size (Inoue *et al.* 2003; Zhu *et al.* 2010) have been obtained or a small litter size has been observed (Burnicka-Turek *et al.* 2009; Quinn *et al.* 2009) if not even infertility (Harding *et al.* 1997; Ramsey *et al.* 2002; Wang *et al.* 2004; Stratikopoulos *et al.* 2008). These findings also make asymmetric selection response plausible.

Litter size was claimed to be a female-linked trait in many studies. In uniparous species like humans the association between the prolific trait and female was rather straightforward. In humans the rate of having a dizygotic twins heavily relied on the ovulation rate and thus was considered as a maternal traits (Meulemans *et al.* 1996; Hoekstra *et al.* 2008). In multiparous species litter size was associated with many components traits as described above, but most components traits are female traits, e.g. ovulation rate. A study reported a two-factorial breeding experiment based on two high fertility lines which were selected for increasing litter size for a long-term and their control line (Langhammer *et al.* 2017). This study showed that the prolific traits depended on the dam's genetics in the mouse line selected for increasing litter size.

In order to understand the female-related feature of litter size the direct genetic effects and maternal genetic effects that the dam contributes and the correlations between these two effects were evaluated in many studies. In a pig study for total number born the maternal heritabilities were estimated to be 0.11 and 0.14 for a Landrace population and a Yorkshire population respectively while their heritability estimates of direct effects were below 0.05 and the correlations between direct and maternal genetic effects for total number born of two populations were -0.39 and -0.41 respectively (Lund *et al.* 2002). In another study for litter size in six Iberian pig strains the direct effect heritabilities were estimated in the range of 0.00 and 0.13 for all strains and the maternal effect heritabilities were estimated to be all smaller than 0.04 (Perez-Enciso and Gianola 1992). Kaufmann *et al.* reported the direct and maternal genetic effects were estimated to be 0.22 and 0.02 respectively for litter size of a Large White pig population and found negative correlations between the maternal genetic effects of litter size and the direct genetic effects of birth weight, weaning weight and litter size, which were -0.14, -0.20 and -0.70 respectively (Kaufmann *et al.* 2000). Interestingly the negative correlation between the maternal genetic effect and the direct genetic effect of litter size were also reported in many studies e.g., by Crump *et al.* (Crump *et al.* 1997), Haley and Lee (Haley and Lee 1992) and Southwood and Kennedy (Southwood and Kennedy 1990). The negative correlation between the maternal genetic effect and the direct genetic effect of litter size implied that female individuals which were born in a larger litter may have smaller litters comparing to female individuals born in a small litter, which was also claimed by Van Der Steen (Van Der Steen 1985). This negative correlation between the maternal genetic effect and the direct genetic effect made litter size more complicated to understand.

Litter size has complex interaction not only with other reproductive traits like ovulation rate, but also with growth traits like body weight. In pigs correlated responses of litter size and body weight were found in a Duroc line selected for body weight at day 200 (Kuhlers and Jungst 1992). A similar correlated, but not significant, response was also found in a Landrace line selected for 200 day weight (Kuhlers and Jungst 1993). Conversely, selection for litter size resulted in pigs with less capacity of lean growth (Estany *et al.* 2002). A study detected the genetic correlation between lean growth and litter traits (number born alive and litter weight at day 21) for four U.S. swine breeds and stated the correlations between these two traits were not significant, but comparable in four swine breeds (Chen *et al.* 2003). No correlated response to the selection for increased litter size was found for growth and carcass traits in a pig line which was selected for increased litter size for 19 generations (Petry *et al.* 2004). In a rabbit population which was selected for the number of young weaned per litter

small genetic correlations between growth traits and litter size at weaning was found (García and Baselga 2002).

In mice Eisen (1978) found a correlated response in litter size in a line selected for 6-week body weight and conversely a correlated response in 3-week body weight was detected in another line selected for birth litter size, which suggested positive pleiotropy of genes related to litter size and body weight. The correlation between litter size and body weight (or other growth traits), however, was not always significantly large in the selection experiment. For instance, in a study of Bradford (1971) selection for body weight gain (measured as difference between body weights at day 21 and at day 42) led to an increase of ovulation rate while litter size did not change significantly as a result of selection. Inconsistencies of results were assumed to be due to small population sizes in some experiments, as response in litter size to selection for postweaning gain was positive in populations with larger effective population size but not if population size was smaller - an effect that was found under the selection for less than 14 generation (Eisen *et al.* 1973) as well as the selection for 24 to 27 generations (Eisen 1975). Also the standardization of litter size after birth was discussed by Bünger *et al.* (1992) as possibly contributing to the uncertainty on the correlation between litter size and body weight in selection experiments. This was underpinned by the observed decline of litter size after selection on body weight at day 42 in a mouse population without standardization of litter size (Bünger *et al.* 1992).

As a result of all these complexities litter size usually exhibits low heritability in the mouse, which was from 0.10 to 0.18 in different mouse populations (Clutter *et al.* 1990; Gion *et al.* 1990; Beniwal *et al.* 1992; Kirby and Nielsen 1993; Falconer and Mackay 1996; Peripato *et al.* 2004; Formoso-Rafferty *et al.* 2017) and around 0.10 (Lund *et al.* 2002) in the range of 0.01 (derived by REML for trait number born alive, Holl and Robison 2003) and 0.22 (Kaufmann *et al.* 2000) in different pig populations.

With the breeder's equations (Walsh and Lynch 2018, p. 150) it is understandable that the short-term selection usually failed to obtain the expected gain of litter size due to its low heritability and infinitesimal genetic model. The low heritability can also bring other problems like low reliability. In this sense the long-term selection becomes necessary and recommended.

Long-term selected mouse lines

In mouse, long-term selection was conducted with mouse populations for increasing the growth traits or litter traits. A selection experiment for high growth rate was conducted for around 100 generations (Allan *et al.* 2005). A mouse population was selected for high fatness for more than 58 generations (Wagener *et al.* 2006), which was subsequently applied in the fine-mapping for fatness using SNP phenotyping data (Schmitt *et al.* 2007). A series of mouse lines which were selected for growth traits have been established by long term selection over 100 generations (Bünger *et al.* 2001; Renne *et al.* 2003). In terms of the selection experiments for litter size there are only few mouse populations published and even less maintained up to date. A gain of 3.69 young in litter size over 29 generation-long selection was reported in a British mouse line (Bakker *et al.* 1978). Crossing from the above-mentioned British mouse line, two Norwegian mouse lines, one selected for increased litter size and another for decreased litter size, were established and published in a series of studies (Holt *et al.* 2004, 2005). After generation 122 the mean number of pups born alive was 22 pups for the upward selected line comparing to 11 pups for the unselected line.

Another two high fecundity mouse lines, FL1 and FL2, were established from a common initial genetic pool (Dietl *et al.* 2004) by selection for a fertility index over 170 generations and have been maintained up to now at the Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany (Langhammer *et al.* 2014, 2017). The initial genetic pool was a systematic crossbreed of four inbred and four outbred founder mouse lines starting in the 1970s (Dietl *et al.* 2004).

Before generation 164 (FL1) and generation 162 (FL2), two high fertility lines were selected with a selection index I , $I = 1.6 \times LS + LW$ where LS is litter size and LW is litter weight. Afterwards the selection was modified to BLUP-selection for litter size with the control of the inbreeding level using Optimal Genetic Contributions to the Next Generation (Meuwissen 1997). The population size was 60 mating pairs per generation for each line. Their control line, FZTDU, was also established from the same pool and maintained with random mating and no selection (Dietl *et al.* 2004). Along with the other FBN selection lines, e.g., the lines DU6, DU6P and DUhTP (which were selected for increasing body mass at day 42, protein amount in the whole body, and male's treadmill length) the FL1 and FL2 provide valuable resources for identifying candidate genes for reproductive traits.

Based on these long-term selection lines a series of studies was implemented in order to explore the mechanisms related to reproduction and fertility. The difference of component fertility traits between these two high fecundity lines was analyzed (Spitschak *et al.* 2007). Increased ovulation number was found in both lines, but the number of ova shed, the rate of loss of ova and pre- and post- implantation conceptuses differed in two lines. A two-factorial breeding study on these two lines indicated that the reproductive performance primarily depends on the females of two lines but was not significantly affected by males of different line-origin (Langhammer *et al.* 2017).

Advanced intercross line for fine-mapping

The two fecundity lines, FL1 and FL2, provide resources to identify candidate genes for reproductive traits. The advanced intercrossed line, which was intercrossed from multiple inbred lines, is proved to provide more accurate estimates of QTL map location than a conventional mapping population (Darvasi and Soller 1995). In the intercross procedure the probability of recombination between any two loci are increased over multiple generations of breeding. As a result, mapping resolution is improved due to the stretched genetic length of the entire genome.

In order to establish a denser QTL map the advanced intercrossed population (AIL) was established by intercrossing the females randomly selected from mouse line FL1 and the males randomly from a branch population (DUKsi) of the original control line FZTDU. The branch population, DUKsi, was extracted from generation 79 of line FZTDU and then maintained independently with random mating and no selection.

The founder generation of the AIL consisted of four females from FL1 and four males from the control line. Each FL1 female mated one male from DUKsi and formed a family. There was no cross between any two families. The four families were bred by full-sib-mating until the F₃ generation, and then only one of four families was continuously bred and maintained till the F₃₀ generation. In total 76 traits were measured in the F₂ generation of four families and later for 28 generations from F₃ to F₃₀ (sample size for each trait differed).

A series of studies were designed and conducted for the AIL population to validate the existence and the approximate locations of causative genes of litter size, to provide the appropriate model and the relationship matrices required by the best linear unbiased prediction (BLUP) method and to evaluate the correlations among traits. These study contents will be unfolded in the following chapters.

Content of the thesis

Chapter one presents a coarse QTL-mapping experiment for the F₂ generation of the AIL. The genotyping data are derived from genotyping 458 F₂ females of line AIL by 77 microsatellite markers. QTLs are mapped for litter traits, e.g. birth litter size and birth litter weight, growth traits, e.g. body mass at day 21, 42 and 63. Several chromosomes, e.g., chromosomes 2 and 4, harbor QTLs for reproductive traits and growth traits in the overlapped regions, which imply the potential existence of candidate genes related to litter size and pleiotropic genes associated with litter size and body mass.

Chapter two describes the method for constructing a new version of the relationship matrix based on the founders' genotypes and the complete pedigree. A modified pedigree-derived relationship matrix $\tilde{\mathbf{A}}$ was constructed using the observed allele frequencies of the founders and the pedigree-derived relationship matrix for autosomal genotypes and X-chromosomal genotypes, respectively. These modified relationship matrices are applied in the genetic analyses for each trait obtained from the AIL population. The models with and without X-chromosomal genetic variance are adopted for each trait in order to evaluate the significance of including the X-chromosomal effect.

Chapter three refines the models used in the genetic analyses of chapter two, tests the significance of X-chromosome-linked effects in detail and evaluates the correlations between traits in order to provide advises for the subsequent fine-mapping experiment. For growth traits the significance of considering a sex difference is tested. The correlations of litter traits between the first and second litters are evaluated. The correlations within growth traits and between reproductive traits and growth traits are detected.

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CHAPTER ONE

Quantitative trait loci for litter size in F₂ female mice from FL1 × DUKsi cross

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Quantitative trait loci for litter size in F₂ female mice from FL1 × DUKsi cross

Abstract

Two mouse models comprising FL1 and FL2 exhibit a high-fertility phenotype (the litter size nearly doubled after long-term selection). Due to their high fecundity, these two lines are valuable materials for analyzing the molecular and cellular mechanisms related to high fertility. To determine the quantitative trait loci (QTL) or genes associated with litter size and their distribution in the genome, QTL analysis was conducted based on interval mapping of the whole genome with 77 microsatellite markers. An F₂ pedigree generated by females from the FL1 line and males from the control line was established for QTL mapping and 68 traits were measured, including the litter size, litter weight, and body mass in different phases. Two P₀ mice, six F₁ females, and 458 F₂ females were genotyped using all of the markers. In total, 213 chromosome-wide significant QTL were identified for 62 traits and 47 genome-wide significant QTL were identified for 31 traits (95% quantile for estimating F-value). QTL related to litter size were mainly identified on chromosomes 2, 4, 9, and 13, QTL for body mass were mostly identified on chromosomes 2, 4, 7, and 8, and QTL for litter weight were mainly located on chromosomes 2, 4, and 13. This study provides preliminary insights into the genetic background of enhanced fertility in mouse line FL1, as well as highlighting several genome regions containing QTL associated with litter size and other target traits such as body mass for subsequent fine mapping.

Key words: QTL mapping, litter size, body mass, advanced intercross mouse line, microsatellite marker

Introduction

In multiparous animals, litter size is a complex quantitative trait with typically low heritability and it is influenced by several components, e.g., the ovulation rate, embryonic survival rate, and maternal body dimension. Its counterpart in uniparous species is the twinning rate, for which some causative genes have already been identified, especially in sheep.

A polymorphism in the bone morphogenetic protein receptor type IB gene (*BMPRI*B), also known as the Booroola mutation (*FecB*), on chromosome 6 in sheep causes an increase in the ovulation rate, which results in twin and triplet births occurring at a higher frequency (Wilson *et al.* 2001; Mulsant *et al.* 2001; Souza *et al.* 2001). Furthermore, a large number of mutations associated with fecundity in sheep have been identified, mainly in three genes comprising the *MPRI*B gene, bone morphogenetic protein 15 (*BMP15* on X chromosome), and growth differentiation factor 9 (*GDF9* on chromosome 5), which are all members of the transforming growth factor β (TGF β) superfamily. Fecundity-related phenotypes *FecX^I* (Galloway *et al.* 2000), *FecX^H* (Galloway *et al.* 2000), *FecX^B* (Hanrahan *et al.* 2004), *FecX^G* (Hanrahan *et al.* 2004), *FecX^L* (Bodin *et al.* 2007), and *FecX^R* (Martinez-Royo *et al.*, 2008; Monteagudo *et al.*, 2009) have been identified for the *BMP15* gene. Phenotypes *FecG^H* (Hanrahan *et al.* 2004), *FecG^T* (Nicol *et al.* 2009), and *FecG^E* (Silva *et al.* 2011) have been identified for the *GDF9* gene. The effects of these mutations on the ovulation rate were detected in sheep populations throughout the world. The differences in the ovulation rate caused by these mutations vary from 10% (not significant) up to 206% (Juengel *et al.* 2013). In terms of the effect on litter size, the *FecB* mutation can contribute 1.0 extra lamb per ewe lambing (heterozygous carriers) and about 1.5 extra lambs per ewe lambing (homozygous carriers) compared with the control ewes, and *FecX* mutations can contribute around 0.6 extra lambs per ewe lambing (Davis 2005). The *FecX^R* heterozygous ewes produce 2.66 lambs per lambing compared with 1.36 lambs per lambing according to the flock mean in the Spanish breed Rasa Aragonesa (Monteagudo *et al.* 2009). In a Small Tail Han sheep population the sheep carried the genotype AA of the gene *GDF9* had 0.30 and 0.77 lambs (least squares means of litter size) more than the genotype AB carries in the first and second parities respectively (Chu *et al.* 2004).

In cattle, quantitative trait loci (QTL) for twinning rate have been identified on BTA14 (Bierman *et al.* 2010), on BTA2, 5, and 14 (Kim *et al.* 2009), and on BTA5, 7, 12, and 23 (Lien *et al.* 2000) by QTL mapping. Candidate genes for the twinning rate were also

identified in local breeds by genome-wide association analysis, e.g., in Maremmana cattle in Italy (Moioli *et al.* 2017) and tropically adapted beef cattle in Australia (Hawken *et al.* 2012).

Pigs are important multiparous livestock for the animal industry. A large number of candidate gene analyses and selective breeding studies have focused on porcine production traits, especially litter size. In the Pig Quantitative Trait Locus Database (PigQTLdb, <https://www.animalgenome.org/cgi-bin/QTLdb/SS/index>), there are 349 litter size-associated QTL (database version: Release 36, August 22, 2018). These litter size-associated QTL are distributed throughout the porcine chromosomes, e.g., on SSC1, SSC5, SSC8, SSC11, SSC13, and SSC18 in a Large White pig population (Sell-Kubiak *et al.* 2015); on chromosomes SSC1 to SSC18 in a subgroup of a Large White × Landrace crossbred line (Onteru *et al.* 2012); and on SSC1, SSC4, SSC13, SSC14, SSC15, and SSC17 in a four bred-cross line (Schneider *et al.* 2012). A variety of candidate genes have been suggested as causal QTL for litter size in pig, such as insulin-like growth factor-binding proteins 2 and 3 on SSC15 (*IGFBP2* and *IGFBP3*, An *et al.*, 2018), insulin-like growth factor 2 on SSC2 (*IGF2*, Stinckens *et al.*, 2010), and estrogen receptor 2 on SSC1 (*ESR2*, Rothschild *et al.*, 1996; Horogh *et al.*, 2005; Laliotis *et al.*, 2017). It should be noted that detecting the candidate genes for litter size can be beneficial in purebred pig lines, but also in crossbred lines, e.g., the crosses between Large White and Meishan (genome-wide linkage analysis; Hernandez *et al.*, 2014), between Iberian and Meishan (QTL and association analyses; Balcells *et al.*, 2011), and between European pig breeds and Meishan (QTL-mapping; Tortereau *et al.*, 2010).

The mouse is the most widely employed mammalian model and it allows the efficient identification of gene functions. Currently (October 04, 2018), the Mammalian Phenotype Ontology Annotation database (<http://www.informatics.jax.org/mp/annotations/MP:0005389>) contains 16,904 annotations for mouse lines/strains with abnormal reproductive phenotypes due to knockout, transgenes, or other gene editing treatments, including 579 annotations for female infertility, 1,079 annotations for male infertility, 38 annotations for decreased ovulation rate, 25 annotations for increased litter size, and 472 annotations for decreased litter size. Many genes are connected to mechanisms that are associated with reproductive traits, such as litter size. For example, a lin-28 homolog A (*Lin28a*) transgenic mouse line has a significantly larger first litter size than the control line ($p < 0.001$; Zhu *et al.*, 2010). In a mouse line disrupted with the gene human membrane cofactor protein (*Mcp* or *Cd46*), the average number of newborn pups for *Cd46*^{-/-} males is significantly greater than that for wild-

type males ($p < 0.02$; Inoue *et al.*, 2003). A p55 knockout (p55^{-/-}) mouse model also produced consistently small litters (Quinn *et al.* 2009).

Mouse models have been shown to be effective for detecting candidate genes related to reproductive traits. The fecundity-associated intervals on MMU1, MMU6, MMU9, MMU13, and MMU17 were identified in a genome-wide association study of a highly fecund inbred mouse strain, QSi5 (Wei *et al.* 2013), where they overlapped with previously reported reproductive performance QTL in the mouse (Liljander *et al.* 2006). The reproduction-related QTL on MMU1, MMU2, MMU8, MMU10, and MMU11 were mapped for a long-term selected mouse line (Rocha *et al.* 2004). In particular, QTL Lfq2 on MMU2 accounts for 13.2% of the phenotypic variance and it overlaps with previously reported positions for QTL (Kirkpatrick *et al.* 1998; Spearow *et al.* 1999). QTL Lsq1 on MMU7 was identified as associated with litter size in a backcross mouse population (SUTO 2015). Two QTL were identified for litter size on MMU7 and MMU12 in a crossbred mouse population (Peripato *et al.* 2004), where they accounted for 7.41% and 6.52% of the phenotypic variance, respectively. In humans, spontaneous dizygotic twinning was reported in different populations as a supernormal reproductive trait (Tong and Short 1998; Busjahn *et al.* 2000; Hoekstra *et al.* 2008). A region on chromosome 3 was identified as a candidate QTL, which yielded a 6.93 LOD (logarithm of the odds ratio) score and it was close to the marker D3S3608 (Busjahn *et al.* 2000 p. 3). Two genetic risk variants comprising one near *FSHB* and another within *SMAD3* were identified as associated with spontaneous dizygotic twinning by a genome-wide association study (Mbarek *et al.* 2016). Due to the abundance of gene ontology annotation projects in different species (<http://www.geneontology.org/>), the candidate genes detected for reproductive traits in mouse lines may provide new insights into those in other species, such as humans and pigs.

Long-term selected mouse models are employed widely in QTL mapping. Compared with short-term selection models, long-term selected models can provide higher resolution and dense single nucleotide polymorphism sequencing for mapping, as well as allowing the identification of the mechanisms responsible for regulating the trait of interest and its components, which do not respond to short-term selection. To the best of our knowledge, only a few previous studies have investigated long-term lines selected for litter size. In particular, a mouse line was maintained and selected for litter size for more than 110 generations (Holt *et al.* 2005). In addition, in a high-fertility mouse model system maintained at the Leibniz Institute for Farm Animal Biology (FBN, Dummerstorf, Germany) comprising two high

fertility mouse lines, FL1 and FL2, the litter size and litter weight doubled after the long-term selection period (Langhammer *et al.* 2014).

The objectives of the present study were to identify the chromosomes related to genetic variations in litter size based on coarse mapping of the QTL for litter size and related traits (e.g., litter weight), as well as investigating the QTL regions for different traits and their overlaps in order to explore the correlations between litter traits and their component traits.

Materials and Methods

Mouse lines

The mouse lines applied in this study were originally derived from a cross of four inbred (CBA/Bln, AB/Bln, C57BL/Bln, and XVII/Bln) and four outbred (NMRI orig., Han:NMRI, CFW, and CF1) lines (Dietl *et al.* 2004), and they were selected for different purposes. FL1 was selected for an index trait combining birth litter size (LS0) and birth litter weight (LW0) at birth in primiparous females, i.e., Index $I = 1.6 \times LS0 + LW0$, up to generation 170 (Langhammer *et al.* 2017). After selection over 131 generations, the fecundity of the mouse population obtained was 1.8-fold higher compared with the mean number of pups born in the control line (Langhammer *et al.* 2014).

The control line, DUKs, was also derived from the same initial genetic pool. This line was maintained by random mating for 79 generations and without any intentional selection pressure. The DUKsi line employed in this study was an inbred line derived from the unselected DUKs line after splitting from DUKs in generation 79. The male founder animals in the experiment were taken after 38 generations of full sib mating in DUKsi. Both lines were maintained with 60 to 100 mating pairs per generation. Females were mated at an age of 63 days with a mating ratio of 1:1. Only in the DUKsi line, the litter size at birth was standardized to nine pups. In general, weaning occurred on the 21st day. The performance metrics for the FL1 and DUKsi lines are compared in Table 1.1.

Table 1.1 Mean and standard deviation (SD) of birth litter size (LS0) and birth litter weight (LW0) for the two founder mouse lines.

Line	Generation	No.	LS0 (mean \pm SD)	LW0 (mean \pm SD)
FL1	121-131	730	17.14 \pm 3.25	27.27 \pm 5.03
DUKsi	28-38	753	9.84 \pm 2.08	14.74 \pm 2.82

In order to evaluate the differences in LS0 and LW0 between breeding without and with selection, the reference animals were collected from 10 generations (around 70 litters per generation) from FL1 and control line DUKsi.

Pedigree structure

Four females from the 131st generation of FL1 and four males from the 38th generation of DUKsi were crossed randomly at a mating ratio of 1:1 to obtain four P0 families and they subsequently generated F₁ litters for each family. Each F₁ litter size was standardized randomly to 12 comprising four males and eight females, and a new litter was reconstituted (if one litter had insufficient pups, pups from other litters were added to the litter). Next, two F₁ females and one male from the same family were selected randomly to generate the F₂ progeny by full sib mating at an age of nine weeks. In order to obtain a sufficiently large F₂ population, all F₁ females were mated at least four times to generate as many F₂ individuals as possible. Each F₂ female mated twice with the same male and generated two litters. The birth litter size and birth litter weight were recorded as the reproductive traits for the dam. Afterwards each litter was standardized to 12 pups. Finally, we obtained 812 F₂ females and we subsequently measured their phenotypes, and 458 females genotyped were employed in this study.

Husbandry and feeding conditions

All mice were maintained under conditions at 22.5°C and a controlled light regime with a 12:12 h light:dark cycle in Macrolon cages measuring 30 × 12.5 × 12.5 cm (Langhammer *et al.* 2014, 2017). All individuals had free access to concentrated pellets and water. A standard breeding diet comprising 21% protein, 0.4% L-methionine, 55% starch, 5% sucrose, 5% fat, 5% cellulose, 2% vitamins, and 6% mineral mixture (Altromin No.1314, Lage, Germany) was fed ad libitum. All animal treatments were conducted with approval of the Animal Care Committee of the Ministry of Nutrition, Agriculture, Forestry, and Fishery, State Mecklenburg-Vorpommern, Germany (LVLMV/310-4/7221.3-1.1-018/03)

Phenotypic measurements

In total, 76 traits were analyzed for F₂ females, where 34 traits were measured and recorded during breeding, and 42 traits were obtained based on calculations from other traits, e.g., average weight per pup = litter weight/litter size. Descriptive statistics for all of the traits are presented in Table S1.1 in the supplementary information.

The number of corpora lutea was counted by microscopy after dissecting both ovaries from each animal and freeing the ovaries from any surrounding tissues. Blood was sampled after decapitation to measure the concentration of progesterone (PROG) in the plasma. Heparinized blood samples were centrifuged (2400 × *g*, 4°C), before separating the plasma and storing at –20°C until their analysis. The PROG concentration was determined using a competitive single-antibody 3H-RIA after extraction in ethyl ether, where 10 µL of plasma was extracted with 1 mL of ethyl ether. The tracer comprising [1, 2, 6, 7-3H]-progesterone was purchased from Amersham Biosciences Europe (Freiburg, Germany). The antibody raised in rabbits was further purified by affinity chromatography on protein A superose (Amersham Pharmacia Biotech). Dose–response curves established for three plasma pools and the PROG standard did not deviate significantly from parallelism over a range of 0–20 µL plasma. The coefficients of variation for the intra- and inter-assays were 9.4% and 10.7%, respectively. The intra-litter variability and the intra-litter standard deviation were calculated as:

$$s^2 = \frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2, \text{ and } s = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (x_i - \bar{x})^2},$$

where n is the number of pups born per litter, x_i is the individual birth weight per pup for individual i , and \bar{x} is the mean birth weight of all pups per litter.

Marker and genotyping

In total, 77 microsatellite markers were employed for genotyping where they covered 965 cM (around 65% of the whole genome according to the Mouse Fact information in the Mouse Genome Informatics (MGI) database, http://www.informatics.jax.org/mgihome/other/mouse_facts1.shtml). The genetic positions of all the markers were extracted from the MGI marker database on August 04, 2014, and checked on October 03, 2018. The distributions and positions of all the markers are presented in Figure 1.1 (see Table S1.2 in the supplementary information for the positions of the markers).

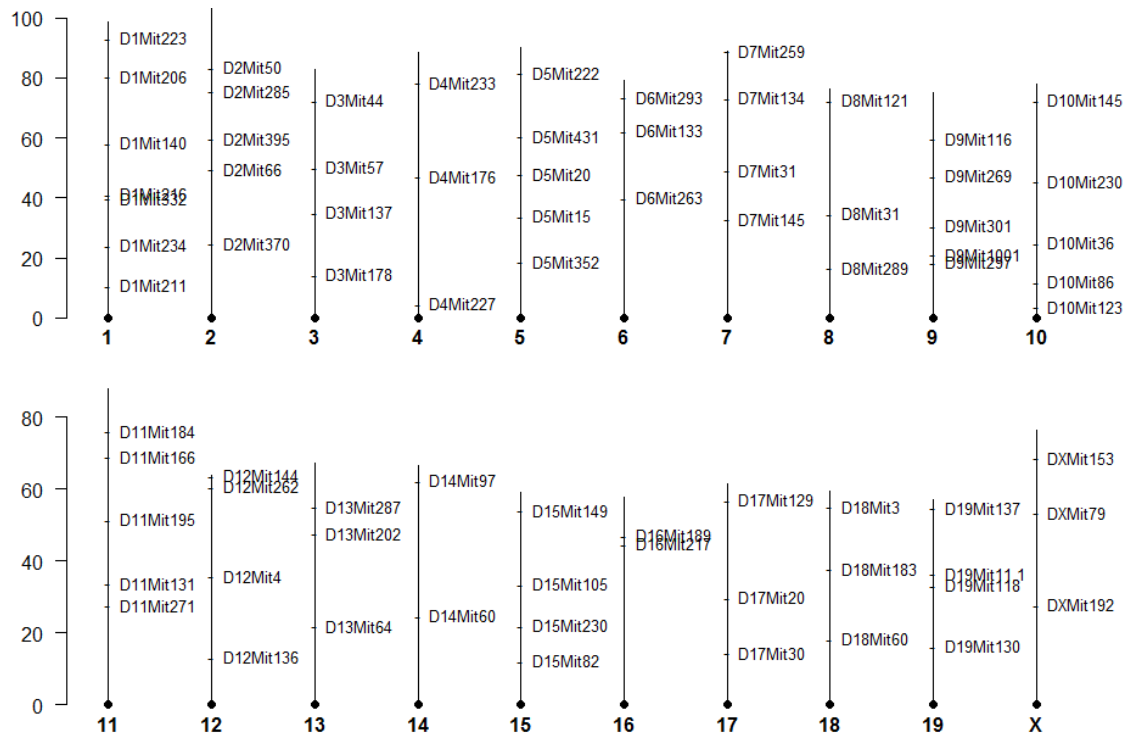


Figure 1.1 Marker positions in centiMorgans (cM) in the whole house mouse (*Mus musculus*) genome, except for chromosome Y.

One female and one male from the founder generation, five F_1 females from four families, and 458 females from 812 F_2 females were genotyped with 77 markers. The genotyping results were recorded as 11, 12, or 22, where the allele "1" represents the same allele as the FL1 founders and the allele "2" represents the same allele as the founders from the control line. All genotyping experiments were conducted at the University of Cologne.

Data analyses

The existence of QTL was examined by regression interval mapping (Haley and Knott 1992; Martínez and Curnow 1992). At each putative QTL position, the model for each trait included the family-specific effect, additive effect, and dominant effect:

$$y_{i,j} = \mu_i + \delta_{1,k} x_{i,j,k} + \delta_{2,k} \omega_{i,j,k} + e_{i,j,k},$$

where μ_i is the fixed effect of family, $i \in \{1, 2, 3, 4\}$, $\delta_{1,k}$ is the regression for the additive effect on the gene content of animal j for family i at position k in the genome, $x_{i,j,k}$ is the QTL probability of the homozygous genotype at position k for animal j in family i , $\delta_{2,k}$ is the regression for the dominant effect on the probability for the heterozygous genotype at

position k , $\omega_{i,j,k}$ is the probability for the heterozygous QTL at position k for animal j in family i , and $e_{i,j,k}$ are the residuals.

The null hypothesis of no QTL ($\mu_0 : \delta_{1,j,k} = \delta_{2,j,k} = 0$) was tested at each position investigated in the genome by using the F-test.

In order to account for multiple testing, genome-wide and chromosome-wide significance thresholds were computed using a permutation test (Churchill and Doerge 1994) with 10,000 permutations for each trait. All computations were conducted with an F_2 version of the ADRQLT program (Reinsch 1999). The minimum investigation unit for QTL mapping was 1 cM, so all genome regions between markers were divided into 975 investigation positions. The genotype frequency was calculated for each position using the genotypes and the genetic distances of markers, before computing the QTL values with the ADRQLT program. The permutation test yielded a chromosome-wide significance threshold for each chromosome and a genome-wide significance threshold for the whole genome in different confidence intervals. The thresholds of the 95% confidence interval were employed for identifying QTL.

The F-value and P-value for each detected locus were derived using the program. The F-value curve was plotted in a figure for each chromosome and each trait. Chromosome- and genome-wide significance thresholds were also labeled in the figures. A region with a higher F-value than the threshold was defined as a QTL region and the peak in this region with the highest F-value was recorded as a QTL. Each QTL was marked with the relevant trait abbreviation, chromosome number, and the order of all the QTL found on the same chromosome. For instance, BM42_03_1 and BM42_03_2 were identified for trait BM42 on chromosome 3. For each QTL, the edges of its QTL region, the peak position, and flanking marker located close to the QTL region were recorded in order to describe the positions of the QTL from different perspectives.

The additive and dominant genetic variance components of the peak position for each genome-wide significant QTL were regressed based on the estimated QTL effects for all individuals at this peak position.

Results

A correlation heat map was produced to represent the correlations among traits (Figure 1.2). The birth litter sizes for the first and second litters (LS0_1, _2) as well as the average of two litters (ALS0), birth litter sizes including stillborn pups for the first and second litters (LST_1, _2) as well as the average of two litters (ALST), birth litter weights for the first and second litters (LW0_1, _2) as well as the average of two litters (ALW0) were significantly correlated, but the average pup weights for the first and second litters (AW0_1, _2) as well as the average of two litters (AAW0) had negative correlations with the litter size and birth litter weight. Body masses at days 21, 42, and 63 as well as when virginal first opened, at first estrus, at mating day, and at slaughter day (BM21, BM42, BM63, BMVO, BMO1, BMM, and BMS) had positive correlations with each other as well as significant correlations with birth litter size, birth litter weight, and body dimension traits such as waist width (WW), belly width (WB), and torso length (TOL), and body weight gains (BWG1, BWG2, and BWG3). The standard deviations of the pup weight in each litter for the whole litter (ILSD_1, _2), male pups (ILSDM_1, _2), and female pups (ILSDF_1, _2) were positively correlated.

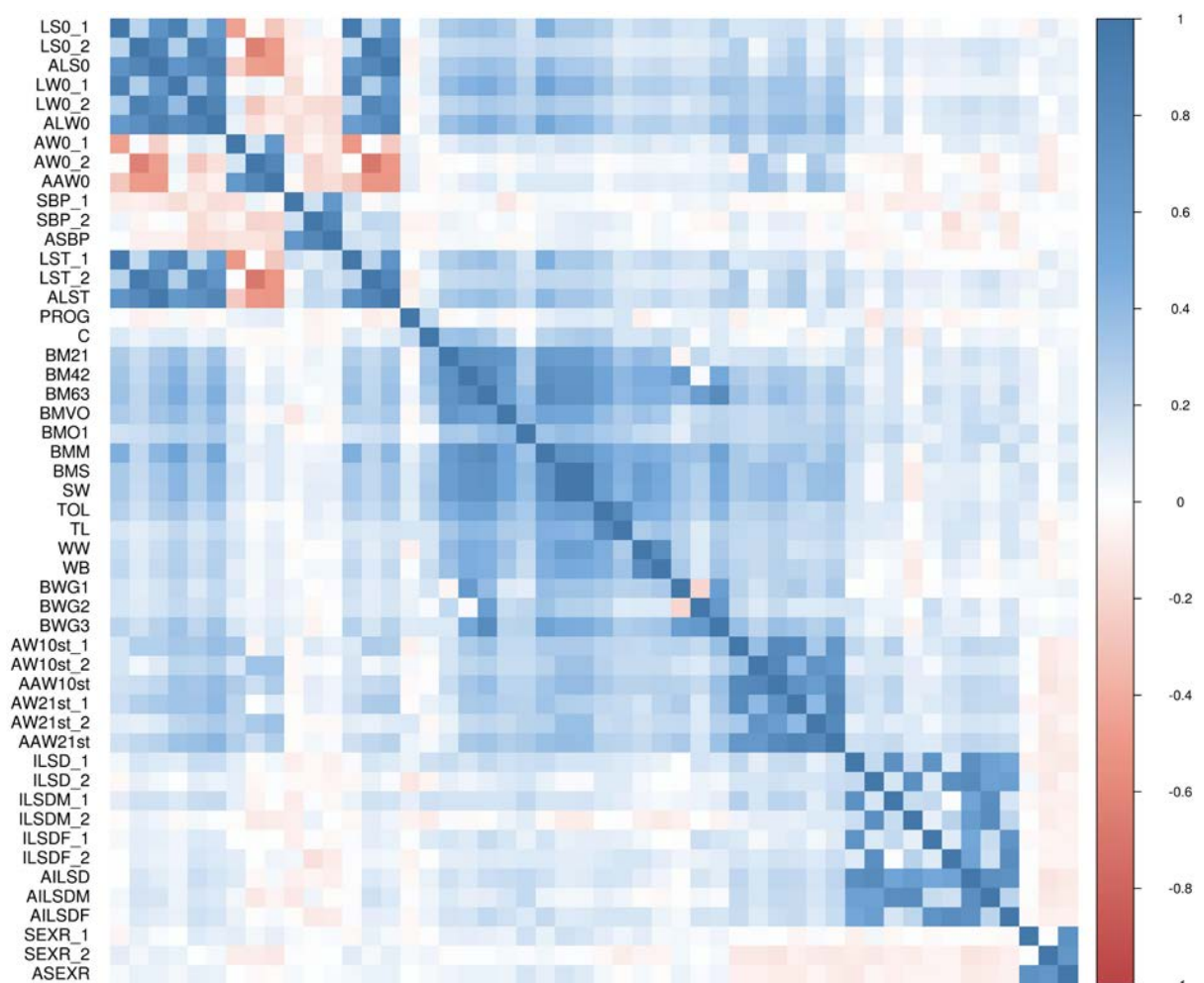


Figure 1.2 Correlations among traits. The 1 and -1 correlations are colored in blue and red, respectively. The other correlation values gradually approach from blue and red to zero, which is colored in white. All traits are ordered as: birth litter size (LS0_1, ..., ALW0), average pup weights (AW0_1, ..., AAW0), number of stillborn pups (SBP_1, ..., ASBP), litter size including number of stillborn pups (LST_1, ..., ALST), reproduction-related traits (PROG and C), body mass at different ages (BM21, ..., BMS), body dimension traits (SW, ..., WB), body weight gain traits (BWG1, 2 and 3), dam nursing traits (AW10st_1, ..., AAW21st), standard deviation of the pup weight in each litter (ILSD_1, ..., AILSDF), and sex ratio traits (SEXR_1, ..., ASEXR).

In total, 47 genome-wide significant QTL were identified for 31 traits and 213 chromosome-wide significant QTL were identified for 63 traits (see all of the genome-wide significant QTL and chromosome-wide significant QTL in Table S1.3 and Table S1.4 in the supplementary information).

For traits related to litter size, 12 genome-wide significant QTL were identified, which were mainly located on MMU2, MMU4 and MMU13. For traits related to litter weight, eight genome-wide significant QTL were identified on MMU2 and MMU4. For traits related to body mass, 13 genome-wide significant QTL were identified on MMU4. Table 1.2 shows the statistics and descriptive information for the genome-wide significant QTL on MMU2, MMU4, and MMU13 for traits related to litter size, litter weight, and body mass.

After comparing all of the putative QTL that affected litter size, several genome-wide significant QTL stacked in a short region on chromosomes 2 (from 45 cM to 55 cM), e.g., LS0_1_02_1 for the birth litter size trait in the first litter (F-value = 8.6499), LS0_2_02_1 for the birth litter size in the second litter (13.3367), ALS0_02_1 for the average birth litter size in two litters (16.8427), and LST_1_02_1 for the birth litter size including stillborn pups in the first litter (10.6806) on MMU2. Other QTL for litter size were mainly located on MMU4 and MMU13.

Table 1.2 Genome-wide significant QTLs detected on chromosomes (Chr.) 2, 4, and 13 for traits comprising: the average birth litter size for two litters (ALS0), average birth litter size including stillborn pups for two litters (ALST), birth litter size for the first litter (LS0_1) and the second litter (LS0_2), birth litter size including stillborn pups for the first litter (LST_1) and the second litter (LST_2), average birth litter weight for two litters (ALW0), birth litter weight for the first litter (LW0_1) and the second litter (LW0_2), tail length (TL); body mass at day 42 (BM42), at day 63 (BM63), at first mating day (BMM), and at slaughter day (BMS); body weight before slaughtering (SW), body weight gain from day 21 to day 42 (BWG1), and waist width (WW).

Chr.	Trait	Peak	F-value (P-value)	Threshold	<i>a</i> (SE)	<i>d</i> (SE)	r%
2	ALS0	52.5	16.84 (< 0.001)	7.77	-1.07 (0.18)	0.07 (0.34)	6.57%
	ALST	51.5	19.65 (0.017)	7.77	-1.14 (0.18)	0.01 (0.33)	7.64%
	LS0_1	44.5	8.60 (< 0.001)	7.69	-0.94 (0.24)	-0.68 (0.48)	3.27%
	LS0_2	51.5	13.34 (< 0.001)	7.78	-1.29 (0.25)	0.39 (0.45)	5.19%
	LST_1	45.5	10.68 (< 0.001)	7.68	-1.03 (0.23)	-0.53 (0.45)	4.10%
	LST_2	50.5	14.77 (< 0.001)	7.77	-1.33 (0.25)	0.27 (0.43)	5.76%
	ALW0	53.5	18.19 (< 0.001)	7.81	-1.68 (0.28)	0.01 (0.53)	7.08%
	LW0_1	51.5	13.13 (< 0.001)	7.80	-1.51 (0.31)	-0.62 (0.56)	5.08%
	LW0_2	53.5	11.80 (< 0.001)	7.80	-1.80 (0.37)	0.45 (0.70)	4.57%
	TL	29.5	8.41 (< 0.001)	7.67	0.15 (0.04)	-0.09 (0.08)	3.20%
4	ALS0	72.4	9.37 (< 0.001)	7.77	-0.86 (0.20)	0.49 (0.48)	3.58%
	LS0_1	63.4	8.60 (< 0.001)	7.69	-1.00 (0.24)	0.89 (0.71)	3.25%
	ALW0	67.4	12.03 (< 0.001)	7.81	-1.58 (0.32)	1.01 (0.88)	4.67%
	LW0_1	60.4	12.25 (< 0.001)	7.80	-1.80 (0.36)	1.14 (1.05)	4.73%
	BM42	40.4	19.03 (< 0.001)	7.69	-1.22 (0.20)	-0.10 (0.50)	7.42%
	BM63	54.4	11.25 (< 0.001)	7.75	-1.17 (0.25)	0.19 (0.64)	4.51%
	BMM	51.4	10.96 (< 0.001)	7.77	-1.36 (0.29)	-0.52 (0.71)	6.04%
	BMS	54.4	16.94 (< 0.001)	7.70	-1.55 (0.27)	-0.60 (0.71)	6.59%
	SW	52.4	15.62 (< 0.001)	7.79	-1.38 (0.25)	-0.29 (0.62)	6.10%
	BWG1	41.4	17.83 (< 0.001)	7.78	-0.76 (0.13)	-0.41 (0.32)	6.96%
	WW	55.4	10.61 (< 0.001)	7.77	-0.09 (0.02)	-0.10 (0.06)	4.08%
	13	ALS0	47.7	8.45 (< 0.001)	7.77	-0.27 (0.18)	1.44 (0.36)
ALST		47.7	8.75 (< 0.001)	7.77	-0.26 (0.18)	1.49 (0.36)	3.32%

Peak: peak positions of the QTL in cM; Threshold: genome-wide significance threshold for each trait and each chromosome; *a* and *d*: additive and dominant QTL effects derived from single marker regression for the peak positions of QTLs, SE in bracket; r%: percentage of phenotypic variance accounted for by QTL.

For all of the body mass traits and body weight gain traits, loci in the region between 40 cM to 50 cM on chromosome 4 were detected for body mass at day 21, day 42, mating day, slaughter day, and body weight gain from day 21 to 42, where the QTL obtained in this region had very high genome-wide significant effects for most traits, e.g., BM42_04_1 for body mass at day 42 (19.0344), BMS_04_1 for body mass at slaughter day (16.9400), and BWG1_04_1 for body weight gain from day 21 to 42 (17.8342). In addition, substantial QTL detected on other chromosomes exhibited genome-wide significant effects, e.g., BMO1_10_1 for body mass when virginal first opened (15.9293) and BMS_07_1 for body mass at slaughter day (24.6000). It was notable that most of the body mass QTL were located in certain regions, such as around 35 cM and 82 cM on MMU2, around 50 cM on chromosome 4, at 40 ± 10 cM on MMU7, and around 50 cM on MMU8.

For litter weight, the QTL detected on MMU2 and MMU4 had very high F-values, e.g., up to 13.1256 and 11.7922 (LW0_1_02_1 for birth litter weight of the first litter and LW0_2_02_1 for birth litter weight of the second litter), and they were also located close together on the chromosomes. The QTL related to the average weight per pup always had high F-values, e.g., AW0_2_09_1 had an F-value of 17.1467. However, QTLs that affected intra-litter variability were rare and they had slightly higher F-values than the chromosome-wide significance threshold.

In addition, six QTL were identified for body dimension traits, e.g., WW, BW, and tail length, where they had genome-wide significant effects on traits. However, for PROG concentration and the number of corpora lutea, which can partly represent the maternal reproductive ability, only one genome-wide significant QTL was found for PROG concentration in plasma and no QTL for the number of corpora lutea.

The F-value curves are shown in Figure 1.3 based on the QTL detected on MMU2, MMU4, MMU7, and MMU13 related to birth litter size with and without stillborn pups (A), birth litter weight and average pup weight (B), and body mass traits (C).

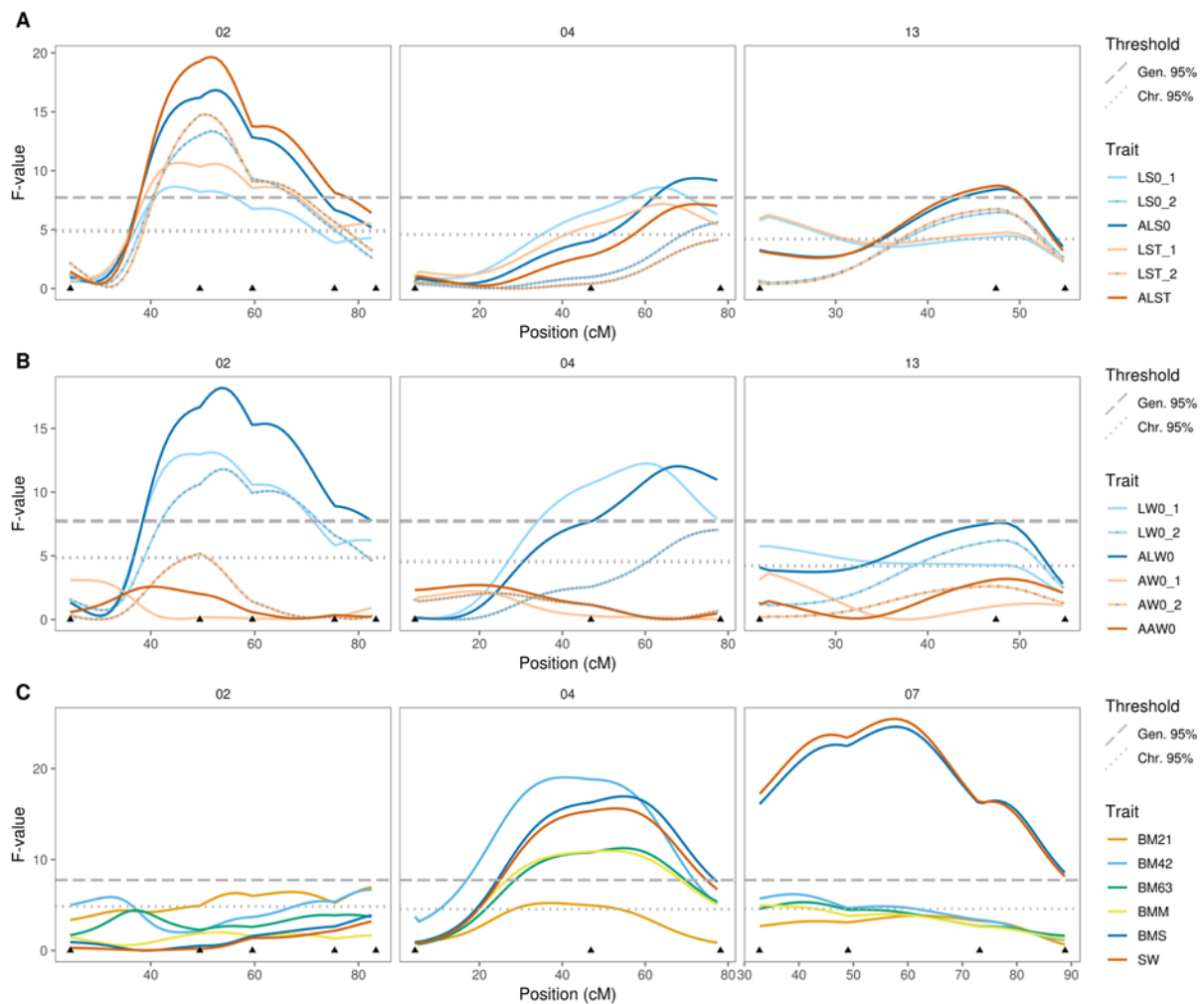


Figure 1.3 QTL effect curves on chromosomes 2, 4, 7 and 13 for: **(A)** birth litter size of the first litter (LS0_1), the second litter (LS0_2), and the average of two litters (ALS0); birth litter size including stillborn pups for the first litter (LST_1), the second litter (LST_2), and the average of two litters (ALST); **(B)** birth litter weight of the first litter (LW0_1), the second litter (LW0_2), and the average of two litters (ALW0); average pup weight for the first litter (AW0_1, LW0_1/ LS0_1), the second litter (AW0_2, LW0_2/ LS0_2), and the average of two litters (AAW0); **(C)** body mass at day 42 (BM42), at day 63 (BM63), at first mating day (BMM), and at slaughter day (BMS), and body weight before slaughtering (SW). The dashed horizontal lines indicate the genome-wide significance threshold for the 95% quantile and the dotted horizontal lines indicate the chromosome-wide significance threshold for the 95% quantile. In **(A)** and **(B)**, the traits for the two litters are colored in light blue and vermillion, and the traits for the second litter are denoted by the “+” symbol. The black triangles labeled on the X axis indicate the marker position on each chromosome.

Discussion

In this study, we aimed to identify QTL related to traits that improved during the selection of FL1, e.g., litter size, litter weight, and body mass, in order to explore the genetic basis for the improvements in these traits and to estimate the side-effects caused by the long-term selection of mouse line FL1. For example, several questions were addressed regarding the correlations among traits, including whether the average pup weight was decreased by selection because of inbreeding depression due to the contradiction between the high litter size and limited abdominal cavity of the mothers, and whether the weight difference was increased between the pups in one litter, i.e., higher intra-litter variability.

The QTL mapping conducted in this study only accurately identified the QTL regions and not candidate genes due to the density of markers and the statistics. The QTL effects detected in this study may be due to a single major gene or a linked group of several QTL with small effects (which will be clarified in further studies, e.g., by fine mapping); thus, the positions with the highest F-values in these regions are not necessarily considered the positions of candidate genes. Instead, we focused on “QTL regions” where each locus in these regions had a higher F-value than the threshold. The range of each QTL region was recorded (see Table S1.3 and Table S1.4 in the supplementary information). In particular, we identified several QTL regions with higher effects than the genome-wide significant threshold. For example, the F-value for QTL BMS_07_1 (24.6000) was three times higher than the genome-wide significance threshold (7.7040) for the body mass at slaughter day trait and five times higher than the chromosome-wide significance threshold for chromosome 7 (4.6289). Therefore, it is reasonable to suggest that essential candidate genes related to litter size are present in this region.

In order to verify the QTL for litter size, we collected two F₃ litters and measured the litter traits for both litters as the reproductive traits for their dams. The results for the two litters were highly similar. Genome-wide significant QTL for LS0_1 and LS0_2 were found on MMU2, MMU4, and MMU13, and the curves for their QTL effects strongly overlapped on these three chromosomes, but also on other chromosomes where we did not find QTL, e.g., MMU1 (see Figure 1.3 A). As shown in Figure 1.3 A and Figure 1.3 B, the peaks in the QTL curves for litter size and litter weight overlapped in terms of their position as well as the F-value at the peak, especially for the litter size and litter weight for the same litter. In order to explore the correlations in the reproductive traits (litter size and litter weight) and growth

traits (body mass and body dimension), we compared the QTL for these traits and found similar overlaps, but it should be noted that the effects of the QTL for litter size and body mass varied on different chromosomes (Figure 1.3).

Genomic regions harboring QTL for both reproductive traits and growth traits were also detected in other QTL studies (Collins *et al.* 1993; Kirkpatrick *et al.* 1998; Spearow *et al.* 1999). In particular, Kirkpatrick *et al.* showed that MMU2 and MMU4 harbored QTL associated with litter size and body weight (Kirkpatrick *et al.* 1998), and Spearow *et al.* and Rocha *et al.* verified that MMU2 harbored QTL for litter size and body weight (Spearow *et al.* 1999; Rocha *et al.* 2004). For example, Rocha *et al.* found two QTL comprising Lfq2 (38.4 cM) and Espq1 (50.1 cM) on MMU2 linked to litter size and body weight (Rocha *et al.* 2004). In human studies, two genetic risk variants were identified for spontaneous dizygotic twinning in a genome-wide association study, with one near *FSHB* and another within *SMAD3* (Mbarek *et al.* 2016). The follicle stimulating hormone beta gene (*Fshb*) is located at 56.02 cM on MMU2 (Glaser *et al.* 1990; Foroni *et al.* 1992). The SMAD family member 3 gene (*Smad3*) is located at 34.22 cM on MMU9 (Jackson Laboratory Mouse Radiation Hybrid Database, <http://www.informatics.jax.org/marker/MGI:1201674>). On MMU2, we also identified two QTL for litter size comprising LS0_1_02_1 (44.5 cM) and LS0_2_02_1 (51.5 cM), and two QTL for body mass comprising BMO1_02_1 (36.5 cM) and BMVO_02_1 (34.5 cM) were also found in the same region, where their QTL regions strongly overlapped with the QTL regions identified in other studies (Rocha *et al.* 2004; Mbarek *et al.* 2016). In particular, the litter trait-related QTL that we identified were close to the genetic position of the *Fshb* gene in mice, which harbors the candidate mutation for spontaneous dizygotic twinning in humans (Mbarek *et al.* 2016). This gene should also be considered as a candidate gene associated with litter traits in mice.

In addition, we observed that the body size (body weight and body dimension) increased in FL1 as a side-effect of the selection for litter size in FL1, which implies that the regions harboring QTL for LS, LW, and BM may contain genes that have important roles in the mechanisms related to reproduction and growth. Identifying and characterizing these genes is essential for determining the mechanisms responsible for reproduction and growth.

We observed that the QTL for birth litter size and birth litter weight strongly overlapped on MMU2 and MMU4 (Figure 1.3), which was partly due to the selection index employed for mouse line FL1 ($I = 1.6 \times LS0 + LW0$), and partly because of the correlation between the birth litter size and birth litter weight. As discussed above, we calculated the average litter

weight per pup at birth to remove the influence of the litter size on the litter weight, and we tested the related QTL. One genome-wide significant QTL for this trait was identified on MMU9 (AW0_2_09_1), and only one chromosome-wide significant QTL (AW0_2_02_1) was located in the region that harbored QTL for both LS0 and LW0. After comparing the QTL curves for birth litter weight and average pup weight (Figure 1.3), the QTL effects of average pup weight were eliminated by removing the influence of high litter size on the birth litter weight. In particular, a chromosome-wide significant QTL effect was detected for the average pup weight in the second litter on MMU2 even after eliminating the influence of litter size, which implies that mechanisms or genes might affect the birth litter weight directly instead of affecting it through the birth litter size.

After birth, each litter was standardized to 12 pups and we then measured the litter weight after standardization (LW0st_1/_2) as well as at day 10 (LW10st_1/_2) and at day 21 (LW21st_1/_2). The distributions of the QTL for LW10st_1/_2 and LW21st_1/_2 suggested that the litter weight may depend on the genes that affect the litter size and body mass. The QTL for LW0st_1/_2 overlapped with the QTL for average pup weight. For example, QTL LW0st_1_16_1 for litter weight after standardization and AW10st_2_16_1 for the average pup weight were located at 46.6 cM on chromosome 16. For litter size, after standardization (LS10st_1/_2 and LS21st_1/_2), most of the QTL were identified in the regions that harbored QTL for body mass. For instance, LW10st_2_02_1, LW21st_2_02_1, and BMVO_02_1 were detected at 32.5 cM, 30.5 cM, and 34.5 cM on MMU2, respectively. In summary, the correlations between litter weight and litter size as well as between litter weight and body mass may be attributable to potential pleiotropic genes and they merit further study.

There were still several interesting correlations between traits, such as the QTL for the PROG concentration in plasma, litter weight, and average pup weigh. The QTL for these two groups of traits were all located in similar regions on MMU 4 and 12, which were between 5.4 cM and 31.4 cM for PROG_04_1, between 4.4 cM and 27.4 cM for AW21st_1_04_1, between 22.0 cM and 30.0 cM for PROG_12_1, and between 26.0 cM and 44.0 cM for AW10st_2_12_1. These results suggest that there are potential correlations between the PROG concentrations in the plasma and embryo and fetal development (where it is related to the average pup weight), as found in other cytological and molecular studies. A previous study demonstrated that PROG can promote fetal development in mouse models (Solano *et al.* 2015). Another study reported that PROG injection without ovarian induction improved embryo survival, implantation rates, and embryo development (Ghaemi *et al.* 2008).

Pleiotropic reproductive abnormalities in mice were reported due to the lack of a PROG receptor (Lydon *et al.* 1995). In pigs, sows with larger litters had higher concentrations of PROG, but pups in the larger litters tended to die in the first few days and they had lower birth weights (Passillé *et al.* 1993), where low milk intake by pigs was a major cause of death and poor growth, and thus a boosted postpartum PROG may inhibit lactogenesis and contribute to poor pig performance. Thus, low lactation due to abnormal PROG concentrations could also explain the correlation between the PROG concentration in plasma and the average pup weight after standardization.

In addition, it is important to note that intra-uterine crowding due to a greater litter size in pigs has a direct negative effect on fetal growth, thereby resulting in a lower average litter birth weight (BtW) and larger variations in BtW within a litter (Rehfeldt *et al.* 2008). This is one reason why we considered these two sets of traits in this study. For the average pup weight, especially the average weight at parturition, the regions on MMU2 that harbored QTL for birth litter weight, birth litter size, and average pup weight overlapped considerably, e.g., LW0_2_02_1 (at around 53.5 cM), LS0_2_02_1 (at 51.5 cM), and AW0_2_02_1 (at 49.5 cM). However, no clear evidence suggested correlations between the intra-litter variability and litter size in this study.

In summary, litter size is indeed a quantitative character with considerable complexity, which is influenced by genes associated with multiple biological mechanisms. The discovery or identification of key genes can boost our understanding of litter size traits. Due to the high fertility of mouse line FL1, we identified a large number of putative QTL related to litter size and other traits, and some key QTL were located in several genomic regions, but especially on chromosome 2 and 4. Similar QTL mapping studies of litter size support our hypothesis that these genomic regions contain essential genes and their mutations may alter the litter size. We expect that characterizing these genes and fine-mapping research will uncover novel insights into the mechanism of high fertility.

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Supplementary Information

Table S1.1 Phenotypic characteristics of the F₂ females with puberty development, growth, litter size and litter weight at different ages and body conformation

Abbreviation	Description	Calculation		N.	Mean (\pm SD)
Litter size and fertility					
LS0_1	live pups in the first litter			458	15.97 \pm 3.40
LS0_2	live pups in the second litter			458	16.49 \pm 3.90
ALSO	average litter size of two litters	$(LS0_1 + LS0_2)/2$		458	16.23 \pm 2.80
LW0_1	total weight of all live pups in the first litter		g	458	27.19 \pm 4.65
LW0_2	total weight of all live pups in the second litter		g	456	28.69 \pm 5.63
ALW0	average litter weight of two litters	$(LW0_1 + LW0_2)/2$	g	456	27.94 \pm 4.32
AW0_1	average pup weight of litter_1	$LW0_1 / LS0_1$	g	458	1.72 \pm 0.16
AW0_2	average pup weight of litter_2	$LW0_2 / LS0_2$	g	456	1.76 \pm 0.18
AAW0	average pup weight of two litters	$(AW0_1 + AW0_2)/2$	g	456	1.74 \pm 0.13
SBP_1	number of stillborn pups in the first litter			458	0.27 \pm 0.76
SBP_2	number of stillborn pups in the second litter			458	0.42 \pm 1.07
ASBP	average of stillborn number	$(SBP_1 + SBP_2)/2$		458	0.34 \pm 0.69
LST_1	litter size of litter_1, including stillborn pups	$LS0_1 + SBP_1$		458	16.24 \pm 3.07
LST_2	litter size of litter_2, including stillborn pups	$LS0_2 + SBP_2$		458	16.91 \pm 3.83
ALST	average litter size of two litters, including stillborn pups	$(LST_1 + LST_2)/2$		458	16.58 \pm 2.81

PROG	concentration of progesterone in plasma		ng/ml	457	36.55 ± 17.38
CL	number of corpora lutea			443	16.84 ± 4.75

Body mass at different ages

BM21	body mass at day 21		g	458	12.18 ± 2.03
BM42	body mass at day 42		g	455	26.58 ± 2.78
BM63	body mass at day 63		g	439	32.90 ± 3.40
BMVO	body mass at vaginal opening		g	456	14.65 ± 2.34
BMO1	body mass at the 1 st oestrus		g	446	20.50 ± 2.94
BMM	body mass at mating day		g	315	33.15 ± 3.76
BMS	body mass at slaughter day		g	457	42.73 ± 3.91
SW	slaughter weight		g	455	42.09 ± 3.67

Body weight gain

BWG1	body weight gain 1	BM42 - BM21	g	455	14.39 ± 1.88
BWG2	body weight gain 2	BM63 - BM42	g	437	6.20 ± 2.00
BWG3	body weight gain 3	BM63 - BM21	g	439	20.63 ± 2.54

Body dimension

TOL	torso length (from neck to the tip of tail)		cm	457	18.67 ± 0.84
TL	tail length		cm	453	9.46 ± 0.51
WW	waist width		cm	457	4.29 ± 0.30
WB	belly width		cm	457	3.99 ± 0.29

Maternal nursing abilities of F2

LS10st_1	litter size at day 10 after standardization_1			447	11.29 ± 1.37
LS10st_2	litter size at day 10 after standardization_2			450	11.46 ± 1.21
LW10st_1	litter weight at day 10 after standardization_1		g	447	65.16 ± 10.82

LW10st_2	litter weight at day 10 after standardization_2		g	450	69.44 ± 10.90
AW10st_1	average weight of standardized litter_1 at day 10	LW10st_1 / LS10st_1	g	447	5.77 ± 0.70
AW10st_2	average weight of standardized litter_2 at day 10	LW10st_2 / LS10st_2	g	450	6.05 ± 0.75
AAW10st	average of average weight of two litters at day 10 after standardization	(AW10st_1+ AW10st_2)/2	g	440	5.91 ± 0.55
LS21st_1	litter size at day 21 after standardization_1			441	11.14 ± 1.49
LS21st_2	litter size at day 21 after standardization_2			446	11.36 ± 1.36
LW21st_1	litter weight at day 21 after standardization_1		g	440	110.99 ± 25.29
LW21st_2	litter weight at day 21 after standardization_2		g	446	121.63 ± 23.95
AW21st_1	average weight of standardized litter_1 at day 21	LW21st_1 / LS21st_1	g	440	9.95 ± 1.90
AW21st_2	average weight of standardized litter_2 at day 21	LW21st_2 / LS21st_2	g	446	10.72 ± 1.77
AAW21st	average of average weight of two litters at day 21 after standardization	(AW21st_1 + AW21st_2)/2	g	429	10.34 ± 1.45
Intra Litter Variability, ILV					
ILV_1	intra litter variability s ² for litter_1			456	0.02 ± 0.02
ILV_2	intra litter variability s ² for litter_2			455	0.03 ± 0.02
ILVM_1	intra litter variability s ² for male pups in litter_1			455	0.02 ± 0.02
ILVM_2	intra litter variability s ² for male pups in litter_2			455	0.03 ± 0.02
ILVF_1	intra litter variability s ² for female pups in litter_1			454	0.02 ± 0.02

ILVF_2	intra litter variability s^2 for female pups in litter_2		451	0.03 ± 0.02
ILSD_1	intra litter standard deviation for litter_1		456	0.15 ± 0.05
ILSD_2	intra litter standard deviation for litter_2		455	0.16 ± 0.05
ILSDM_1	intra litter standard deviation for male pups in litter_1		455	0.14 ± 0.06
ILSDM_2	intra litter standard deviation for male pups in litter_2		455	0.14 ± 0.06
ILSDF_1	intra litter standard deviation for female pups in litter_1		454	0.14 ± 0.06
ILSDF_2	intra litter standard deviation for female pups in litter_2		451	0.15 ± 0.06
AILSD	average intra litter standard deviation		453	0.15 ± 0.04
AILSDM	average intra litter standard deviation for male pups		452	0.14 ± 0.04
AILSDF	average intra litter standard deviation for female pups		447	0.14 ± 0.04
other traits				
MPL_1	male pups in litter_1		458	8.93 ± 2.60
MPL_2	male pups in litter_2		456	8.93 ± 2.83
FPL_1	female pups in litter_1		458	7.04 ± 2.34
FPL_2	female pups in litter_2		456	7.63 ± 2.75
SEXR_1	sex ratio for the first litter	FPL_1 / MPL_1	458	0.91 ± 0.58
SEXR_2	sex ratio for the second litter	FPL_2 / MPL_2	456	0.97 ± 0.55
ASEXR	average sex ratio of two litters	$(SEXR_1 + SEXR_2)/2$	456	0.94 ± 0.39

Table S1.2 Marker positions in centiMorgan (cM) on the whole genome of house mouse (*Mus musculus*), except chromosome Y. The positions were checked on Oct. 03, 2018 with the MGI database (<http://www.informatics.jax.org/>).

Marker	Position	Marker	Position	Marker	Position	Marker	Position
D1Mit211	10.59	D5Mit20	47.77	D10Mit36	24.87	D15Mit149	53.94
D1Mit234	23.93	D5Mit431	60.40	D10Mit230	45.28		
D1Mit332	39.58	D5Mit222	81.53	D10Mit145	72.35	D16Mit217	44.58
D1Mit216	40.97					D16Mit189	46.83
D1Mit140	58.18	D6Mit263	39.71	D11Mit271	27.44		
D1Mit206	80.33	D6Mit133	62.18	D11Mit131	33.48	D17Mit30	14.26
D1Mit223	93.21	D6Mit293	73.44	D11Mit195	51.34	D17Mit20	29.73
				D11Mit166	68.73	D17Mit129	56.83
D2Mit370	24.51	D7Mit145	32.75	D11Mit184	75.93		
D2Mit66	49.45	D7Mit31	49.01			D18Mit60	18.04
D2Mit395	59.57	D7Mit134	73.18	D12Mit136	13.00	D18Mit183	37.57
D2Mit285	75.41	D7Mit259	88.85	D12Mit4	35.51	D18Mit3	54.83
D2Mit50	83.37			D12Mit262	60.56		
		D8Mit289	16.47	D12Mit144	63.48	D19Mit130	16.14
D3Mit178	14.27	D8Mit31	34.43			D19Mit118	33.10
D3Mit137	34.91	D8Mit121	72.27	D13Mit64	21.73	D19Mit11.1	36.26
D3Mit57	50.09			D13Mit202	47.43	D19Mit137	54.60
D3Mit44	72.43	D9Mit297	18.24	D13Mit287	54.94		
		D9Mit1001	20.98			DXMit192	27.71
D4Mit227	4.43	D9Mit301	30.43	D14Mit60	24.60	DXMit79	53.17
D4Mit176	46.89	D9Mit269	47.15	D14Mit97	62.20	DXMit153	68.46
D4Mit233	78.17	D9Mit116	59.58				
				D15Mit82	11.95		
D5Mit352	18.40	D10Mit123	3.44	D15Mit230	21.75		
D5Mit15	33.68	D10Mit86	11.87	D15Mit105	33.42		

Table S1.3 Genome-wide significant QTL.

QTL	Chr.	Trait	Forward marker	Backward marker	Peak	F-value	P-value	<i>a</i>	SE	<i>d</i>	SE	r%
SBP_1_01_1	1	SBP_1	D1Mit206	D1Mit223	86.6	9.7044	0.0001	0.2275	0.0534	0.0961	0.1013	3.70%
ALS0_02_1	2	ALS0	D2Mit66	D2Mit395	52.5	16.8427	0.0000	-1.0625	0.1829	0.0734	0.3401	6.57%
ALST_02_1	2	ALST	D2Mit66	D2Mit395	51.5	19.6589	0.0173	-1.1375	0.1814	0.0126	0.3288	7.64%
LS0_1_02_1	2	LS0_1	D2Mit370	D2Mit66	44.5	8.6499	0.0002	-0.9380	0.2403	-0.6836	0.4806	3.27%
LS0_2_02_1	2	LS0_2	D2Mit66	D2Mit395	51.5	13.3367	0.0000	-1.2864	0.2504	0.3853	0.4538	5.19%
LST_1_02_1	2	LST_1	D2Mit370	D2Mit66	45.5	10.6806	0.0000	-1.0267	0.2305	-0.5304	0.4451	4.10%
LST_2_02_1	2	LST_2	D2Mit66	D2Mit395	50.5	14.7701	0.0000	-1.3337	0.2457	0.2749	0.4318	5.76%
ALS0_04_1	4	ALS0	D4Mit176	D4Mit233	72.4	9.3692	0.0001	-0.8586	0.2010	0.4930	0.4820	3.58%
LS0_1_04_1	4	LS0_1	D4Mit176	D4Mit233	63.4	8.5973	0.0002	-0.9997	0.2449	0.8930	0.7060	3.25%
ASBP_11_1	11	ASBP	D11Mit271	D11Mit131	30.4	7.9335	0.0004	0.1743	0.0439	-0.0318	0.0915	2.97%
ALS0_13_1	13	ALS0	D13Mit202	D13Mit287	47.7	8.4515	0.0002	-0.2732	0.1752	1.4358	0.3604	3.20%
ALST_13_1	13	ALST	D13Mit202	D13Mit287	47.7	8.7451	0.0002	-0.2628	0.1772	1.4871	0.3646	3.32%
ALW0_02_1	2	ALW0	D2Mit66	D2Mit395	53.5	18.1868	0.0000	-1.6803	0.2787	0.0105	0.5274	7.08%
LW0_1_02_1	2	LW0_1	D2Mit66	D2Mit395	51.5	13.1256	0.0000	-1.5141	0.3061	-0.6200	0.5551	5.08%
LW0_2_02_1	2	LW0_2	D2Mit66	D2Mit395	53.5	11.7922	0.0000	-1.8026	0.3718	0.4515	0.7035	4.57%
ALW0_04_1	4	ALW0	D4Mit176	D4Mit233	67.4	12.0340	0.0000	-1.5770	0.3237	1.0119	0.8794	4.67%
LW0_1_04_1	4	LW0_1	D4Mit176	D4Mit233	60.4	12.2545	0.0000	-1.7984	0.3645	1.1370	1.0498	4.73%
AAW0_09_1	9	AAW0	D9Mit269	D9Mit116	57.2	18.0596	0.0000	-0.0502	0.0083	-0.0034	0.0159	7.03%
AW0_2_09_1	9	AW0_2	D9Mit269	D9Mit116	53.2	17.1467	0.0000	-0.0718	0.0123	0.0138	0.0243	6.68%
PROG_15_1	15	PROG	D15Mit82	D15Mit230	12.0	8.1409	0.0003	-1.6059	1.0968	7.4796	1.9813	3.06%
BMS_01_1	1	BMS	D1Mit206	D1Mit223	80.6	7.7485	0.0005	0.9626	0.2475	-0.3243	0.4275	2.90%
BMVO_02_1	2	BMVO	D2Mit370	D2Mit66	34.5	8.1105	0.0003	-0.3026	0.2153	-2.0170	0.5348	3.06%
BM42_04_1	4	BM42	D4Mit227	D4Mit176	40.4	19.0344	0.0000	-1.2224	0.1982	-0.1028	0.4993	7.42%
BM63_04_1	4	BM63	D4Mit176	D4Mit233	54.4	11.2459	0.0000	-1.1704	0.2466	0.1850	0.6434	4.51%

BMM_04_1	4	BMM	D4Mit176	D4Mit233	51.4	10.9558	0.0000	-1.3563	0.2938	-0.5173	0.7110	6.04%
BMS_04_1	4	BMS	D4Mit176	D4Mit233	54.4	16.9400	0.0000	-1.5455	0.2727	-0.6040	0.7109	6.59%
SW_04_1	4	SW	D4Mit176	D4Mit233	52.4	15.6191	0.0000	-1.3767	0.2489	-0.2851	0.6180	6.10%
BMS_07_1	7	BMS	D7Mit31	D7Mit134	57.8	24.6000	0.0000	-1.8116	0.2731	1.1830	0.5720	9.46%
SW_07_1	7	SW	D7Mit31	D7Mit134	57.8	25.4599	0.0000	-1.7272	0.2577	1.2039	0.5384	9.81%
BMO1_08_1	8	BMO1	D8Mit289	D8Mit31	25.5	9.9829	0.0001	0.7218	0.2037	1.2917	0.5077	3.92%
BMS_08_1	8	BMS	D8Mit289	D8Mit31	24.5	8.5872	0.0002	0.8492	0.2558	1.4872	0.6335	3.25%
BMO1_10_1	10	BMO1	D10Mit123	D10Mit86	6.4	15.9293	0.0000	1.1041	0.1956	-0.0711	0.3631	6.34%
BM42_11_1	11	BM42	D11Mit131	D11Mit195	49.4	7.9904	0.0004	0.7003	0.1761	-0.1691	0.3451	3.01%
BWG1_03_1	3	BWG1	D3Mit178	D3Mit137	34.3	10.0981	0.0001	-0.5308	0.1186	-0.0131	0.2114	3.89%
BWG1_04_1	4	BWG1	D4Mit227	D4Mit176	41.4	17.8342	0.0000	-0.7554	0.1299	-0.4085	0.3204	6.96%
TL_02_1	2	TL	D2Mit370	D2Mit66	29.5	8.4061	0.0003	0.1466	0.0370	-0.0878	0.0813	3.20%
WW_04_1	4	WW	D4Mit176	D4Mit233	55.4	10.6144	0.0000	-0.0947	0.0223	-0.1021	0.0613	4.08%
WW_08_1	8	WW	D8Mit31	D8Mit121	56.5	9.5657	0.0001	0.1131	0.0259	-0.0461	0.0776	3.65%
WB_11_1	11	WB	D11Mit131	D11Mit195	45.4	8.1884	0.0003	0.0747	0.0195	-0.0549	0.0416	3.08%
TL_11_1	11	TL	D11Mit131	D11Mit195	42.4	8.4435	0.0003	0.1196	0.0323	-0.1241	0.0715	3.22%
TL_19_1	19	TL	D19Mit130	D19Mit118	23.1	8.6318	0.0002	-0.1434	0.0365	0.0879	0.0819	3.30%
LW10st_2_02_1	2	LW10st_2	D2Mit370	D2Mit66	32.5	8.2658	0.0003	0.3092	0.9584	9.2992	2.2949	3.16%
LW21st_2_02_1	2	LW21st_2	D2Mit370	D2Mit66	30.5	8.0520	0.0004	3.0480	1.9270	15.9420	4.3560	3.10%
AW10st_2_03_1	3	AW10st_2	D3Mit137	D3Mit57	42.3	9.9854	0.0001	0.1753	0.0541	-0.3752	0.1088	3.88%
AAW10st_03_1	3	AAW10st	D3Mit137	D3Mit57	41.3	8.2599	0.0003	0.1131	0.0394	-0.2536	0.0788	3.23%
AW21st_2_06_1	6	AW21st_2	D6Mit263	D6Mit133	39.7	7.9580	0.0004	-0.3454	0.1099	-0.5613	0.2294	3.06%
FPL_2_02_1	2	FPL_2	D2Mit66	D2Mit395	51.5	9.4431	0.0001	-0.8170	0.1887	-0.0644	0.3419	3.61%

Peak: peak positions of the QTL in cM; *a* and *d*: additive and dominant QTL effects derived from single marker regression for the peak positions of the QTLs, SE in bracket; *r*%: percentage of phenotypic variance accounted for by QTL.

Table S1.4 Chromosome-wide significant QTL.

QTL	Chr.	Trait	Forward	Backward	Peak	F-value	P-value
ALS0_01_1	1	ALS0	D1Mit216	D1Mit140	53.6	6.7616	0.0013
SBP_1_01_1	1	SBP_1	D1Mit206	D1Mit223	86.6	9.7044	0.0001
LS0_1_02_1	2	LS0_1	D2Mit370	D2Mit66	44.5	8.6499	0.0002
LS0_2_02_1	2	LS0_2	D2Mit66	D2Mit395	51.5	13.3367	0.0000
LST_1_02_1	2	LST_1	D2Mit370	D2Mit66	45.5	10.6806	0.0000
LST_2_02_1	2	LST_2	D2Mit66	D2Mit395	50.5	14.7701	0.0000
ALS0_02_1	2	ALS0	D2Mit66	D2Mit395	52.5	16.8427	0.0000
ALST_02_1	2	ALST	D2Mit66	D2Mit395	51.5	19.6589	0.0173
SBP_1_03_1	3	SBP_1	D3Mit178	D3Mit137	22.3	5.0333	0.0069
ASBP_03_1	3	ASBP	D3Mit178	D3Mit137	25.3	5.0333	0.0069
LS0_1_04_1	4	LS0_1	D4Mit176	D4Mit233	63.4	8.5973	0.0002
LS0_2_04_1	4	LS0_2	D4Mit176	D4Mit233	77.4	5.6102	0.0039
LST_1_04_1	4	LST_1	D4Mit176	D4Mit233	64.4	7.1901	0.0008
ALS0_04_1	4	ALS0	D4Mit176	D4Mit233	72.4	9.3692	0.0001
ALST_04_1	4	ALST	D4Mit176	D4Mit233	72.4	7.1591	0.0009
LS0_1_05_1	5	LS0_1	D5Mit15	D5Mit20	40.4	6.0230	0.0026
LST_1_05_1	5	LST_1	D5Mit15	D5Mit20	39.4	6.2094	0.0022
ALST_05_1	5	ALST	D5Mit15	D5Mit20	43.4	5.3709	0.0050
LST_2_09_1	9	LST_2	D9Mit269	D9Mit116	51.2	5.9811	0.0027
SBP_2_09_1	9	SBP_2	D9Mit269	D9Mit116	47.2	7.3589	0.0007
ALS0_09_1	9	ALS0	D9Mit301	D9Mit269	31.2	4.6670	0.0099
ALST_09_1	9	ALST	D9Mit1001	D9Mit301	31.2	4.9025	0.0078
ASBP_09_1	9	ASBP	D9Mit301	D9Mit269	46.2	5.9730	0.0028
SBP_2_11_1	11	SBP_2	D11Mit271	D11Mit131	29.4	7.7815	0.0005
LS0_1_13_1	13	LS0_1	D13Mit64	D13Mit202	22.7	6.0887	0.0025
LS0_2_13_1	13	LS0_2	D13Mit202	D13Mit287	47.7	6.4758	0.0017
LST_1_13_1	13	LST_1	D13Mit64	D13Mit202	22.7	6.2633	0.0021
LST_2_13_1	13	LST_2	D13Mit202	D13Mit287	47.7	6.7532	0.0013
ALS0_13_1	13	ALS0	D13Mit202	D13Mit287	47.7	8.4515	0.0002
ALST_13_1	13	ALST	D13Mit202	D13Mit287	47.7	8.7451	0.0002
SBP_2_15_1	15	SBP_2	D15Mit230	D15Mit105	28.0	5.9736	0.0028
SBP_1_17_1	17	SBP_1	D17Mit20	D17Mit129	41.3	5.8274	0.0032
ASBP_17_1	17	ASBP	D17Mit20	D17Mit129	30.3	4.7774	0.0088
ALW0_01_1	1	ALW0	D1Mit216	D1Mit140	57.6	5.8867	0.0003
LW0_1_02_1	2	LW0_1	D2Mit66	D2Mit395	51.5	13.1256	0.0000
LW0_2_02_1	2	LW0_2	D2Mit66	D2Mit395	53.5	11.7922	0.0000
ALW0_02_1	2	ALW0	D2Mit66	D2Mit395	53.5	18.1868	0.0000
LW0_1_04_1	4	LW0_1	D4Mit176	D4Mit233	60.4	12.2545	0.0000
LW0_2_04_1	4	LW0_2	D4Mit176	D4Mit233	77.4	7.0490	0.0010
ALW0_04_1	4	ALW0	D4Mit176	D4Mit233	67.4	12.0340	0.0000
LW0_1_05_1	5	LW0_1	D5Mit15	D5Mit20	37.4	5.2452	0.0056
LW0_1_13_1	13	LW0_1	D13Mit64	D13Mit202	22.7	5.7623	0.0034

LW0_2_13_1	13	LW0_2	D13Mit202	D13Mit287	47.7	6.2187	0.0022
ALW0_13_1	13	ALW0	D13Mit202	D13Mit287	47.7	7.6139	0.0006
LW0_1_15_1	15	LW0_1	D15Mit105	D15Mit149	53.0	5.1178	0.0063
ALW0_15_1	15	ALW0	D15Mit105	D15Mit149	52.0	6.1792	0.0023
LW0_1_16_1	16	LW0_1	D16Mit217	D16Mit189	46.6	5.1178	0.0063
LW0_2_16_1	16	LW0_2	D16Mit217	D16Mit189	46.6	3.1274	0.0448
ALW0_16_1	16	ALW0	D16Mit217	D16Mit189	46.6	6.1639	0.0023
AW0_2_02_1	2	AW0_2	D2Mit66	D2Mit395	49.5	5.1654	0.0061
AW0_2_05_1	5	AW0_2	D5Mit431	D5Mit222	71.4	4.7242	0.0093
AW0_1_09_1	9	AW0_1	D9Mit301	D9Mit269	33.2	5.5627	0.0041
AW0_2_09_1	9	AW0_2	D9Mit269	D9Mit116	53.2	17.1467	0.0000
AAW0_09_1	9	AAW0	D9Mit269	D9Mit116	57.2	18.0596	0.0000
AW0_2_12_1	12	AW0_2	D12Mit136	D12Mit4	33.0	6.1443	0.0023
AW0_1_10_1	10	AW0_1	D10Mit86	D10Mit36	24.4	5.5933	0.0040
PROG_04_1	4	PROG	D4Mit227	D4Mit176	17.4	7.1367	0.0009
PROG_12_1	12	PROG	D12Mit136	D12Mit4	26.0	4.7858	0.0088
PROG_15_1	15	PROG	D15Mit82	D15Mit230	12.0	8.1409	0.0003
BM63_01_1	1	BM63	D1Mit206	D1Mit223	80.6	5.3320	0.0052
BMS_01_1	1	BMS	D1Mit206	D1Mit223	80.6	7.7485	0.0005
SW_01_1	1	SW	D1Mit206	D1Mit223	81.6	7.1940	0.0008
BM21_02_1	2	BM21	D2Mit285	D2Mit50	82.5	6.9562	0.0011
BM42_02_1	2	BM42	D2Mit285	D2Mit50	82.5	6.7085	0.0013
BMO1_02_1	2	BMO1	D2Mit370	D2Mit66	36.5	5.0563	0.0067
BMVO_02_1	2	BMVO	D2Mit370	D2Mit66	34.5	8.1105	0.0003
BM21_03_1	3	BM21	D3Mit57	D3Mit44	59.3	5.5659	0.0041
BM42_03_1	3	BM42	D3Mit57	D3Mit44	57.3	6.5752	0.0015
BM21_04_1	4	BM21	D4Mit227	D4Mit176	36.4	5.2165	0.0058
BM42_04_1	4	BM42	D4Mit227	D4Mit176	40.4	19.0344	0.0000
BM63_04_1	4	BM63	D4Mit176	D4Mit233	54.4	11.2459	0.0000
BMM_04_1	4	BMM	D4Mit176	D4Mit233	51.4	10.9558	0.0000
BMS_04_1	4	BMS	D4Mit176	D4Mit233	54.4	16.9400	0.0000
SW_04_1	4	SW	D4Mit176	D4Mit233	52.4	15.6191	0.0000
BMVO_05_1	5	BMVO	D5Mit431	D5Mit222	68.4	6.3481	0.0019
BM42_06_1	6	BM42	D6Mit133	D6Mit293	66.7	4.3168	0.0139
BM42_07_1	7	BM42	D7Mit145	D7Mit31	38.8	6.1907	0.0022
BM63_07_1	7	BM63	D7Mit145	D7Mit31	40.8	5.3124	0.0053
BMM_07_1	7	BMM	D7Mit145	D7Mit31	32.8	4.9709	0.0075
BMO1_07_1	7	BMO1	D7Mit145	D7Mit31	45.8	6.1126	0.0024
BMS_07_1	7	BMS	D7Mit31	D7Mit134	57.8	24.6000	0.0000
SW_07_1	7	SW	D7Mit31	D7Mit134	57.8	25.4599	0.0000
BM42_08_1	8	BM42	D8Mit31	D8Mit121	63.5	4.7056	0.0095
BM63_08_1	8	BM63	D8Mit31	D8Mit121	34.5	7.1897	0.0008
BMO1_08_1	8	BMO1	D8Mit289	D8Mit31	25.5	9.9829	0.0001
BMS_08_1	8	BMS	D8Mit289	D8Mit31	24.5	8.5872	0.0002

SW_08_1	8	SW	D8Mit31	D8Mit121	44.5	6.9368	0.0011
BMO1_10_1	10	BMO1	D10Mit123	D10Mit86	6.4	15.9293	0.0000
SW_10_1	10	SW	D10Mit230	D10Mit145	72.4	5.1505	0.0061
BM42_11_1	11	BM42	D11Mit131	D11Mit195	49.4	7.9904	0.0004
BM63_11_1	11	BM63	D11Mit131	D11Mit195	39.4	5.4546	0.0046
BMO1_12_1	12	BMO1	D12Mit136	D12Mit4	13.0	5.2982	0.0053
BMM_13_1	13	BMM	D13Mit202	D13Mit287	47.7	4.2510	0.0152
BM21_15_1	15	BM21	D15Mit105	D15Mit149	50.0	5.0473	0.0068
BM21_16_1	16	BM21	D16Mit217	D16Mit189	45.6	4.9385	0.0076
BMVO_16_1	16	BMVO	D16Mit217	D16Mit189	46.6	3.4927	0.0312
BM21_19_1	19	BM21	D19Mit130	D19Mit118	19.1	7.4761	0.0006
SW_19_1	19	SW	D19Mit130	D19Mit118	30.1	4.6081	0.0104
BWG2_01_1	1	BWG2	D1Mit211	D1Mit234	13.6	5.3971	0.0048
BWG3_01_1	1	BWG3	D1Mit234	D1Mit332	28.6	6.0381	0.0026
BWG1_03_1	3	BWG1	D3Mit178	D3Mit137	34.3	10.0981	0.0001
BWG1_04_1	4	BWG1	D4Mit227	D4Mit176	41.4	17.8342	0.0000
BWG3_04_1	4	BWG3	D4Mit176	D4Mit233	60.4	7.5932	0.0006
BWG1_07_1	7	BWG1	D7Mit145	D7Mit31	37.8	5.1503	0.0061
BWG2_08_1	8	BWG2	D8Mit31	D8Mit121	34.5	5.0588	0.0067
BWG3_08_1	8	BWG3	D8Mit31	D8Mit121	38.5	7.1529	0.0009
BWG1_11_1	11	BWG1	D11Mit131	D11Mit195	50.4	7.5689	0.0006
BWG3_11_1	11	BWG3	D11Mit131	D11Mit195	36.4	6.6900	0.0014
BWG1_12_1	12	BWG1	D12Mit4	D12Mit262	52.0	4.8766	0.0080
BWG2_19_1	19	BWG2	D19Mit130	D19Mit118	24.1	4.7400	0.0092
BWG1_X_1	X	BWG1	DXMit192	DXMit79	27.7	5.6913	0.0036
BWG3_X_1	X	BWG3	DXMit192	DXMit79	27.7	5.7644	0.0034
TL_01_1	1	TL	D1Mit234	D1Mit332	26.6	6.0348	0.0026
WW_01_1	1	WW	D1Mit206	D1Mit223	83.6	5.6854	0.0036
TL_02_1	2	TL	D2Mit370	D2Mit66	29.5	8.4061	0.0003
BW_04_1	4	WB	D4Mit227	D4Mit176	33.4	7.3160	0.0007
TOL_04_1	4	TOL	D4Mit227	D4Mit176	29.4	5.6649	0.0037
WW_04_1	4	WW	D4Mit176	D4Mit233	55.4	10.6144	0.0000
TL_06_1	6	TL	D6Mit263	D6Mit133	54.7	4.7367	0.0092
WW_07_1	7	WW	D7Mit145	D7Mit31	32.8	4.9908	0.0072
WB_08_1	8	WB	D8Mit289	D8Mit31	23.5	5.3635	0.0050
WW_08_1	8	WW	D8Mit31	D8Mit121	56.5	9.5657	0.0001
TL_09_1	9	TL	D9Mit297	D9Mit1001	18.2	5.9655	0.0028
WB_11_1	11	WB	D11Mit131	D11Mit195	45.4	8.1884	0.0003
TL_11_1	11	TL	D11Mit131	D11Mit195	42.4	8.4435	0.0003
TOL_11_1	11	TOL	D11Mit131	D11Mit195	45.4	7.0796	0.0009
TL_14_1	14	TL	D14Mit60	D14Mit97	24.6	5.4519	0.0046
TOL_14_1	14	TOL	D14Mit60	D14Mit97	36.6	4.1929	0.0157
TOL_17_1	17	TOL	D17Mit30	D17Mit20	21.3	5.3359	0.0051
TL_19_1	19	TL	D19Mit130	D19Mit118	23.1	8.6318	0.0002
TOL_19_1	19	TOL	D19Mit130	D19Mit118	23.1	6.3352	0.0019

LS10st_1_04_1	4	LS10st_1	D4Mit176	D4Mit233	57.4	4.5154	0.0115
LS21st_1_04_1	4	LS21st_1	D4Mit176	D4Mit233	53.4	5.3273	0.0052
LS10st_2_06_1	6	LS10st_2	D6Mit263	D6Mit133	43.7	4.6039	0.0105
LS21st_1_10_1	10	LS21st_1	D10Mit86	D10Mit36	12.4	5.8007	0.0033
LS10st_1_16_1	16	LS10st_1	D6Mit217	D16Mit189	46.6	3.1084	0.0457
LS21st_1_16_1	16	LS21st_1	D16Mit217	D16Mit189	46.6	3.9411	0.0201
LS21st_2_18_1	18	LS21st_2	D18Mit183	D18Mit3	51.0	4.9774	0.0073
LS21st_1_X_1	X	LS21st_1	DXMit192	DXMit79	46.7	4.3160	0.0139
LW10st_2_02_1	2	LW10st_2	D2Mit370	D2Mit66	32.5	8.2658	0.0003
LW21st_2_02_1	2	LW21st_2	D2Mit370	D2Mit66	30.5	8.0520	0.0004
LW10st_1_03_1	3	LW10st_1	D3Mit57	D3Mit44	66.3	4.7793	0.0088
LW10st_2_03_1	3	LW10st_2	D3Mit137	D3Mit57	39.3	5.5748	0.0041
LW21st_1_03_1	3	LW21st_1	D3Mit57	D3Mit44	59.3	4.6376	0.0102
LW10st_1_04_1	4	LW10st_1	D4Mit176	D4Mit233	58.4	7.1746	0.0009
LW21st_1_04_1	4	LW21st_1	D4Mit176	D4Mit233	55.4	7.3737	0.0007
LW0st_2_05_1	5	LW0st_2	D5Mit431	D5Mit222	74.4	6.0585	0.0025
LW21st_1_06_1	6	LW21st_1	D6Mit133	D6Mit293	67.7	4.8201	0.0085
LW0st_1_09_1	9	LW0st_1	D9Mit301	D9Mit269	33.2	7.4432	0.0007
LW0st_1_10_1	10	LW0st_1	D10Mit86	D10Mit36	24.4	5.9357	0.0029
LW10st_2_11_1	11	LW10st_2	D11Mit166	D11Mit184	74.4	4.9050	0.0078
LW21st_2_11_1	11	LW21st_2	D11Mit166	D11Mit184	75.4	5.9745	0.0028
LW0st_2_12_1	12	LW0st_2	D12Mit136	D12Mit4	35.0	8.6520	0.0002
LW10st_2_12_1	12	LW10st_2	D12Mit4	D12Mit262	36.0	4.6027	0.0105
LW10st_1_X_1	X	LW10st_1	DXMit79	DXMit153	58.7	5.2335	0.0057
LW21st_1_X_1	X	LW21st_1	DXMit79	DXMit153	60.7	5.6363	0.0038
AAW21st_01_1	1	AAW21st	D1Mit206	D1Mit233	80.6	5.1094	0.0064
AW10st_2_02_1	2	AW10st_2	D2Mit370	D2Mit66	31.5	6.7259	0.0013
AW21st_2_02_1	2	AW21st_2	D2Mit370	D2Mit66	29.5	6.1958	0.0022
AW10st_2_03_1	3	AW10st_2	D3Mit137	D3Mit57	42.3	9.9854	0.0001
AW21st_2_03_1	3	AW21st_2	D3Mit137	D3Mit57	44.3	6.0664	0.0025
AAW21st_03_1	3	AAW21st	D3Mit57	D3Mit44	58.3	6.8437	0.0012
AW21st_1_04_1	4	AW21st_1	D4Mit227	D4Mit176	16.4	7.5038	0.0006
AAW21st_04_1	4	AAW21st	D4Mit227	D4Mit176	18.4	5.5856	0.0040
AW21st_2_06_1	6	AW21st_2	D6Mit263	D6Mit133	39.7	7.9580	0.0004
AAW21st_06_1	6	AAW21st	D6Mit263	D6Mit133	39.7	5.1658	0.0061
AAW21st_06_2	6	AAW21st	D6Mit133	D6Mit293	67.7	4.4329	0.0124
AW10st_1_07_1	7	AW10st_1	D7Mit134	D7Mit259	88.8	6.1509	0.0023
AW21st_1_07_1	7	AW21st_1	D7Mit134	D7Mit259	84.8	4.6552	0.0100
AAW10st_07_1	7	AAW10st	D7Mit134	D7Mit259	88.8	6.6403	0.0014
AW21st_2_08_1	8	AW21st_2	D8Mit289	D8Mit31	17.5	5.1689	0.0060
AAW10st_08_1	8	AAW10st	D8Mit289	D8Mit31	17.5	5.6491	0.0038
AAW21st_08_1	8	AAW21st	D8Mit289	D8Mit31	16.5	6.2271	0.0022
AW21st_1_11_1	11	AW21st_1	D11Mit131	D11Mit195	37.4	5.1115	0.0064
AW21st_2_11_1	11	AW21st_2	D11Mit166	D11Mit184	75.4	5.2180	0.0058

AAW21st_11_1	11	AAW21st	D11Mit271	D11Mit131	29.4	5.8211	0.0032
AW10st_2_12_1	12	AW10st_2	D12Mit136	D12Mit4	35.0	6.9651	0.0011
AW10st_2_16_1	16	AW10st_2	D16Mit217	D16Mit189	46.6	3.4298	0.0333
AW10st_1_19_1	19	AW10st_1	D19Mit130	D19Mit118	16.1	5.0154	0.0070
AAW10st_19_1	19	AAW10st	D19Mit130	D19Mit118	17.1	5.3799	0.0049
AW10st_1_X_1	X	AW10st_1	DXMit79	DXMit153	62.7	5.0059	0.0071
AW21st_1_X_1	X	AW21st_1	DXMit79	DXMit153	67.7	4.7513	0.0091
AAW10st_X_1	X	AAW10st	DXMit79	DXMit153	61.7	4.7333	0.0093
AAW21st_X_1	X	AAW21st	DXMit79	DXMit153	63.7	4.4352	0.0124
ILV_1_02_1	2	ILV_1	D2Mit285	D2Mit50	82.5	4.9498	0.0075
ILV_2_02_1	2	ILV_2	D2Mit66	D2Mit395	50.5	4.8420	0.0083
ILVF_1_02_1	2	ILVF_1	D2Mit285	D2Mit50	82.5	4.8827	0.0080
ILVF_2_02_1	2	ILVF_2	D2Mit66	D2Mit395	49.5	5.6412	0.0083
ILV_2_13_1	13	ILV_2	D13Mit64	D13Mit202	28.7	5.3558	0.0050
ILV_2_14_1	14	ILV_2	D14Mit60	D14Mit97	24.6	4.0970	0.0172
ILVM_2_X_1	X	ILVM_2	DXMit79	DXMit153	67.7	4.9633	0.0074
ILSDF_2_02_1	2	ILSDF_2	D2Mit66	D2Mit395	49.5	5.9283	0.0029
AILSD_02_1	2	AILSD	D2Mit285	D2Mit50	82.5	6.8287	0.0012
AILSDF_02_1	2	AILSDF	D2Mit66	D2Mit395	49.5	5.1624	0.0061
AILSD_03_1	3	AILSD	D3Mit178	D3Mit137	29.3	4.9556	0.0074
ILSD_2_13_1	13	ILSD_2	D13Mit64	D13Mit202	26.7	4.9938	0.0072
ILSDF_2_13_1	13	ILSDF_2	D13Mit64	D13Mit202	26.7	4.5420	0.0112
AILSD_13_1	13	AILSD	D13Mit64	D13Mit202	30.7	4.2565	0.0148
AILSD_14_1	14	AILSD	D14Mit60	D14Mit97	27.6	4.6556	0.0100
ILSDM_02_X_1	X	ILSDM_2	DXMit79	DXMit153	67.7	4.5925	0.0106
AILSMDM_X_1	X	AILSMDM	DXMit79	DXMit153	64.7	6.1346	0.0024
FPL_2_02_1	2	FPL_2	D2Mit66	D2Mit395	51.5	9.4431	0.0001
MPL_1_04_1	4	MPL_1	D4Mit176	D4Mit233	54.4	4.8886	0.0079
MPL_1_09_1	9	MPL_1	D9Mit301	D9Mit269	36.2	4.8455	0.0083
MPL_2_11_1	11	MPL_2	D11Mit271	D11Mit131	27.4	4.7112	0.0094
MPL_1_13_1	13	MPL_1	D13Mit64	D13Mit202	22.7	5.0412	0.0068
MPL_2_13_1	13	MPL_2	D13Mit64	D13Mit202	38.7	4.6271	0.0103
FPL_2_14_1	14	FPL_2	D14Mit60	D14Mit97	61.6	6.3263	0.0020
SEXR_2_14_1	14	SEXR_2	D14Mit60	D14Mit97	61.6	5.3224	0.0052
ASEXR_14_1	14	ASEXR	D14Mit60	D14Mit97	61.6	7.2149	0.0008

Chr.: chromosome; Forward and Backward: forward and backward flanking markers; Peak: peak positions of the QTL in cM.

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CHAPTER TWO

Turning observed founder alleles into expected relationships in an intercross population

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Turning observed founder alleles into expected relationships in an intercross population

Abstract

Pedigree-derived relationships for individuals from an intercross of several lines cannot easily account for the segregation variance that is mainly caused by loci with alternative alleles fixed in different lines. However, when all founders are genotyped for a large number of markers, such relationships can be derived for descendants as expected genomic relationships conditional on the observed founder allele frequencies. A tabular method was derived in detail for autosomes and the X chromosome. As a case study, we analyzed litter size and body weights at three different ages in an advanced mouse intercross (29 generations, total pedigree size 19,266) between a line selected for high litter size (FL1) and a highly inbred control line (DUKsi). Approximately 60% of the total genetic variance was due to segregation variance. Estimated heritability values were 0.20 (0.03), 0.34 (0.04), 0.23 (0.03), 0.41 (0.03) and 0.47 (0.02) for litter size, litter weight and body weight at ages of 21, 42 and 63 days, respectively (standard errors in brackets). These values were between 12% and 65% higher than observed in analyses that treated founders as unrelated. Fields of applications include experimental populations (selection experiments or advanced intercross lines) with a limited number of founders, which can be genotyped at a reasonable cost. In principle any number of founder lines can be treated. Additional genotypes from individuals in later generations can be combined into a joint relationship matrix by capitalizing on previously published approaches.

Keywords: founder genomic relationships; X-chromosomal genomic relationships; sex-linked inheritance; litter size; growth traits; selection experiments

Data Repository

Data is temporally stored in the Research Data Repository (RADAR) maintained by the FIZ Karlsruhe – Leibniz Institute for Information Infrastructure, Germany, for reviewing.

Introduction

The founders of a pedigreed population are the very first individuals with no further recorded ancestors. They are usually treated as unrelated and non-inbred for setting up relationship matrices. However, treating founders of a genealogy as related has been shown to be a useful concept (Legarra *et al.* 2015) when genomic relationships (VanRaden 2008) and pedigree information are to be combined into a joint relationship matrix (Legarra *et al.* 2009; Aguilar *et al.* 2010). This has led to the notion that identity by descent (IBD) of founder alleles arises with a certain probability as a consequence of a limited effective population size. The main achievement of taking founder relatedness into account is a suitable scaling of pedigree relationships (Legarra *et al.* 2015), which makes them compatible with genomic relationships. Other benefits are reasonably interpretable estimates of genetic variance components and the prediction of genetic trends (Legarra *et al.* 2015). Founder relationships can be estimated from marker data of genotyped individuals (Christensen 2012; Legarra *et al.* 2015; Colleau *et al.* 2017), which are usually only available for younger generations in ongoing breeding programs.

In the context of crossbreeding, founders comprise individuals from two or more genetically distinct populations. This requires relationship coefficients for each single population, in addition to a combination of populations (Legarra *et al.* 2015). Here, the aim is to model relationships between purebreds and also between purebreds and crossbreds, most frequently from the F_1 generation. Applications are in genetic evaluations, where purebred and crossbred performances are treated as genetically correlated traits (Aguilar *et al.* 2010; Pszczola *et al.* 2012; de los Campos *et al.* 2013; Garcia-Baccino *et al.* 2017). For this purpose, the interest is in the genetic (co-)variance components for these traits in the purebred populations, where selection takes place.

A somewhat different focus exists when composite populations are generated, e.g. for selection experiments in laboratory animals (Holt *et al.* 2005) or when building advanced intercross lines (AIL, Darvasi and Soller 1995) for fine-mapping purposes. In this case, two or more genetically distinct lines, in some cases inbred lines, are intercrossed. The population is further developed from generation to generation by inter-mating crossbreds. Only performance traits of the intercross are entered into the analyses of, for example, selection experiments. This is an undertaking for which the use of mixed models, with an appropriate relationship matrix, has been recommended (Walsh and Lynch 2018, p. 631-668). The genetic

variance in the intercross generations later than F_1 includes the so-called segregation variance (Lande 1981; Lo *et al.* 1993), which is caused by loci that are fixed for different alleles in the founder lines but begin to segregate from the F_2 generation onwards. The proportion to which the segregation variance contributes to the total genetic variance in the F_2 and later generations can, in principle, vary between zero and one. However, this proportion cannot be derived from pedigree data alone.

As a solution, we propose a relationship matrix that takes account of known marker allele frequencies of founders. Those markers that are fixed for alternative alleles in different lines largely determine the extent of the role of segregation variance at an average locus. Rules for the Mendelian transmission of these relationships to later generations were derived for both autosomal and X-chromosomal relationships. These matrices can then be combined with information on observed genotypes, which may include non-founders, and be used for the estimation of variance components and genetic trends. The associated genetic variance is thereby defined as the variance among unrelated individuals in the first generation of the composite population (i.e. the F_2 in a two line cross). In addition, an application is presented to obtain litter size and growth traits from an advanced intercross between a long-term selected, high fecundity mouse line and a highly inbred control line.

Theory

Underlying assumptions

We assume two distinct founder populations, A and B, that contribute to a composite crossbred population. All founders are assumed to be genotyped and line specific founder allele frequencies are known. For each marker, i , founder frequencies are denoted as p_i^A and p_i^B . Under the condition that all founders contribute equally to the composite population, the expected allele frequency in the F_2 generation, \bar{p}_i , is fully determined as the average, $\bar{p}_i = \frac{1}{2}(p_i^A + p_i^B)$, of the two line specific frequencies.

Observed genomic founder relationships

The genotypes of the founders can be summarized into a centered genotype matrix, \mathbf{Z}_0 , with one row per individual and one column per marker.

Autosomes: For autosomal markers, entries into the \mathbf{Z}_0 matrix are $z_i^k \in (2 - 2\bar{p}_i, 1 - 2\bar{p}_i, 0 - 2\bar{p}_i)$ for genotypes AA, Aa and aa, respectively. The observed genomic relationship matrix, \mathbf{G}_0 , between founders is

$$\mathbf{G}_0 = \mathbf{Z}_0 \mathbf{Z}_0' / S \quad (1)$$

where S is the scaling factor; $S = 2 \sum_i \bar{p}_i (1 - \bar{p}_i)$. \mathbf{G}_0 is a standard genomic relationship matrix, except when using \bar{p}_i for centering and scaling, as previously described by Van Raden (2008).

X chromosome: The observed X-chromosomal genomic relationship matrix is set up in accordance with the rules for autosomal markers. Extra details apply to the definitions of average gene frequencies and the treatment of male (hemizygous) individuals. For X chromosome markers we define the mean allele frequency \bar{p}_i , again, as $\bar{p}_i = \frac{1}{2}(p_i^A + p_i^B)$. However, on the X chromosome this is only equal to the gene frequency to be ultimately reached in later generations if the two founder lines contribute equally through males and females to the genetic makeup of the composite population.

Genotype codes have to be transformed to gene counts; $c_i^k \in (1, 0)$, for male founders and then centered by \bar{p}_i instead of $2\bar{p}_i$. For matrix \mathbf{Z}_0 the entries for X chromosome markers are $z_i^k \in (1 - \bar{p}_i, 0 - \bar{p}_i)$, for genotypes A and a, respectively. The X chromosome genotypes for female founders are, in contrast, treated in the same way as autosomal markers. The observed X-chromosomal genomic relationship matrix can then be calculated by formula (1), using X-chromosomal gene counts and the scaling factor S , as defined above.

Expected founder genomic relationships

Autosomes: An expectation of \mathbf{G}_0 (denoted $E(\mathbf{G}_0)$) can be derived under the assumption that alleles at independent loci are randomly sampled from each founder line's particular gene

pool (Binomial sampling), as defined by their known founder allele frequencies. We consider two gametes randomly chosen from base population A. The 2×2 matrix \mathbf{R}^{AA} has the expected sum of squared centered coefficients z_i^A ($z_i^A \in (1 - \bar{p}_i, 0 - \bar{p}_i)$), for alleles A and a, respectively) for each of the gametes on its diagonal and the corresponding expected sum of cross products on its off diagonal,

$$\mathbf{R}^{AA} = \begin{bmatrix} d^{AA} & r^{AA} \\ r^{AA} & d^{AA} \end{bmatrix} = \frac{1}{S} \begin{bmatrix} \sum_i [p_i^A(1-p_i^A) + (p_i^A - \bar{p}_i)^2] & \sum_i (p_i^A - \bar{p}_i)^2 \\ \sum_i (p_i^A - \bar{p}_i)^2 & \sum_i [p_i^A(1-p_i^A) + (p_i^A - \bar{p}_i)^2] \end{bmatrix}. \quad (2)$$

For base population B, the equivalent matrix \mathbf{R}^{BB} can be derived from its line specific allele frequencies p_i^B . In the following matrices, \mathbf{R}^{AA} and \mathbf{R}^{BB} are referred to as the expected covariance matrices between gametes from the same founder line.

Furthermore, we can set up \mathbf{R}^{AB} as the equivalent relationship matrix of two randomly chosen gametes from populations A and B:

$$\mathbf{R}^{AB} = \begin{bmatrix} d^{AA} & r^{AB} \\ r^{AB} & d^{BB} \end{bmatrix} = \frac{1}{S} \begin{bmatrix} \sum_i [p_i^A(1-p_i^A) + (p_i^A - \bar{p}_i)^2] & \sum_i (p_i^A - \bar{p}_i)(p_i^B - \bar{p}_i) \\ \sum_i (p_i^A - \bar{p}_i)(p_i^B - \bar{p}_i) & \sum_i [p_i^B(1-p_i^B) + (p_i^B - \bar{p}_i)^2] \end{bmatrix}. \quad (3)$$

From the distinct elements in \mathbf{R}^{AA} and \mathbf{R}^{AB} (also \mathbf{R}^{BB}) we can compute all necessary expected relationships between individuals, which may occur in $E(\mathbf{G}_0)$. First, we have the expected self-relationship of an individual from a particular founder line (e.g. line A)

$$\omega^A = 2d^{AA} + 2r^{AA}. \quad (4)$$

The expected relationship between two individuals from the same founder line is

$$\omega^{AA} = 4r^{AA}. \quad (5)$$

The expected relationship between two individuals from two different founder lines is

$$\omega^{AB} = 4r^{AB}. \quad (6)$$

X chromosome: With regards to expected X-chromosomal relationships, different combinations between either males or females may occur, in addition to the same or different founder lines. In total, there are eight different kinds of possible relationships: the self-relationship of a male originating from a certain line, ω_x^{AA} ; the expected relationship

between two males from the same line, $\omega_{x,x}^{AA}$, or $\omega_{x,x}^{AB}$ from different lines; the self-relationship, ω_{xx}^{AA} , of a female from a certain line; the relationships $\omega_{xx,xx}^{AA}$ and $\omega_{xx,xx}^{AB}$ between two females from the same line and different lines, respectively, and finally the relationships $\omega_{x,xx}^{AB}$ and $\omega_{xx,x}^{AB}$ between a male and a female from different lines. Formulas for all eight cases are summarized in Table 2.1.

Table 2.1 Formulas for calculating the different types of expected X-chromosomal relationships of males and females in a crossbred population derived from two founder lines A and B

	Relationship ^a	Formula ^b
Self-relationship	ω_x^A	$\frac{1}{S} \sum_i [p_i^A (1 - p_i^A) + (p_i^A - \bar{p}_i)^2] = d^A$
	ω_{xx}^A	$2\omega_x^A + 2\omega_{x,x}^{AA} = 2d^A + 2r^A$
Relationships between individuals	$\omega_{x,x}^{AA}$	$\frac{1}{S} \sum_i [(p_i^A - \bar{p}_i)^2] = r^A$
	$\omega_{x,x}^{AB}$	$\frac{1}{S} \sum_i [(p_i^A - \bar{p}_i)(p_i^B - \bar{p}_i)] = r^{AB}$
	$\omega_{xx,xx}^{AA}$	$4\omega_{x,x}^{AA} = 4r^A$
	$\omega_{xx,xx}^{AB}$	$r^A + r^B + 2r^{AB}$
	$\omega_{x,xx}^{AA}$	$2r^A$
	$\omega_{x,xx}^{AB}$ or $\omega_{xx,x}^{AB}$	$2r^{AB}$

^a ω_x^A and ω_{xx}^A are the expected self-relationships of males and females, respectively; $\omega_{x,x}^{AA}$ and $\omega_{x,x}^{AB}$ are the expected relationships between two males, which are all from line A, or from different lines (A and B respectively); $\omega_{xx,xx}^{AA}$ and $\omega_{xx,xx}^{AB}$ are the expected relationships between two females, which are all from line A, or from different lines (A and B respectively); $\omega_{x,xx}^{AA}$, $\omega_{x,xx}^{AB}$ and $\omega_{xx,x}^{AB}$ are the expected relationships between a male and a female, which are all from line A, or from different lines (A and B respectively).

^b p_i^A and p_i^B are the line-specific allele frequencies of lines A and B for locus i ; \bar{p}_i is the mean of p_i^A and p_i^B for locus i ; d^A and d^B are the expected self-relationships of gametes inherited from line A, or from line B; r^A , r^B and r^{AB} are the expected relationships between two gametes, that are from the same line (line A or line B) or from different lines.

Extending expected genomic relationships to later generations

Expected founder genomic relationships can be extended to all descendants by following the paths of Mendelian transmission, as specified in the pedigree. The resulting expected genomic relationship matrix is denoted by $\tilde{\mathbf{A}}$. The diagonal elements $g_{k,k}^E$ and off-diagonal elements $g_{k1,k2}^E$ of matrix $\tilde{\mathbf{A}}$ are computed by a modified version of the tabular method (Emik and Terrill 1949; Cruden 1949).

Autosomes: The expected autosomal self-relationships (diagonal elements of $\tilde{\mathbf{A}}$) consist of three parts; the expected self-relationships of gametes inherited from the sire, from the dam and the gametic relationship between these parental gametes. Relationships between individuals (off-diagonal elements) are an average of the relationships of one candidate and the parents of another, as known from the tabular method. The expected self-relationships and relationships are:

$$g_{k,k}^E = s_{sire} + s_{dam} + \frac{1}{2} g_{sire,dam}^E \quad (7)$$

$$g_{k1,k2}^E = \frac{1}{2} (g_{sire1,k2}^E + g_{dam1,k2}^E) \quad (8)$$

where *sire* and *dam* are the parents of individual *k*, *sire1* and *dam1* are the parents of individual *k1*, s_{sire} and s_{dam} are the expected self-relationships of gametes that individual *k* inherits from their parents (see supplement for a derivation of formulas (7) and (8)).

These formulas are applicable from the F_1 generation onwards, whereby their components depend on generation number. For F_1 individuals $s_{sire} = \frac{1}{2}(2d^{AA}) = d^{AA}$ and $s_{dam} = \frac{1}{2}(2d^{BB}) = d^{BB}$ if we assume a male founder from line A and a female founder from line B. The $g_{sire,dam}^E$ is the expected relationship between two founders, i.e. the parents of an F_1 individual, which in our case gives $g_{sire,dam}^E = \omega^{AB} = 4r^{AB}$. Generally, for an F_1 individual the expected self-relationship is $g_{k,k}^E = d^{AA} + d^{BB} + 2r^{AB}$. Individuals from the F_2 generation receive gametes from each parent with a 50% probability for line A and line B alleles. Therefore, in the F_2 , $s_{sire} = \frac{1}{2}(d^{AA} + d^{BB})$ and $s_{dam} = \frac{1}{2}(d^{AA} + d^{BB})$.

With the two founder lines used in our case, the sum of d^{AA} and d^{BB} is equal to 1 but d^{AA} and d^{BB} are usually different (see supplement). The expected self-relationship for a gamete

that an F_2 individual inherits from one of its parents is always equal to 0.5 and $g_{k,k}^E = 1 + \frac{1}{2} g_{sire,dam}^E$. The same applies to later generations.

X chromosome: For the X chromosome, formulas (7) and (8) are modified for females to give

$$g_{k,k}^E = s_{sire} + s_{dam} + g_{sire,dam}^E \quad (9)$$

$$g_{k1,k2}^E = g_{sire1,k2}^E + \frac{1}{2} g_{dam1,k2}^E \quad (10)$$

and for males to give

$$g_{k,k}^E = s_{dam} \quad (11)$$

$$g_{k1,k2}^E = \frac{1}{2} g_{dam1,k2}^E \quad (12)$$

Note that Fernando and Grossman (1990) introduced similar equations for calculating the pedigree-derived relationships for the X chromosome, where the self-relationship for males, s_{sire} , is 0.5 in all generations, meaning s_{dam} must also be 0.5. The underlying assumption is that allele frequencies of both sexes are equal, as is the case if the population is in an equilibrium state (Self and Liang 1987). Equations (9) to (11), in contrast, allow s_{sire} and s_{dam} to fluctuate along with male and female marker frequencies in early generations, after mating male and female founders with differing allele frequencies.

Expected genomic co-variances in an infinitely large F_2 population

In a cross between two populations, A and B, the gametes that an individual receives in the F_2 generation are of types A and B, with equal probability. Such an individual will have a probability of 0.25 of inheriting two A gametes, a probability of 0.25 of inheriting two B gametes and a probability of 0.5 of inheriting one A gamete and one B gamete.

The average self-relationship of an individual in an infinitely large F_2 population is, therefore

$$\begin{aligned} & \frac{1}{4}(2d^{AA} + 2r^{AA}) + \frac{1}{4}(2d^{BB} + 2r^{BB}) + \frac{1}{2}(d^{AA} + d^{BB} + 2r^{AB}) \\ &= \frac{1}{4}(2d^{AA} - 2r^{AB}) + \frac{1}{4}(2d^{BB} - 2r^{AB}) + \frac{1}{2}(d^{AA} + d^{BB} + 2r^{AB}) \\ &= d^{AA} + d^{BB} \\ &= 1 \end{aligned}$$

as $r^{AA} = r^{BB} = -r^{AB}$ in a cross of two lines.

The average covariance between F_2 individuals can be derived as the weighted average of covariances in nine possible combinations of two individuals, were each of them may carry two (AA), one (AB) or no (BB) gametes from the A line. These nine single pair-wise covariances can be expressed in terms of relationships between gametes, i.e. r^{AA} , r^{BB} and r^{AB} . The weights are the probabilities of the occurrence of all these combinations:

$$\begin{aligned} & \frac{1}{16}4r^{AA} + \frac{2}{16}(2r^{AA} + 2r^{AB}) + \frac{1}{16}2r^{AB} + \\ & \frac{2}{16}(2r^{AA} + 2r^{AB}) + \frac{4}{16}(r^{AA} + r^{BB} + 2r^{AB}) + \frac{2}{16}(2r^{BB} + 2r^{AB}) + \\ & \frac{1}{16}4r^{AB} + \frac{2}{16}(2r^{AB} + 2r^{BB}) + \frac{1}{16}4r^{BB} = 0 \end{aligned}$$

The expected covariance matrix $\tilde{\mathbf{A}}$ for unrelated and non-inbred F_2 individuals is, therefore, an identity matrix. This means that such a hypothetical population can be viewed as a reference for the actual composite population derived from the genotyped founders. Variance components estimated with either an $\tilde{\mathbf{A}}$ or an \mathbf{H} matrix can, after proper adjustment for founder relationships (Legarra *et al.* 2015), be interpreted as the genetic variance in such a population. When defined in this way the genetic variance includes the segregation variance, i.e. the difference between the genetic variance in the F_2 and the F_1 (Lande 1981; Lo *et al.* 1993). The segregation variance can be expressed as the difference between expected self-relationships of non-inbred animals in the F_2 and the F_1 generations:

$$\left[1 - (d^{AA} + d^{BB} + 2r^{AB})\right] \sigma_a^2 = -2r^{AB} \sigma_a^2 .$$

Note that in the case of a cross between two inbred lines $d^{AA} = d^{BB} = -r^{AB} = \frac{1}{2}$ and the last formula correctly flags all genetic variance as segregation variance.

Accounting for observed genotypes

Combined relationship matrix \mathbf{H} : Expected genomic founder relationships will generally differ from those observed. This can be taken into account by applying a previously developed theory (Legarra *et al.* 2009; Christensen and Lund 2010; Aguilar *et al.* 2010) for combining pedigree-derived relationships (\mathbf{A}) and genomic relationships into a joint matrix, \mathbf{H} . In our case we used \mathbf{H} as a modification of $\tilde{\mathbf{A}}$ that is corrected for the observed founder

relationships in \mathbf{G}_0 . We denote $\tilde{\mathbf{A}}_{11}$ as the expected genomic relationships of founders. In terms of the inverse of \mathbf{H} (Christensen and Lund 2010; Aguilar *et al.* 2010), we then get

$$\mathbf{H}^{-1} = \tilde{\mathbf{A}}^{-1} + \begin{bmatrix} \mathbf{G}_0^{-1} - \tilde{\mathbf{A}}_{11}^{-1} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} \end{bmatrix} = \tilde{\mathbf{A}}^{-1} + \begin{bmatrix} \mathbf{G}_0^{-1} - \mathbf{E}(\mathbf{G}_0)^{-1} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} \end{bmatrix}.$$

The inverse of matrix $\tilde{\mathbf{A}}$ is described in the supplement. The observed founder genomic relationship matrix, \mathbf{G}_0 , may be singular. In this case, one may capitalize on the idea of blending (Garcia-Baccino *et al.* 2017). We used $\mathbf{G}_0^{BLD} = 0.98 \times \mathbf{G}_0 + 0.02 \times \mathbf{E}(\mathbf{G}_0)$, instead of \mathbf{G}_0 , when computing \mathbf{H}^{-1} .

Joint relationships from simulated genotypes: For the sake of comparison with \mathbf{H} , a joint relationship matrix, \mathbf{G} , was generated from observed genotypes of founders plus simulated genotypes of non-founders. The alleles were randomly sampled from observed founder genotypes and simulated marker genotypes of offspring in later generations were derived by gene-drop. The expected self-relationships for autosomes were calculated as

$$g_{k1,k2}^E = \frac{1}{S} \sum_i \mathbf{E}[(c_i^{k1} - 2\bar{p}_i)(c_i^{k2} - 2\bar{p}_i)] \quad (13)$$

where $g_{k1,k2}^E$ is the expected relationship between individuals $k1$ and $k2$, c_i^{k1} and c_i^{k2} are the gene counts of individuals $k1$ and $k2$ at locus i . For self-relationship we used $g_{k,k}^E = \frac{1}{S} \sum_i \mathbf{E}[(c_i^k - 2\bar{p}_i)^2]$. Expectations were obtained by averaging over 10000 replicates of the gene-drop simulation.

For X chromosomes, the sex of the descendants must also be considered. For female descendants formula (13) remains. For male descendants formula (13) will be modified by applying the centered X-chromosomal genotypes ($z_i^k \in (1 - \bar{p}_i, 0 - \bar{p}_i)$), which gives

$$g_{k,k}^E = \frac{1}{S} \sum_i \mathbf{E}[(c_i^k - \bar{p}_i)^2].$$

Application Example

Animals, pedigree, phenotypes and genotypes

The advanced intercross mouse line (AIL) bred in the Leibniz Institute for Farm Animal Biology (FBN) was established by randomly choosing and intercrossing four females from the

long-term selected, high-fecundity line, FL1 (Langhammer *et al.* 2014), and four males from a highly inbred (theoretical inbreeding coefficient > 0.999) control line, DUKsi (Alm *et al.* 2010). Both lines were derived from the same initial gene pool (Dietl *et al.* 2004).

The high fecundity FL1 line was selected for an index trait that combines litter size (LS0) and litter weight (LW0) at birth in primiparous females (Index $I = 1.6 \times LS0 + LW0$) up to generation 131. As a result of selection over 131 generations an average of 17.14 ± 3.25 pups per litter had been reached. This is a 1.8 fold higher fecundity than observed in the control line (see Table 1.2). An outbred control line, DUKs, was maintained at approximately the same population size for 79 generations by random mating and without any selection pressure. The inbred derivative DUKsi was split from DUKs in generation 79.

Four male founders were chosen for the experiment after 38 generations of full sib mating in the DUKsi line. Each of four females from generation 131 of the FL1 selection line was mated with one male from the control line. The F_1 litters were standardized to four male and eight female pups immediately after birth in order to maintain a surplus of females for further reproduction. Full sibs from the four initial F_1 families were then repeatedly (at least four times) inter-mated by rotating males and females within the family. Thus, each of the four pairs of founder parents constitutes a family of its own, with descendants up to generation F_3 . Offspring of only one these families were then maintained and became the ancestors of all further generations of the AIL.

A total of 19,266 mice (9,453 males and 9,813 females) were used for this study. They were distributed unevenly across all generations; 44 in F_1 , 1,483 in F_2 , 5,235 in F_3 , 1,025, 1,058 and 1,070 in F_{23} , F_{24} and F_{25} , respectively, and between 312 and 431 for other generations.

Reproductive ability was measured as litter size at birth (LS0) and litter weight at birth (LW0). The litter traits were recorded for 4,430 females (from 9,813 females) for their first litter. Amongst these females, 1,481 also had a record for their second litter (there were no second litter records for generations from F_3 to F_{21}). Growth traits that were recorded for all generations were body weight at day 21, 42 and 63 (BM21, BM42, BM63), in addition to body weight at first mating (BMM).

The six fertility and growth traits (see Table 2.2 for summary statistics) were analyzed using different kinds of relationship matrices, as described below.

Table 2.2 Descriptive statistics for traits related to litter size and growth. Litter size and litter weight was recorded in first (LS0_1, LW0_1) and partially also in second (LS0_2, LW0_2) parity females. Body mass of both sexes was measured at days 21 (BM21), 42 (BM42), 63 (BM63) after birth as well as at day of mating (BMM).

Trait	N	Generation ^a	Mean (SD)
LS0_1	4,430	F ₂ -F ₂₉	16.025 (3.286)
LS0_2	1,481	F ₂ , F ₂₂ -F ₂₅	16.315 (3.766)
LW0_1	4,410	F ₂ -F ₂₉	27.456 (4.807)
LW0_2	1,474	F ₂ , F ₂₂ -F ₂₅	28.627 (5.530)
BM21	19,080	F ₂ -F ₃₀	10.878 (2.013)
BM42	14,586	F ₂ -F ₃₀	29.439 (3.787)
BM63	13,910	F ₂ -F ₃₀	35.185 (3.801)
BMM	7,416	F ₂ -F ₂₉	36.418 (4.167)

^a Generation, generations from which phenotypic records were available. For instance, for trait LW0_1 data were recorded from generations from F₂ to F₂₉ and for trait LW0_2 data were only recorded from five generations F₂, F₂₂, F₂₃, F₂₄ and F₂₅.

All eight founders of this intercross line were genotyped with the JAX Mouse Diversity Genotyping Array (Yang *et al.* 2009) at the genotyping facility of the Jackson Laboratory, The Jackson Laboratory, Bar Harbor, Maine, USA.

Comparative estimation of variance components

Two fertility and four growth traits were comparatively analyzed with mixed models that comprised of different kinds of relationship matrices. Fertility traits (LS0, LW0) were analyzed as traits of females (Langhammer *et al.* 2017). For both traits, the model for the i th observation ($i = 1, 2$) of animal a was

$$y_{gacpi} = \mu + \beta_g + \gamma w_a + a_a + x_a + u_c + u_p + e_{gacpi} \quad (14)$$

where β_g is the fixed generation effect for females born in generations from F₂ to F₂₉ ($g=1, \dots, 28$) and γ is the linear regression of the body weight w_a at mating of each female's mother. The random part of the model comprised the additive autosomal and X-chromosomal genetic effects a_a and x_a of animal a ($a=1, \dots, 19266$), the common litter environmental

effect u_c ($c=1, \dots, 2420$), the permanent environmental effect u_p ($p=1, \dots, 4430$) and the residual e_{gacpi} . The covariance matrices of the common litter environmental and permanent environmental effects were assumed as equal to an identity matrix of proper size times the respective variance component.

Observations of growth traits (BM21, BM42, BM63, and BMM) were made from males and females from generations F_2 to F_{30} . Therefore, the fixed part of the model also included an additional sex effect, β_s ($s=1,2$), and the number of levels was 29 for the generation effect. For BM21 and BMM no permanent environmental effect could be fitted because these traits were only measured once for all animals. Table 2.3 gives more details of the model we applied.

Table 2.3 Numbers of observations (N) and number of levels for fixed and random effects in models fitted to litter size (LS0), litter weight (LW0) and body mass traits at ages of 21, 42, 63 days and at mating (BM21, BM42, BM63, BMM). Fixed effects are generation number, linear regression on mother's weight at mating, litter number and sex; random effects are additive genetic, litter and permanent environmental effects.

Trait	N	Gen.	Dam's Weight	Litter No.	Sex	Additive Genetic Effect	Litter env.	Permanent env.
LS0	5,911	28	1	2	1	4,430	2,420	1,481
LW0	5,884	28	1	2	1	4,410	2,397	1,474
BM21	19,080	29	1	-	2	19,080	4,362	-
BM42	14,586	29	1	-	2	13,959	3,132	627
BM63	13,910	29	1	-	2	13,283	3,044	627
BMM	7,416	28	1	-	2	7,416	2,626	-

All random effects were assumed to be mutually independent. Three different kinds of relationship matrices were compared for autosomal and X-chromosomal genetic effects: pedigree-derived relationship matrices that assumed unrelated founders, \mathbf{H} matrices, as explained above, and \mathbf{G} matrices, based on gene-drop simulations (denoted as "A", "H" and "G", respectively). Model variants "A_a", "G_a" and "H_a" include only an autosomal relationship matrix of one of the specified types. Model variants "A_{a+x}", "G_{a+x}" and "H_{a+x}" additionally include the X-chromosomal relationship matrix of the same type. The Restricted maximum likelihood (REML) estimates of all variance components were obtained from the

ASReml package (Gilmour *et al.* 2015). All estimated genetic variance components from model variants, which included founder relationships, were corrected for non-independence of founders by multiplication with a correction factor, D_k (Searle 1982. p. 355; Legarra 2016):

$$D_k = \frac{\sum_{i=1}^f g_0(i,i)}{f} - \frac{\sum_{i=1}^f \sum_{j=1}^f g_0(i,j)}{f^2}$$

where $g_0(i,j)$ are elements of either the observed founder relationship matrix, \mathbf{G}_0 (after blending), or a respective diagonal matrix for pedigree-derived relationships, and $f = 8$ is the number of founders.

The significance of X-chromosomal genetic effects was evaluated by comparing the full model for each trait with a reduced model without X-chromosomal genetic effects. Error probabilities were derived via restricted likelihood ratio tests (RLRT), with a single degree of freedom (Wiencierz *et al.* 2011).

Data availability

Marker genotypes of founders, a pedigree file for all AIL animals and a data file with observed phenotypes for the six analyzed traits can be found in the RADAR research data repository (DOI: 10.22000/88).

Animal welfare declaration

The animal experiments were performed following national and international guidelines and were approved by the local authorities (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern, Germany).

Results and Discussion

Founder relationship matrices

Marker data: The number of polymorphic autosomal SNPs (single nucleotide polymorphisms) was 140,532 in all founders (see Table 2.4). The number that segregated in the FL1 line alone was 44,827, whilst 67,450 segregated within the DUKsi control line. The numbers on the X chromosome (non-pseudoautosomal, Perry *et al.* 2001) were 2,009 for all founders, 191 in the FL1 line and 1,055 in the control line. Opposite alleles with frequencies

at hundred and zero per cent in the two lines (line-specific alleles) occurred with 38.9% on autosomes as well as the X chromosome. Polymorphic markers were evenly distributed across the genome (see Figure S2.1 in supplement file) and the density (number per 1 Mbp) was between 34.0 and 70.4 (see Table S2.1 in supplement file).

Table 2.4 Numbers of SNP-markers which were segregating within all eight founders, and within both founder lines (FL1, control). Line-specific SNPs do not segregate within founders of the same line but have alternative kinds of alleles in each line.

Chromosomal Location	All Founders	FL1	Control	Line-specific SNP
autosomal	140,532	44,827	67,450	54,654
X-chromosomal	2,009	191	1,055	869

Observed genomic relationships: The observed 8×8 genomic founder relationship matrices are shown in Figure 2.1 as triangular matrices for autosomes (above) and X chromosomes (below).

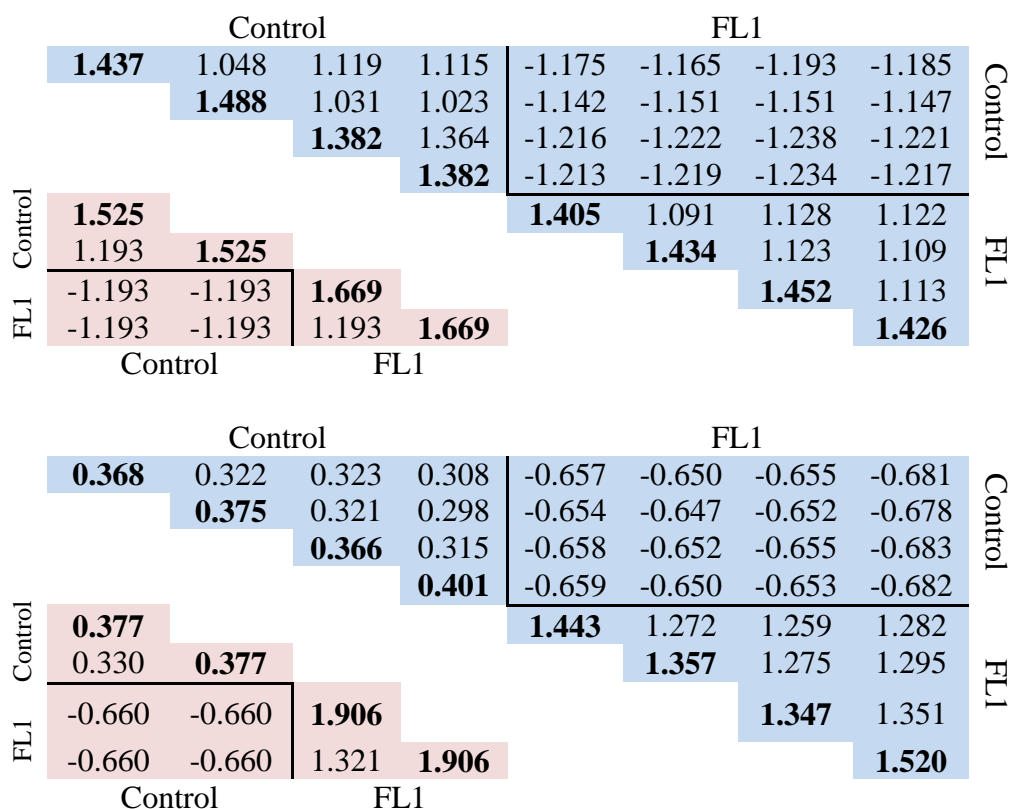


Figure 2.1 Comparison of the observed and expected founder genomic relationship matrices

The upper panel shows the autosomal observed genomic founder relationship matrix \mathbf{G} (marked in blue) and the autosomal expected founder relationship matrix \mathbf{G}^E (marked in red). The lower panel shows X-chromosomal \mathbf{G} (marked in blue) and X-chromosomal \mathbf{G}^E (marked in red). The diagonal elements are in bold.

Observed autosomal self-relationships were fairly uniform in both the control (between 1.382 and 1.488) and the FL1 line (between 1.405 and 1.452). The expected self-relationships (lower triangle of 4×4 matrix, same panel) were 1.525 and 1.669 for the control and FL1 founders, respectively. The lower observed relationships (approximately 7% in controls and 14% in the FL1 line) indicate an excess of heterozygosity in both lines relative to within line Hardy-Weinberg proportions at observed allele frequencies. These deviations can be explained by the sampling of rare alleles, which are more likely to occur in a heterozygous condition when compared with more frequent alleles. Observed self-relationships are, however, considerably larger than one due to elevated homozygosity relative to the non-inbred F_2 individuals that define the base population. Despite some fluctuations, relationships between founders of the same line (expected: 1.193) and different lines (expected: -1.193) barely deviate from the expectations. On the X chromosome, the observed self-relationships of female control founders (range: 1.347 - 1.520) deviate more (about 26%) from the

expectation of 1.906, whilst in contrast, observed self-relationships of male control founders and all observed X-chromosomal relationships between male and female founders agree well with the expectations. On both kinds of chromosomes, large negative relationships between individuals from different lines are predominantly a result of SNPs with line-specific alleles. Their high proportion and the resulting negative between-line relationships reflect the long lasting separation of the two lines and their selection for different goals. Consequently, the expectation of $\omega^{AB} = 4r^{AB} = -1.193$ translates into a proportion of $-2r^{AB} = 2 \times 0.29825 \approx 0.6$ of the total genetic variance that can be attributed to segregation variance.

Both the autosomal and X-chromosomal observed genomic relationship matrices are singular, with rank seven. In the case of the autosomal markers, this is caused by the relationship of 1.36 between the third and fourth founders ($g_{3,4}$), which is close to the self-relationship of the same animals (1.38). This translates into a very close correlation of almost one. The background is that the inbred control line actually consisted of several sublines that can be traced back to the same pair of ancestors. Sublines were generated by branching the main line in different generations and maintained by repeated full-sib mating. Unintentionally, two male founders were sampled from the same subline and the other male founders from two different sublines. In the case were all control founders had been drawn from the same subline, the rank for the observed relationship matrix is expected to be five, as almost no genetic variation is expected within the subline.

As a consequence of this rank deficiency, the observed relationship matrices are not invertible. This was solved by blending them with their expected counterparts (see Theory). Alternatively, one could have averaged the two columns and rows of the highly correlated animals and used this average as a replacement in a 7×7 relationship matrix, thereby assigning a single genetic effect to both founders (e.g. Tuchscherer *et al.* 2004).

Evolution of self-relationships over generations: The mean self-relationships, as derived from different kinds of relationship matrices, develop differently over generations (Figure 2.2). The classical pedigree-derived matrix \mathbf{A} has diagonal elements of one in the base generations and the F_1 , followed by a jump to 1.25, which indicates inbreeding of F_2 animals due to full-sib mating in the F_1 . From then on there is only a very slight increase of the mean inbreeding coefficient. This pattern is present for autosomal relationships (Figure 2.2, upper left), as well as for X-chromosomal self-relationships in females (lower left panel). Self-relationships from $\tilde{\mathbf{A}}$, in contrast, show strong fluctuations from high position values in founders to high

negative values in the F_1 (same panels). Autosomal self-relationships from the $\tilde{\mathbf{A}}$ matrix reach an average of larger than one in the F_2 , which increases only slightly in further generations (upper left panel). The $\tilde{\mathbf{A}}$ matrix is scaled in such a way that non-inbred F_2 animals would have a self-relationship of one. Therefore, larger values are a sign of a higher expected homozygosity when compared with this reference population. The fluctuations of average generational autosomal self-relationships come to an end from generation F_2 onwards (upper left), whilst they continue, albeit with decreasing amplitudes, for X-chromosomal self-relationships in males (middle left) and females (lower left). The underlying reason is that the genetic equilibrium is reached after two generations for autosomal markers, when initial allele frequencies differ in males and females (Crow and Kimura 1970). This process takes longer for X-chromosomal loci (Li 1976, p. 137). In line with this, the amplitudes for male X-chromosomal self-relationships have the opposite sign to those for females.

The mean X-chromosomal self-relationships of males stabilize at around 0.54, which is somewhat larger than 0.5. The reason is that the actual equilibrium allele frequencies approach $\bar{p}_i = \frac{2}{3} p_i^{FL1} + \frac{1}{3} p_i^{control}$ instead of $\bar{p}_i = \frac{1}{2} (p_i^{FL1} + p_i^{control})$, since all founders from the FL1 line were females with two alleles and all founders from the control line had only a single allele at each X chromosome locus. The X-chromosomal $\tilde{\mathbf{A}}$ matrix was, however, computed under the assumption of equal contributions of both founder lines to the F_2 , which would require equal numbers of male and female founders from both lines. Values of 0.54 therefore indicate somewhat more X chromosome varieties, as in a reference population were $\bar{p}_i = \frac{1}{2} (p_i^{FL1} + p_i^{control})$.

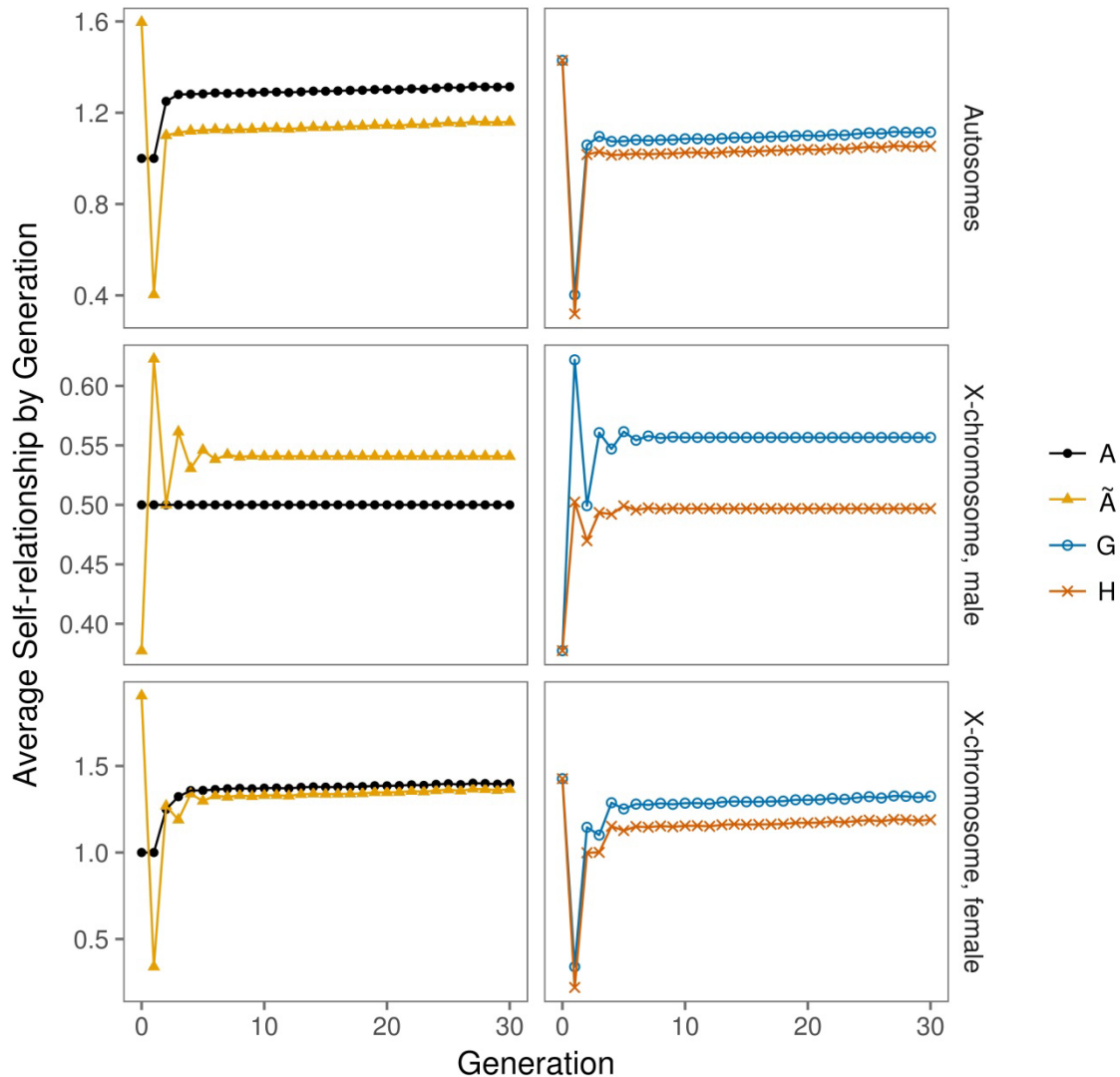


Figure 2.2 Comparison of the generation mean of the self-relationships for the relationship matrices derived in the study. \mathbf{A} is the pedigree-derived relationship matrix. $\tilde{\mathbf{A}}$ is the relationship matrix derived from the pedigree and the allele frequencies of all founder lines. \mathbf{G} is the pedigree-genotype-combined relationship matrix derived by the method introduced in the study. \mathbf{H} is the relationship matrix derived by Legarra's method (2008) using matrix $\tilde{\mathbf{A}}$ instead of matrix \mathbf{A} . The X-chromosomal self-relationships are divided by sex. The oscillatory approach of the allele frequency of the X-linked markers can be observed in the first few generations of the curves for matrices $\tilde{\mathbf{A}}$, \mathbf{G} and \mathbf{H} , when compared with the matrix \mathbf{A} and the autosomal cases.

Average self-relationships from \mathbf{H} and \mathbf{G} types of relationship matrices can be seen on the panels to the right of Figure 2.2. The initial fluctuation patterns already described for $\tilde{\mathbf{A}}$ are also present in the average self-relationships of these two matrices. The three curves for the \mathbf{G}

matrices are similar but not identical to $\tilde{\mathbf{A}}$. In comparison, averages for the \mathbf{H} matrices are lower in all cases, which is a result of correcting $\tilde{\mathbf{A}}$ to lower observed homozygosity than expected, under the assumptions made for the construction of $\tilde{\mathbf{A}}$.

Genetic parameters: The genetic variance components and heritability for six selected traits can be found in the Table 2.5 and Table S2.2. The X-chromosomal genetic variance proved to be significant at the 5% level for the three growth traits; BM21, BM42 and BM63, regardless of what type of relationship matrix was used as part of the model (Table S2.3). The additive genetic variance component for the sex chromosome was almost zero for BMM (see Table S2.2) and was also not significant for litter traits LS0 and LW0 (Table S2.3).

The proportion of the total genetic variance that was attributed to the sex chromosome was approximately 3% for BM21 in all analyses (data in Table 2.5). When founders were assumed to be related, the same proportion was 9% and 12% for BM42 and BM63, respectively. These were lower than the comparative values of 12% and 17%, when the assumption of unrelatedness was applied. Over all traits, a standard pattern emerged (Table 2.5) where genetic variance components and heritability were larger when either an \mathbf{H} or a \mathbf{G} matrix was part of the model, compared to an \mathbf{A} matrix analysis. In contrast, results from \mathbf{H} and \mathbf{G} matrices were almost equal for all traits. Estimated residual and common litter environmental variances were barely affected by the choice of genetic relationship matrix for any of the traits analyzed. The same was true for the permanent environmental variance component for BM42 and BM63, whereas both litter traits displayed lower estimates for the permanent environmental variance component from \mathbf{H} and \mathbf{G} matrices, compared with \mathbf{A} matrices. This was accompanied by considerably larger estimates for autosomal additive genetic variances. Consequently, heritability for LS0 was 19% when founder relationships were taken into account, versus 12% when they were not taken into account (Table 2.5). For LW0 and growth traits, the respective comparisons were 34% and 32% vs. 23% (LW0), 23% and 22% vs. 17% (BM21), 41% and 40% vs. 35% (BM42), 47% and 45% vs. 41% (BM63), and 52% and 50% vs. 45% (BMM). For LS0, in particular, the increase in the estimates of the genetic variance using \mathbf{H} and \mathbf{G} matrices is larger than 60% (64% and 78%). To that effect estimated genetic standard deviations changed and the corresponding range of genotypes with very low and very high litter size, defined as six times the estimated genetic standard deviation, rose from seven to approximately nine pups per litter. In essence, the results from Table 2.5 demonstrate that the chosen scaling of \mathbf{H} and \mathbf{G} matrices provided higher estimates for the genetic variance

components for all traits. This increase can be interpreted as caused by correctly including the segregation variance, which is part of the genetic variance from generation F₂ onwards and is expected to be prominent for the trait LS0, on which the FL1 line has been selected.

Table 2.5 Estimates of genetic parameters for all traits obtained by three different kinds of relationship matrices.

Trait	Relation-ship	σ_a^2	σ_x^2	σ_c^2	σ_p^2	σ_e^2	h^2
LS0	A _a	1.37 (0.30)		0.31 (0.16)	2.06 (0.33)	7.79 (0.27)	.12 (.03)
	G _a	2.25 (0.42)		0.30 (0.16)	1.76 (0.34)	7.81 (0.27)	.19 (.03)
	H _a	2.44 (0.45)		0.31 (0.16)	1.70 (0.34)	7.81 (0.27)	.20 (.03)
LW0	A _a	5.76 (0.83)		0.53 (0.33)	3.86 (0.70)	14.5 (0.5)	.23 (.03)
	G _a	8.46 (1.10)		0.49 (0.33)	3.15 (0.72)	14.5 (0.5)	.32 (.03)
	H _a	9.09 (1.17)		0.50 (0.33)	3.02 (0.73)	14.6 (0.5)	.34 (.04)
BM21	A _{a+x}	0.54 (0.07)	0.02 (0.01)	2.15 (0.06)		0.61 (0.03)	.17 (.02)
	G _{a+x}	0.73 (0.09)	0.02 (0.01)	2.13 (0.06)		0.59 (0.03)	.22 (.02)
	H _{a+x}	0.77 (0.10)	0.03 (0.02)	2.12 (0.06)		0.59 (0.03)	.23 (.03)
BM42	A _{a+x}	2.47 (0.23)	0.35 (0.11)	1.71 (0.09)	2.82 (0.11)	0.68 (0.04)	.35 (.02)
	G _{a+x}	3.09 (0.29)	0.30 (0.10)	1.70 (0.09)	2.82 (0.11)	0.68 (0.04)	.40 (.03)
	H _{a+x}	3.28 (0.31)	0.34 (0.11)	1.70 (0.09)	2.82 (0.11)	0.68 (0.04)	.41 (.03)
BM63	A _{a+x}	3.89 (0.33)	0.77 (0.20)	1.19 (0.10)	4.73 (0.17)	0.80 (0.05)	.41 (.02)
	G _{a+x}	4.89 (0.42)	0.64 (0.16)	1.19 (0.10)	4.73 (0.17)	0.80 (0.05)	.45 (.02)
	H _{a+x}	5.17 (0.44)	0.72 (0.19)	1.19 (0.10)	4.73 (0.17)	0.80 (0.05)	.47 (.02)
BMM	A _a	5.59 (0.46)		1.24 (0.16)		5.74 (0.24)	.45 (.03)
	G _a	7.01 (0.57)		1.23 (0.16)		5.75 (0.24)	.50 (.03)
	H _a	7.42 (0.61)		1.23 (0.16)		5.75 (0.24)	.52 (.03)

Traits: litter size (LS0), litter weight (LW0), body weights at ages of 21, 42, 63 days and at mating (BM21, BM42, BM63, BMM). Kinds of relationship matrices: pedigree-derived numerator relationship matrix (A), gene-drop derived (G), combined expected and observed genomic relationships (H); subscripts indicate that autosomal relationships only (a) or both autosomal and X-chromosomal relationships (a+x) were part of the model. Genetic parameters: σ_a^2 : autosomal additive genetic variance, σ_x^2 : X-chromosomal additive genetic variance, σ_c^2 : common litter environmental variance, σ_p^2 : permanent environmental variance, σ_e^2 : residual variance, h^2 : heritability, standard errors in brackets.

Our own estimated heritability values for LS0 are within the wide range of results reported in older studies. Falconer (1960) reported 8.3% heritability for upward selection and 22.9% for downward selection. Bradford (1968) presented realized heritability from 0.13 to 0.39 for litter size in several lines, while Bakker *et al.* (1978) found a realized heritability of 0.11. More recent investigations tend towards lower values, compared with our result of 19%, e.g. Beniwal *et al.* (1992) reported a comparatively low heritability for litter size (0.181 ± 0.093 for control and 0.166 ± 0.043 overall), as did Peripato *et al.* (2004), with $h^2=12\%$. Similarly, Gutiérrez *et al.* (2006) published heritability values for litter size from 0.099 to 0.101. Gutiérrez *et al.* (2006) also reported considerably lower heritability, from 0.112 to 0.148 (derived with different models), for litter weight.

Estimated heritability values for body weight traits in mice vary widely in the literature. Falconer (1953) reported the heritability for body weight measured at day 60 as approximately 20% for upward selection and 50% for downward selection. Interestingly, Wilson *et al.* (1971) found that the realized heritability for body weight at day 60 declined from 0.32 in the first 10 generations to 0.08 between generation 61 and 70, in a selection experiment. Eisen (1978) reported a heritability of 0.44 (or 0.55, depending on the method of estimation) for six week body weight. Heath *et al.* (1995) reported a heritability of 0.25 before selection and a mean heritability of 0.216 ± 0.0077 (from 0.191 ± 0.016 to 0.242 ± 0.014 for different pairs of lines) for six week body weight. In an intercross-population, Kramer *et al.* (1998) estimated high heritability values of 0.54 ± 0.24 for three week body weight, 0.76 ± 0.04 for six week body weight and 0.81 ± 0.01 for nine week body weight in a cross-fostering experiment. Although they are within the upper ranks, our estimates of approximately 40% to 50% at ages from 42 to 63 days may be seen as well within the range of literature values.

Estimated genetic trends: Figure 2.3 shows estimated genetic trends for LS0 and BM42 from models with different relationship matrices. The upper panels (Figure 2.3A) show genetic trends from models with autosomal relationships only, while the other panels (Figure 2.3B) depict those from applying the autosomal and X-chromosomal relationship matrices, simultaneously. In general, trends are very similar by shape and also by level, in accordance with the absence of any serious trend. An exception is the jump at generation F_3 for LS0. This reflects the fact that from generation F_3 onwards only the descendants of a single family were maintained in order to breed the later generations, which makes the differences between the four founder families manifest. See also Figure S2.2 for genetic trends in other traits.

Underlying assumptions: In deriving \tilde{A} , the expected overall heterozygosity, as described by the scale parameter S , is taken as being fully determined by the observed allele frequencies of the genotyped founders and the assumption that both lines contribute equally to the new composite population. The latter can easily be adapted to more than two founder lines, even with unequal contributions, by an alteration of the definition of the average allele frequency \bar{p} . With more than two founder lines, the equilibrium state with foreseen autosomal heterozygosity, $S = \sum \bar{p}_i (1 - \bar{p}_i)$, may also be reached later than in the second crossbred generation. This depends on the mating scheme that generates the new composite population and, as with two founder lines, is an asymptotic process for X chromosome markers. The initial numbers of male and female founders may be different in each line to be crossed later.

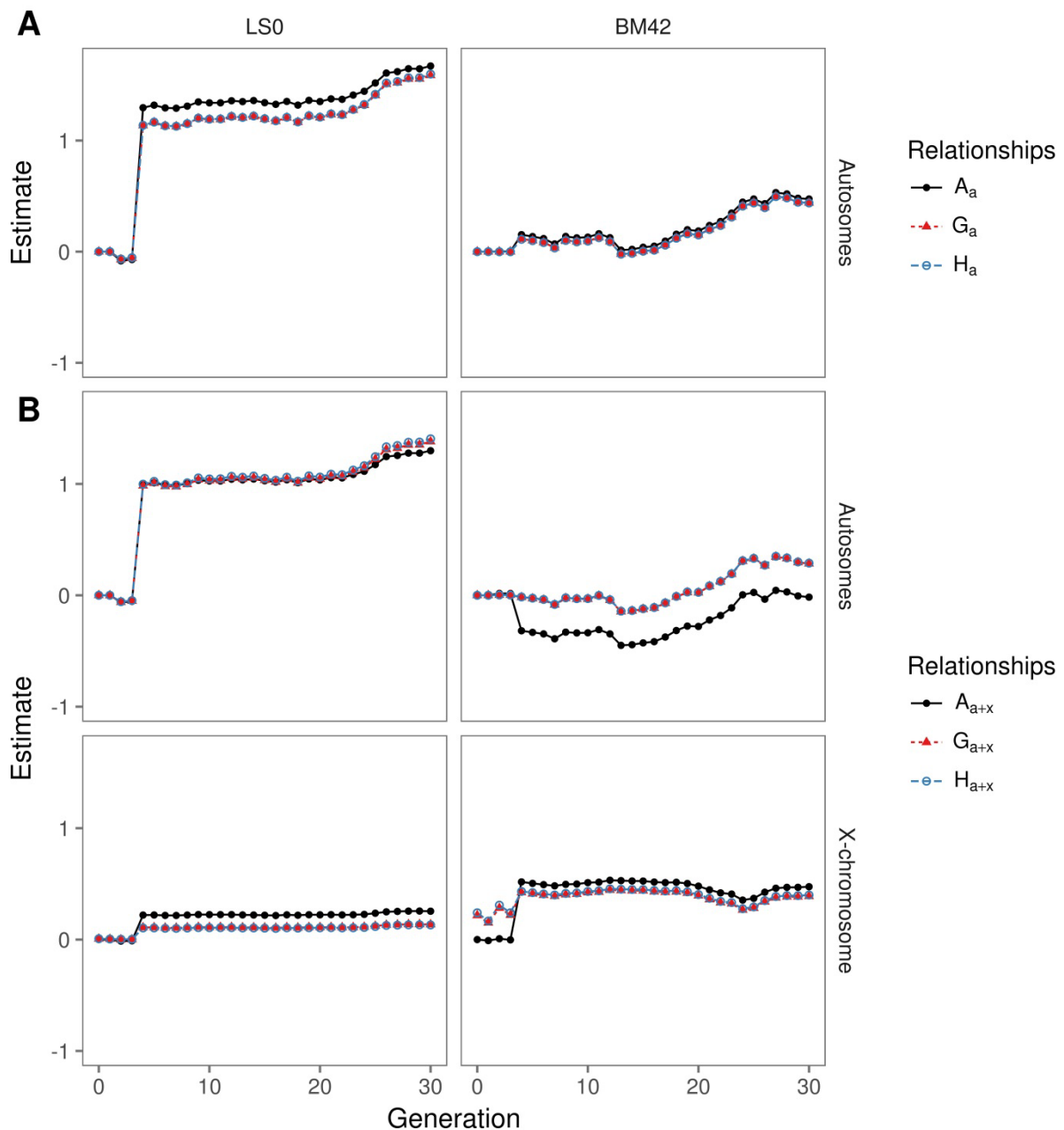


Figure 2.3 Autosomal (above and middle) and X-chromosomal (below) genetic trends for litter size (LS0, left) and body mass at day 42 (BM42, right) obtained by different kinds* of relationship matrices (A, G and H) and models with (subscript a+x) and without (subscript a) taking X-chromosomal genetic variation into account. Kinds of relationship matrices: pedigree-derived numerator relationship matrix (A), gene-drop derived (G), combined expected and observed genomic relationships (H); subscripts indicate that autosomal relationships only (a) or both autosomal and X-chromosomal relationships (a+x) were fitted.

Autosomal and X chromosome reference populations should be comparable, especially in the interpretation of genetic parameters. Therefore, it seems reasonable to define \bar{p} for X chromosome markers, and hence S , as if males and females from all founder lines contribute equally. However, this does not need to be the case, as demonstrated by our mouse example. A further assumption entered into $\tilde{\mathbf{A}}$ is that founder genotype frequencies meet line-specific Hardy-Weinberg equilibrium. Observed founder genomic self-relationships that deviate from their expected counterparts indicate an excess or lack of heterozygosity relative to this assumption. Actual marker heterozygosity values are then accounted for using the combined relationship matrix \mathbf{H} .

Matrix $\tilde{\mathbf{A}}$ reflects average genomic relationships that result from repeatedly sampling alleles at observed line-specific frequencies from founders and their forward transmission to later generations, in accordance with the pedigree. No attempts were made to estimate IBD-based founder relationships (e.g. Powell *et al.* 2010) within lines. There are typically only a few founders of experimental populations, as in our mouse example, and they may provide information on within-line IBD-relationships only with high sampling errors, unless a larger sample is genotyped. However, we did not treat founders as a sample from their respective lines but their genotypes were treated as a complete inventory of all possible alleles that can be further transmitted to the F_2 generation and beyond. Therefore, they fully determine the genetic makeup of the later crossbred population, as mirrored by the construction of $\tilde{\mathbf{A}}$. The usefulness of $\tilde{\mathbf{A}}$, and hence \mathbf{H} , for estimating the genetic variance will depend on how well the frequency spectrum of markers reflect the frequency spectrum of QTLs (quantitative trait loci) for a trait (Walsh and Lynch 2018, p. 631-668). The latter requirement is likely to be largely fulfilled in a cross of two divergently selected lines, where a large contribution by segregation variance can be expected to be picked up by a large proportion of markers with line-specific alleles.

Fields of application: In the analysis of selection experiments, mixed models have largely replaced other methods due to their flexibility (Walsh and Lynch 2018, p. 631-668). Data from AIL lines can be seen as a special case, with no artificial selection applied. Pedigree-based relationship matrices traditionally treat founders as unrelated and non-inbred. As only a limited number of founders are often genotyped, due to cost, this unrealistic assumption can be overcome by taking genomic founder relationships into account. Using mixed models, with the described $\tilde{\mathbf{A}}$ based version of \mathbf{H} , accounts for the initial

disequilibrium in allele and genotype frequencies, which exists for more generations on the X chromosome, compared with only two generations for autosomal loci. Meaningful estimates of genetic variances and heritability may be calculated in crosses of two lines in which line-specific alleles can be expected to prevail both at marker loci and QTL due to divergent selection histories. Moreover, in contrast to a gene-drop derived matrix, additional genotypes from later generations can easily be integrated by setting up a joint genomic relationship matrix for all genotyped individuals and joining it with $\tilde{\mathbf{A}}$ into \mathbf{H} . Thus $\tilde{\mathbf{A}}$ leads to more realistic assumptions and more flexibility in the analysis of selection experiments if all founders are genotyped.

Conclusion

An approach for constructing expected autosomal and X-chromosomal genomic relationship matrices for founders from an arbitrary number of founder lines was developed. Extension to non-genotyped individuals in later generations can be performed by an adapted version of the tabular method using pedigree information. The resulting matrix $\tilde{\mathbf{A}}$ expresses relationships as average genomic relationships, as one would expect from repeated random sampling of alleles from founders at the observed frequencies. Implicitly, $\tilde{\mathbf{A}}$ accounts for any proportion of segregation variance between zero and one, which is not possible using only pedigree data. Observed marker data of founders and non-founders can then be combined into a joint relationship matrix, \mathbf{H} , and its inverse can be used in mixed models for estimating the genetic variance in the crossbred population.

Acknowledgments

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Supplementary Information

Table S2.1 SNP density and number of SNPs by chromosome. For each chromosome the number of polymorphic SNPs per Mbp, the total number of genotyped SNPs per Mbp, and the absolute numbers of polymorphic and genotyped SNPs are given.

Chr.	Polymorphic SNP (per Mbp)	SNP loci (per Mbp)	Polymorphic SNP	SNP loci
Chr1	69.9	262.8	13,633	51,251
Chr2	53.0	232.1	9,641	42,248
Chr3	62.2	243.0	9,948	38,865
Chr4	54.2	234.1	8,517	36,748
Chr5	54.0	246.8	8,201	37,506
Chr6	59.1	247.6	8,870	37,137
Chr7	55.0	241.2	7,973	34,980
Chr8	50.0	248.5	6,393	32,059
Chr9	70.4	249.2	8,796	31,154
Chr10	34.0	224.1	4,446	29,356
Chr11	62.0	233.8	7,543	28,520
Chr12	56.3	254.2	6,750	30,507
Chr13	56.5	247.1	6,783	29,652
Chr14	58.0	211.5	7,254	26,441
Chr15	58.6	252.7	6,092	26,279
Chr16	54.5	254.6	4,960	23,170
Chr17	68.2	268.1	6,480	25,474
Chr18	46.4	259.3	4,224	23,599
Chr19	65.8	265.2	4,015	16,179
ChrX	11.8	127.9	2,022	21,870
Overall	54.3	237.2	142,541	622,995

Table S2.2 Estimates of genetic parameters for all traits obtained by three different kinds of relationship matrices.

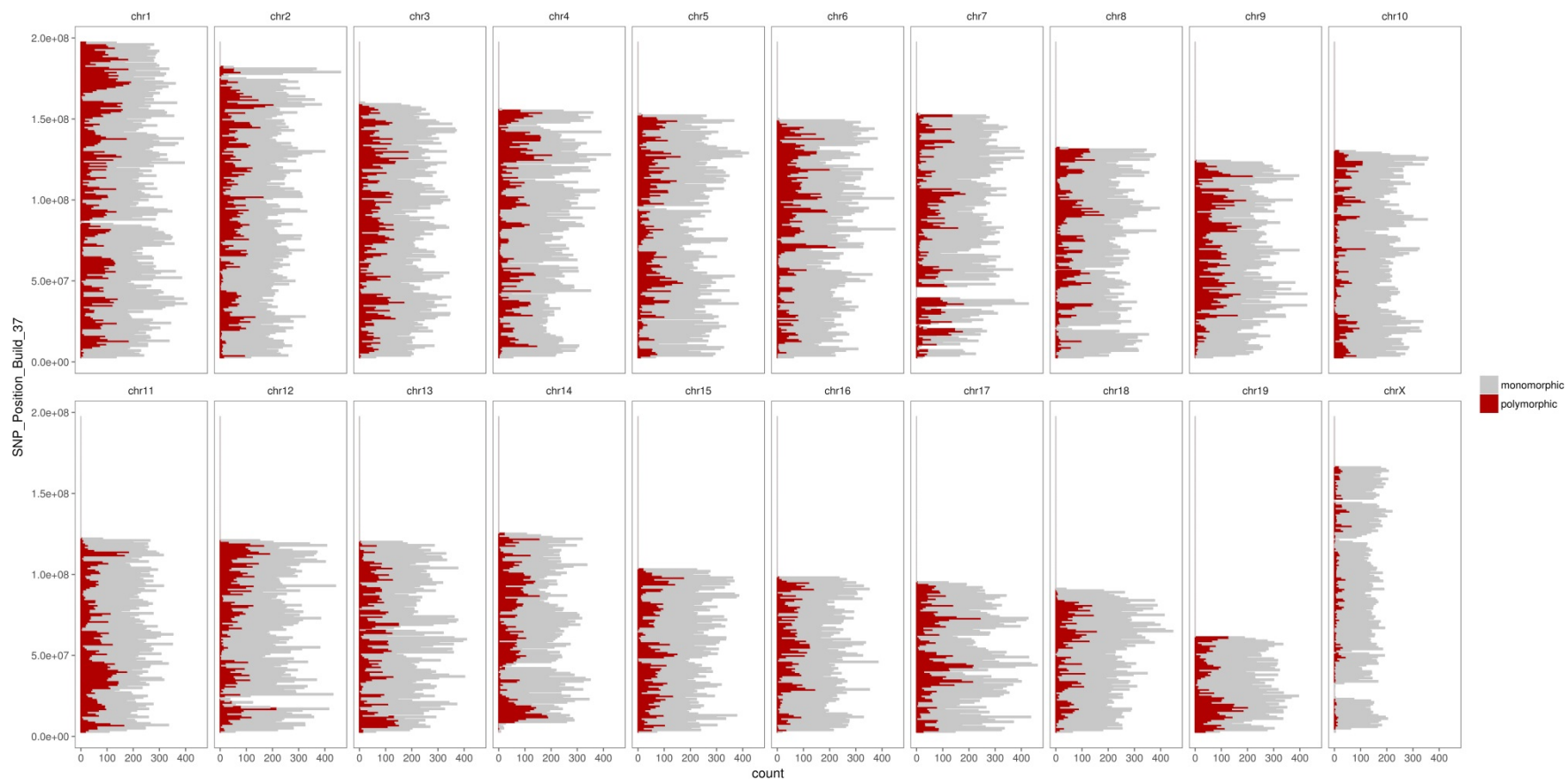
Trait	Relationship	σ_a^2	σ_x^2	σ_c^2	σ_p^2	σ_e^2	h^2
LS0	A _{a+x}	1.10 (0.48)	0.18 (0.27)	0.30 (0.16)	2.12 (0.34)	7.79 (0.27)	.11 (0.03)
	G _{a+x}	1.95 (0.64)	0.13 (0.23)	0.29 (0.16)	1.82 (0.36)	7.80 (0.27)	.17 (0.04)
	H _{a+x}	2.14 (0.69)	0.15 (0.28)	0.29 (0.16)	1.76 (0.36)	7.81 (0.27)	.19 (0.04)
LW0	A _{a+x}	5.70 (1.20)	0.04 (0.62)	0.53 (0.33)	3.87 (0.73)	14.5 (0.5)	.23 (0.03)
	G _{a+x}	8.44 (1.54)	0.01 (0.52)	0.49 (0.33)	3.15 (0.75)	14.5 (0.5)	.32 (0.04)
	H _{a+x}	8.97 (1.65)	0.06 (0.62)	0.49 (0.33)	3.04 (0.75)	14.6 (0.5)	.33 (0.04)
BM21	A _a	0.55 (0.07)		2.14 (0.06)		0.61 (0.03)	.17 (0.02)
	G _a	0.75 (0.09)		2.12 (0.06)		0.59 (0.03)	.22 (0.02)
	H _a	0.80 (0.10)		2.12 (0.06)		0.59 (0.03)	.23 (0.03)
BM42	A _a	2.64 (0.22)		1.69 (0.10)	2.90 (0.11)	0.68 (0.04)	.33 (0.02)
	G _a	3.32 (0.28)		1.68 (0.10)	2.90 (0.11)	0.68 (0.04)	.39 (0.03)
	H _a	3.52 (0.29)		1.68 (0.10)	2.90 (0.11)	0.68 (0.04)	.40 (0.03)
BM63	A _a	4.26 (0.31)		1.16 (0.10)	4.90 (0.16)	0.80 (0.05)	.38 (0.02)
	G _a	5.36 (0.40)		1.16 (0.10)	4.90 (0.16)	0.80 (0.05)	.44 (0.02)
	H _a	5.67 (0.42)		1.16 (0.10)	4.90 (0.16)	0.80 (0.05)	.45 (0.02)
BMM	A _{a+x}	5.59 (0.46)	0.00 (0.00)	1.24 (0.16)		5.73 (0.24)	.45 (0.03)
	G _{a+x}	7.01 (0.57)	0.00 (0.00)	1.23 (0.16)		5.75 (0.24)	.50 (0.03)
	H _{a+x}	7.42 (0.61)	0.00 (0.00)	1.23 (0.16)		5.75 (0.24)	.52 (0.03)

Traits: litter size (LS0), litter weight (LW0), body weights at ages of 21, 42, 63 days and at mating (BM21, BM42, BM63, BMM). Kinds of relationship matrices: pedigree-derived numerator relationship matrix (A), gene-drop derived (G), combined expected and observed genomic relationships (H); subscripts indicate that autosomal relationships only (a) or both autosomal and X-chromosomal relationships (a+x) were part of the model. Genetic parameters: σ_a^2 : autosomal additive genetic variance, σ_x^2 : X-chromosomal additive genetic variance, h^2 : heritability, σ_c^2 : common litter common litter environmental effect, σ_p^2 : permanent environmental effect, σ_e^2 : residual, standard errors in brackets.

Table S2.3 Details for the restricted likelihood ratio tests of the null hypothesis of no significant X-chromosomal genetic variance by trait and kind of relationship matrix. Traits are litter size (LS0), litter weight (LW0) and body mass at ages of 21, 42, 63 days and at mating (BM21, BM42, BM63, BMM). Kinds of relationship matrices were pedigree-derived (A), computed by gene-drop (G) and combined expected and observed genomic relationships (H). The full (a+x) and the reduced (a) model were identical except of the X-chromosomal genetic variance and were compared by their respective log-likelihoods (LogL), resulting in the RLRT test-statistics and error probabilities (P-value).

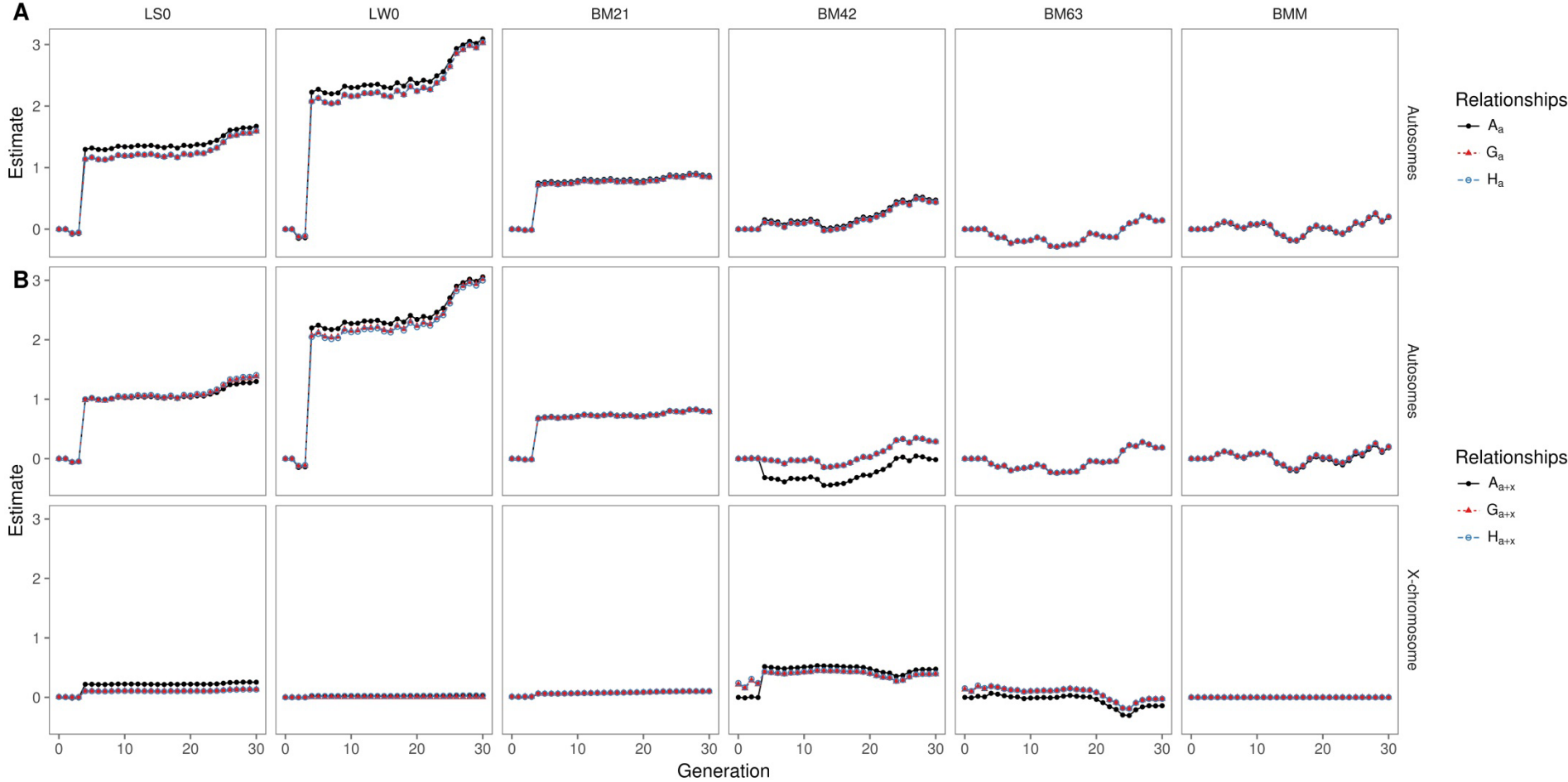
Trait	Relationship	LogL (a)	LogL (a+x)	RLRT	P-value
LS0	A_a / A_{a+x}	-9918.92	-9918.69	0.46	0.4976
	G_a / G_{a+x}	-9919.05	-9918.88	0.34	0.5598
	H_a / H_{a+x}	-9918.45	-9918.30	0.30	0.5839
LW0	A_a / A_{a+x}	-11908.37	-11908.36	0.02	0.8875
	G_a / G_{a+x}	-11905.99	-11905.99	0.00	1.0000
	H_a / H_{a+x}	-11904.94	-11904.93	0.02	0.8875
BM21	A_a / A_{a+x}	-11549.06	-11546.66	4.80	0.0285
	G_a / G_{a+x}	-11548.70	-11546.36	4.68	0.0305
	H_a / H_{a+x}	-11549.22	-11546.63	5.18	0.0228
BM42	A_a / A_{a+x}	-18038.47	-18031.09	14.76	0.0001
	G_a / G_{a+x}	-18037.29	-18030.61	13.36	0.0003
	H_a / H_{a+x}	-18036.76	-18030.06	13.40	0.0003
BM63	A_a / A_{a+x}	-19516.24	-19505.23	22.02	2.687E-06
	G_a / G_{a+x}	-19516.11	-19505.14	21.94	2.836E-06
	H_a / H_{a+x}	-19515.93	-19504.96	21.94	2.830E-06
BMM	A_a / A_{a+x}	-10663.10	-10663.10	0.00	1.0000
	G_a / G_{a+x}	-10663.00	-10663.00	0.00	1.0000
	H_a / H_{a+x}	-10663.10	-10663.10	0.00	1.0000

Figure S2.1 SNP distribution on the whole genome



The range of each bar is 1Mbp, bars colored in red are the counts of polymorphic SNP in each 1Mbp-long genomic segment and bar colored in grey are the counts of monomorphic SNP in each 1Mbp-long genomic segment. So the height of each bar (polymorphic SNP plus monomorphic SNP) represents the overall number of all SNP markers in the 1Mbp-long genomic segment.

Figure S2.2 Comparison of the genetic trends for six important traits



S1: d^{AA} and d^{BB}

In the article we demonstrate

$$d^{AA} = \frac{1}{S} \sum_i [p_i^A(1-p_i^A) + (p_i^A - \bar{p}_i)^2]$$

$$d^{BB} = \frac{1}{S} \sum_i [p_i^B(1-p_i^B) + (p_i^B - \bar{p}_i)^2]$$

With $\bar{p}_i = \frac{1}{2}(p_i^A + p_i^B)$ and $S = 2 \sum_i \bar{p}_i(1 - \bar{p}_i)$ we can get

$$\begin{aligned} d^{AA} + d^{BB} &= \frac{1}{S} \sum_i [p_i^A(1-p_i^A) + (p_i^A - \bar{p}_i)^2 + p_i^B(1-p_i^B) + (p_i^B - \bar{p}_i)^2] \\ &= \frac{1}{S} \sum_i [p_i^A - (p_i^A)^2 + (p_i^A)^2 - 2p_i^A\bar{p}_i + \bar{p}_i^2 + p_i^B - (p_i^B)^2 + (p_i^B)^2 - 2p_i^B\bar{p}_i + \bar{p}_i^2] \\ &= \frac{1}{S} \sum_i [p_i^A + p_i^B - 2\bar{p}_i(p_i^A + p_i^B) + 2\bar{p}_i^2] \\ &= \frac{1}{S} \sum_i [2\bar{p}_i - 4\bar{p}_i^2 + 2\bar{p}_i^2] \\ &= 1 \end{aligned}$$

This indicates that for autosomes the sum of the gametic self-relationships inherited from parents is equal to 1. So the inbreeding coefficient can be derived by expected self-relationship subtracting 1 which follows the same rule with the calculation of the pedigree-derived inbreeding coefficient.

It should be noted that d^{AA} doesn't have to be equal to d^{BB} especially when the inbreeding degrees of these two lines are different.

S2: Direct inversion of $\tilde{\mathbf{A}}$

The inverse of the relationship matrix $\tilde{\mathbf{A}}$ can be set up directly, starting with the inverse expected relationship matrix for all founders, which usually will be small and therefore is easy to invert. Then for each other individual in the pedigree an additional column and row is added, as known for the inverse of the numerator relationship matrix (Henderson 1976). For this step we need to know the Mendelian sampling variance among full-sibs of the candidate.

For autosomes: The Mendelian Sampling term (MS) refers to the random within-family deviation of a candidate's breeding value from parent average. For autosomes we have

$$BV_k = \frac{1}{2}BV_s + \frac{1}{2}BV_d + MS_k \quad (\text{S15})$$

where BV_k is the breeding value of candidate k , BV_s and BV_d are the breeding values of parents s and d of individual k and MS_k is the Mendelian Sampling term. The variance $Var(MS_k)$ of MS_k can then be computed from the 3x3 relationship matrix of the candidate and its parents

$$Var(MS_k) = \begin{bmatrix} -\frac{1}{2} & -\frac{1}{2} & 1 \end{bmatrix} \begin{bmatrix} g_{s,s} & g_{s,d} & g_{s,k} \\ g_{s,d} & g_{d,d} & g_{d,k} \\ g_{s,k} & g_{d,k} & g_{k,k} \end{bmatrix} \begin{bmatrix} -\frac{1}{2} \\ -\frac{1}{2} \\ 1 \end{bmatrix}, \quad (S16)$$

which simplifies to

$$Var(MS_k) = -\frac{1}{4}g_{s,s} - \frac{1}{4}g_{d,d} - \frac{1}{2}g_{s,d} + g_{k,k}.$$

In the particular case of a population bred from only two founder lines, as in our case, the term $g_{k,k}$ will be equal to $1 + \frac{1}{2}g_{s,d}$ as soon as allele frequencies reach their equilibrium, which is from generation F2 on in our case. Then the above equation can be further simplified to

$$Var(MS_k) = 1 - \frac{1}{4}(g_{s,s} + g_{d,d})$$

for the later generations. Thus the variance of the Mendelian sampling term can be calculated from the diagonal elements of $\tilde{\mathbf{A}}$ and the pedigree, provided that values for $g_{s,d}$ are also known for early generations. Note that in crosses of more than two lines it may take more generations until equilibrium of allele frequencies is reached.

Thus, for any non-founder candidate with index k an additional row and column with zeroes is appended to the inverse and multiples of $Var(MS_k)^{-1}$ are added to the expanded matrix at nine different positions. Indices and multiples are given by

$$\begin{cases} (k,k, Var(MS_k)^{-1}) & (s,k, -\frac{1}{2}Var(MS_k)^{-1}) & (d,k, -\frac{1}{2}Var(MS_k)^{-1}) \\ (s,s, \frac{1}{4}Var(MS_k)^{-1}) & (s,d, \frac{1}{4}Var(MS_k)^{-1}) & (d,d, \frac{1}{4}Var(MS_k)^{-1}) \end{cases} \quad (S17)$$

where MS_k^{-1} is the Mendelian Sampling value of individual k , s and d are the parents of individual k . In order to get all nine-positions indices of off-diagonal elements have to be exchanged.

For X chromosome: With some modifications similar rules can also be applied to the X chromosome. First, X-chromosomal Mendelian sampling terms for female and male individuals can be derived from the following equations:

$$BV_k^x = BV_s^x + \frac{1}{2}BV_d^x + MS_k^x, \text{ if } k \text{ is female} \quad (\text{S18})$$

$$BV_k^x = \frac{1}{2}BV_d^x + MS_k^x, \text{ if } k \text{ is male} \quad (\text{S19})$$

where BV_k^x is the breeding value of individual k , BV_s^x and BV_d^x are the breeding value of the parents of individual k and the term MS_k^x is the Mendelian Sampling term of individual k . Then we can calculate variances of the Mendelian sampling terms by adopting (S4) and (S5)

$$\text{Var}(MS_k^x) = \begin{bmatrix} -1 & -\frac{1}{2} & 1 \end{bmatrix} \begin{bmatrix} g_{s,s} & g_{s,d} & g_{s,k} \\ g_{s,d} & g_{d,d} & g_{d,k} \\ g_{s,k} & g_{d,k} & g_{k,k} \end{bmatrix} \begin{bmatrix} -1 \\ -\frac{1}{2} \\ 1 \end{bmatrix}, \text{ if } k \text{ is female} \quad (\text{S20})$$

$$\text{Var}(MS_k^x) = \begin{bmatrix} 0 & -\frac{1}{2} & 1 \end{bmatrix} \begin{bmatrix} g_{s,s} & g_{s,d} & g_{s,k} \\ g_{s,d} & g_{d,d} & g_{d,k} \\ g_{s,k} & g_{d,k} & g_{k,k} \end{bmatrix} \begin{bmatrix} 0 \\ -\frac{1}{2} \\ 1 \end{bmatrix}, \text{ if } k \text{ is male} \quad (\text{S21})$$

which simplifies to

$$\text{Var}(MS_k^x) = g_{k,k} - g_{s,s} - \frac{1}{4}g_{d,d} - g_{s,d}, \text{ if } k \text{ is female}$$

$$\text{Var}(MS_k^x) = g_{k,k} - \frac{1}{4}g_{d,d}, \text{ if } k \text{ is male.}$$

Allele frequencies of X-chromosomal alleles will fluctuate around their equilibrium frequencies for a series of generations. In case all founder lines have equal genetic contributions through males and females and equilibrium frequencies have been reached $g_{k,k}$ will eventually stabilize at a value of 0.5. This was, however, not the case in our application example. Likewise, $g_{s,d}$ will be affected by these fluctuations. Under the same conditions as diagonal elements for males reach 0.5 the elements $g_{k,k}$ for females will ultimately equal to $1 + \frac{1}{2}g_{s,d}$.

The rules (S3) for autosomes are modified to

$$\begin{aligned}
&\text{for male, } \begin{cases} (k, k, MS_k^{-1}) & (s, k, -\frac{1}{2}MS_k^{-1}) & (d, k, -\frac{1}{2}MS_k^{-1}) \\ (s, s, \frac{1}{4}MS_k^{-1}) & (s, d, \frac{1}{4}MS_k^{-1}) & (d, d, \frac{1}{4}MS_k^{-1}) \end{cases} \\
&\text{for female, } \begin{cases} (k, k, MS_k^{-1}) & (d, k, -\frac{1}{2}MS_k^{-1}) & (d, d, \frac{1}{4}MS_k^{-1}) \end{cases}
\end{aligned} \tag{S22}$$

Except from the computation of the Mendelian sampling variances the rule (S8) are identical to those given by Fernando and Grossman (1990) for a pedigree-derived relationship matrix of a single population that has allele frequencies at their equilibrium already in the founder generation.

S3: conversion between gametic and individual genomic founder relationships

In the case of a two-line cross the expected gametic and individual genomic founder relationships can be transformed back and forth by

$$\begin{bmatrix} 2 & 0 & -2 \\ 0 & 2 & -2 \\ 0 & 0 & 4 \end{bmatrix} \begin{bmatrix} d^A \\ d^B \\ r^{AB} \end{bmatrix} = \begin{bmatrix} \omega^{AA} \\ \omega^{BB} \\ \omega^{AB} \end{bmatrix}$$

and

$$\begin{bmatrix} d^A \\ d^B \\ r^{AB} \end{bmatrix} = \begin{bmatrix} 2 & 0 & -2 \\ 0 & 2 & -2 \\ 0 & 0 & 4 \end{bmatrix}^{-1} \begin{bmatrix} \omega^{AA} \\ \omega^{BB} \\ \omega^{AB} \end{bmatrix} = \begin{bmatrix} 1/2 & 0 & 1/4 \\ 0 & 1/2 & 1/4 \\ 0 & 0 & 1/4 \end{bmatrix} \begin{bmatrix} \omega^{AA} \\ \omega^{BB} \\ \omega^{AB} \end{bmatrix}.$$

For X chromosome the expected gametic and individual genomic founder relationships have conversion equations

$$\begin{bmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \\ 2 & 0 & -2 \\ 0 & 2 & -2 \\ 0 & 0 & -2 \\ 0 & 0 & -2 \\ 0 & 0 & -2 \\ 0 & 0 & -4 \\ 0 & 0 & -4 \\ 0 & 0 & 2 \\ 0 & 0 & -2 \\ 0 & 0 & -2 \\ 0 & 0 & 2 \\ 0 & 0 & 2 \end{bmatrix} \begin{bmatrix} d^A \\ d^B \\ r^{AB} \end{bmatrix} = \begin{bmatrix} \omega_x^A \\ \omega_x^B \\ \omega_{xx}^A \\ \omega_{xx}^B \\ \omega_{x,x}^{AA} \\ \omega_{x,x}^{BB} \\ \omega_{x,x}^{AB} \\ \omega_{xx,xx}^{AA} \\ \omega_{xx,xx}^{BB} \\ \omega_{xx,xx}^{AB} \\ \omega_{x,xx}^{AA} \\ \omega_{x,xx}^{BB} \\ \omega_{x,xx}^{AB} \\ \omega_{xx,x}^{AB} \end{bmatrix} .$$

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CHAPTER THREE

Genetic parameters for litter- and body mass-related traits in an advanced intercross mouse population

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Genetic parameters for litter- and body mass-related traits in an advanced intercross mouse population

Abstract

Litter size- and body mass-related traits from all generations of the AIL population were analyzed. Founder relationships were taken into account by using the previously developed relationship matrices. In particular the question of a significant X-chromosomal genetic variance component was revisited. To this end body mass in both sexes were treated as genetically different traits in a series of bivariate analyses. Further, relationships between litter traits in the first and second parity were investigated as well as relationships between body mass in males and females and first-parity litter characteristics. When body mass in males and females were treated as different traits in multivariate models no significant X-chromosomal genetic variability could be detected. The genetic and phenotypic correlations of the litter traits between the first and second parities were considerably higher for litter weight (.84 and .47) and compared to litter size (.52 and .35). Body mass at day 42 had relatively high genetic and phenotypic correlations with litter weight of the first parity while had minor correlations with litter size. The results derived in this study suggest that no X-chromosomal QTLs for body weight traits are to be expected in subsequent fine-mapping. Further, there is room for plenty of genetic variation in body mass traits that is independent of litter-size-related QTLs, in contrast to pleiotropic QTLs with large effects on both traits. Finally, litter weights measured in the first and second parity seem to be controlled by widely the same loci, in contrast to litter size, where independent genetic variation seems to exist for the size of the second litter.

Keywords: genetic correlation, litter size, body mass, advanced intercross mouse line

Introduction

An advanced intercross line (AIL) consisting of 30 generations has been developed as a cross between the long-term selected high fecundity mouse line FL1 (Langhammer *et al.* 2014) and a special highly inbred control line. Coarse QTL mapping for litter size-related and body size-related traits, among others, has already been conducted in the F₂-generation (see chapter one). For further insight in the details of genetic variation of these traits in the AIL population an estimation of certain genetic parameters of special interest was done by capitalizing on all phenotypic data as they were recorded over 30 generations. Further, we made use of our previously derived autosomal and X-chromosomal relationship matrices that take marker-derived founder relationships into account. More specifically these analyses were related to the possible existence of X-linked genetic variability, the genetic equivalence of litter size-related traits recorded in different parities, and finally, the genetic co-variances between litter size-related and body mass-related traits. All these research questions are linked to the goal of fine mapping of already coarsely mapped QTLs or additionally, but not yet detected ones. In particular, it might happen that QTLs are X-linked, have different effects on litter size in different parities, or are acting pleiotropically on litter size and body mass.

X-linked genetic variance is known to exist for litter size-related traits in sheep (*FecX*; Davis *et al.* 2006), yet has not been extensively investigated for other traits, e.g. growth traits, in other livestock species. Rance *et al.* reported the different response in growth rate between males and females from reciprocal crosses between two selected lines and identified an X-linked QTL for 10 week weight (Rance *et al.* 1997). Dragani *et al.* identified two X-linked QTLs for body weight at 40 weeks in two crossbred mouse populations (Dragani *et al.* 1995). However no causative mutations have been found for these X-linked QTLs. Own results from another long-term selection line suggest that estimates of X-chromosomal genetic variances are sensitive to the choice of the model (unpublished results), especially whether female and male body mass traits are considered to be the same genetically. Therefore it was investigated if previous results (see chapter two) on the significance of X-chromosomal variability could be confirmed in multivariate analyses for male and female body mass traits.

The question whether litter size in different parities can be regarded as the same trait genetically has already been investigated both in mice and pigs. In terms of genetic parameters such a difference means either different genetic variances for e.g. first and second litter, or their genetic correlation to be smaller than one, or both at the same time. In mice the

litter traits of the first litter were concerned in many studies. Bayon *et al.* established two selected mouse populations, $L+$ and $L-$, which were selected for increasing and decreasing the sum of litter size of first three litters respectively (Bayon *et al.* 1988). They observed the litter sizes of the first three litters were diverse in two selected mouse populations, which were 11.89, 11.11 and 11.63 young in $L+$ and 10.08, 8.87 and 9.06 young in $L-$, respectively, suggested to consider the litter sizes of different litters when selecting litter size, but did not report the genetic correlations of litter sizes of the first three litters. In pigs the genetic correlations of litter sizes of different litters were reported in the range of 0.28 to 0.99 (Fernández *et al.* 2008) and around 0.5 (Irgang *et al.* 1994; Alfonso *et al.* 1997) in average. Several studies suggested that the litter traits of different parities should be treated as different traits (Irgang *et al.* 1994; Alfonso *et al.* 1997; Noguera *et al.* 2002a, 2002b).

Similarly, genetic correlations between litter traits and body mass traits in mouse matter in our investigations because of the possible existence of pleiotropic QTLs with effects on both traits at the same time. In mice genetic correlations of 0.59 (Joakimsen and Baker 1977) and 0.52 (Eisen 1978) between litter size and body weight were reported. Eisen and Johnson reported the realized genetic correlations of 0.52 between body weight and litter size in a litter size selected mouse line (Eisen and Johnson 1981). Beniwal *et al.* reported the genetic correlation between litter size and body weight was about 0.25 in a mouse line which was index-selected for increased lean mass traits (Beniwal *et al.* 1992). Hanrahan and Eisen reported correlations between litter size and 3-, 6-, and 8- week body weights of 0.18, 0.36, 0.34, respectively, in a random-bred mouse population (Hanrahan and Eisen 1974). Bakker *et al.* did not find any significant correlated response between body weight and litter size after selection for increased litter size (Bakker *et al.* 1978). It should be noted Butler *et al.* observed a significant reduction of litter size due to selection on increased 8-week body weight (Butler *et al.* 1984). Together these studies suggest positive correlations between litter size and body mass in laboratory mice. In the light of the uniformly positive direction QTLs with pleiotropic effects on both traits can to be assumed common in mice.

To find answers to the research questions as outlined above different kinds of statistical analyses were carried out. In particular the significance of X-chromosomal genetic variability for body mass traits was tested for multivariate models, genetic correlations between litter size-related traits in different parities was investigated, and genetic relationships between body-mass and litter size were analyzed.

Materials and Methods

Advanced intercross mouse population

The phenotypic data analyzed in this study are identical to the data set already considered in the application of the modified genotype-pedigree-combined relationship matrices. Here Table 3.1 presents detailed descriptive statistics of litter size-related and growth-related traits from the underlying experiment.

Table 3.1 Descriptive statistics for the traits litter size (LS), litter weight (LW) and body mass (BM). Litter size and litter weight were recorded in first (_1) and second (_2) parity females. Body mass was measured at day 21 (BM21), 42 (BM42), 63 (BM63) after birth, and at the day of mating (BMM). Body mass was separated by sex (_M = male; _F = female).

Trait	N	Generation ^a	Mean	SD
LS0_1	4,430	F ₂ -F ₂₉	16.02	3.29
LS0_2	1,481	F ₂ , F ₂₂ -F ₂₅	16.32	3.77
LW0_1	4,410	F ₂ -F ₂₉	27.46	4.81
LW0_2	1,474	F ₂ , F ₂₂ -F ₂₅	28.63	5.53
BM21	19,080	F ₂ -F ₃₀	10.88	2.01
BM21_M	9,386	F ₂ -F ₃₀	11.00	2.05
BM21_F	9,694	F ₂ -F ₃₀	10.76	1.97
BM42	13,959	F ₂ -F ₃₀	29.46	3.77
BM42_M	6,838	F ₂ -F ₃₀	31.88	3.28
BM42_F	7,121	F ₂ -F ₃₀	27.14	2.57
BM63	13,283	F ₂ -F ₃₀	35.21	3.79
BM63_M	6,496	F ₂ -F ₃₀	37.05	3.42
BM63_F	6,787	F ₂ -F ₃₀	33.45	3.24
BMM	7,416	F ₂ -F ₂₉	36.42	4.17
BMM_M	3,554	F ₂ -F ₂₉	38.15	3.84
BMM_F	3,862	F ₂ -F ₂₉	34.82	3.80

^a Generations from which phenotypic records were available.

Modified genomic relationship matrices \mathbf{H}_a and \mathbf{H}_x

Variance components were estimated by different linear mixed models. Autosomal and X-chromosomal relationships took account for founder relationships as previously derived. The autosomal relationship matrix \mathbf{H}_a and its X-chromosomal counterpart \mathbf{H}_x served as covariance matrices for the respective additive genetic effects. The model with only \mathbf{H}_a and with both \mathbf{H}_a and \mathbf{H}_x are fitted for the analyses of the sex-specific body mass traits in order to test the significance of including X-chromosomal effect in the model. In the rest multivariate models only the matrix \mathbf{H}_a is used, e.g. the bivariate analyses for the litter traits of two litters.

Body mass traits

All body mass traits were modeled as bivariate with the trait in males as the first and that in females as the second. A series of bivariate analyses was performed in order to additive genetic estimate variances and for both sex-specific traits as well as their mutual additive-genetic co-variance.

The full model for each single trait was as follows:

$$y_{ijk} = \mu + \alpha_i + c_k + u_k^a + u_k^x + e_{ijk}$$

where y_{ijk} is the body-mass trait of animal k of litter j in generation i, μ is the general mean, α_i is the fixed effect of generation i, c_k is the random common litter environmental effect k, u_k^a and u_k^x are the random autosomal and X-chromosomal additive genetic effects of animal k, and e_{ijk} is the residual.

For males and females their two by two covariance matrices for autosomal and X-chromosomal additive genetic effects ($\mathbf{A}_a, \mathbf{A}_x$), common litter effects (\mathbf{C}) and residuals (\mathbf{R}) were assumed to be

$$\mathbf{A}_a = \begin{pmatrix} \sigma_{am}^2 & \sigma_{amf} \\ \sigma_{amf} & \sigma_{af}^2 \end{pmatrix}; \mathbf{A}_x = \begin{pmatrix} \sigma_{xm}^2 & \sigma_{xmf} \\ \sigma_{xmf} & \sigma_{xf}^2 \end{pmatrix} \text{ and}$$

$$\mathbf{C} = \begin{pmatrix} \sigma_{cm}^2 & \sigma_{cmf} \\ \sigma_{cmf} & \sigma_{cf}^2 \end{pmatrix}; \mathbf{R} = \begin{pmatrix} \sigma_{em}^2 & 0 \\ 0 & \sigma_{ef}^2 \end{pmatrix}.$$

Restricted maximum likelihood (REML) estimates of all variance components were obtained by using the ASReml package (Gilmour *et al.* 2015). All genetic variance components are corrected by the parameter D_k (Searle 1982; Legarra 2016), which can be computed by

$$D_k = \frac{\sum_{i=1}^f g_0(i,i)}{f} - \frac{\sum_{i=1}^f \sum_{j=1}^f g_0(i,j)}{f^2}$$

where $g_0(i,j)$ are elements of the observed genomic founder relationship matrix, and $f = 8$ is the number of founders. For matrices \mathbf{H}_a and \mathbf{H}_x the specific parameters D_k^a and D_k^x were derived.

The Null hypothesis of no significant X-chromosomal genetic variation was tested by comparing the log-likelihood of the full model with the log-likelihood of the reduced model with all covariance parameters in A_x assumed to be zero. The corresponding error probability of the restricted likelihood ratio-test (RLRT) was computed from a Chi-square distribution with three degrees of freedom for bivariate analyses (Wiencierz *et al.*, 2011).

The genetic and phenotypic correlations between two sex-specific traits are computed by

$$r_g = \frac{\text{cov}_g^{A,B}}{\sqrt{\sigma_g^A \times \sigma_g^B}}, \text{ and } r_p = \frac{\text{cov}_p^{A,B}}{\sqrt{\sigma_p^A \times \sigma_p^B}}$$

where $\text{cov}_g^{A,B}$ and $\text{cov}_p^{A,B}$ are the genetic and phenotypic covariances between traits A and B, σ_g^A and σ_g^B are the genetic variances of traits A and B, σ_p^A and σ_p^B are the phenotypic variances of traits A and B and r_g and r_p are the genetic and phenotypic correlations.

A multivariate analysis was conducted for traits BM21, BM42 and BM63 to explore the genetic correlations among them. The variance components and the covariance between each two traits were estimated with the model that only included an autosomal effect.

Litter traits in different parities

Previously the litter traits of the first and second parities were considered as two repeated records instead of two traits. Bivariate analyses were carried out for separately for litter size (LS0_1 and LS0_2) and litter weight (LW0_1 and LW0_2).

For the AIL the first mating was arranged for each dam when its first oestrus occurred. The second mating varied depending on the dams' body conditions after recovering. Thus the age at mating and the age at the parturition differed. The age ranged from 57 to 121 day-old at first mating, and from 77 to 153 day-old at second mating. The time interval between two parturitions ranged from 21 to 61 days. Due to these varieties the litter traits of two litters are fitted with the age at the first parturition for LS0_1 and LW0_1 and with the age at the second parturition for LS0_2 and LW0_2. The model for each single trait was:

$$y_{ijk} = \mu + \alpha_i + c_k + u_k^a + e_{ijk}$$

where y_{ijk} is the litter trait (e.g. LS0 or LW0) of animal k of litter j in generation i, μ is the general mean, α_i is the fixed effect of generation i, c_k is the random common litter environmental effect k, u_k^a is the random autosomal additive genetic effect of animal k, and e_{ijk} is the residual.

The different kinds of covariance matrices for traits in the first and second parity were \mathbf{A} for additive genetic effects, \mathbf{C} for common litter effects and \mathbf{R} for residuals (\mathbf{R}) and were assumed satisfy

$$\mathbf{A} = \begin{pmatrix} \sigma_{a1}^2 & \sigma_{a12} \\ \sigma_{a12} & \sigma_{a2}^2 \end{pmatrix}, \mathbf{C} = \begin{pmatrix} \sigma_{c1}^2 & \sigma_{c12} \\ \sigma_{c12} & \sigma_{c2}^2 \end{pmatrix} \text{ and } \mathbf{R} = \begin{pmatrix} \sigma_{e1}^2 & \sigma_{e12} \\ \sigma_{e12} & \sigma_{e2}^2 \end{pmatrix}.$$

Correlation among litter traits and body mass traits

In order to address genetic correlations between litter size-related and body mass-related traits several the bivariate analyses were conducted: for BM42_M and LS0_1, BM42_M and LW0_2, BM42_F and LS0_1 and BM42_F and LW0_2. The genetic and phenotypic correlations for each group of traits were computed by the formulas presented above. The effects in the model applied in the bivariate analyses and the multivariate analyses were as already specified above. For the sake of simplicity only litter size traits from the first parity

were taken into account consequently no permanent environmental variance component could be estimated.

Results and Discussion

Body mass traits separated by sex

The significance of including the X-chromosomal relationships was tested for each pair of sex-specific body mass traits by RLRT test. Table 3.2 presents the RLRT scores and the P-values of the univariate analyses for body mass traits omitting the sex and the bivariate analyses for each pair of sex-specific body mass traits.

In all univariate analyses the data of each body mass was considered as one variate without distinguishing sex. In all bivariate analyses each body mass is separated into two sets, *_M* and *_F* for males and females respectively, which are considered as two variates.

Table 3.2 Restricted likelihood ratio tests of the null hypothesis of no significant X-chromosomal genetic variance for body mass at day 21 (BM21), 42 (BM42), 63 (BM63) and at mating (BMM). The full (a+x) and the reduced (a) model were identical except of the X-chromosomal genetic variance and were compared by their respective log-likelihoods (LogL), resulting in the RLRT test-statistics and error probabilities (P-value).

Trait	Analysis	LogL (a)	LogL (a+x)	RLRT	P-value
BM21	Univariate	-11549.22	-11546.63	5.18	0.0228
BM21_M,_F	Bivariate	-13873.37	-13870.37	6.00	0.1116
BM42	Univariate	-18036.76	-18030.06	13.40	0.0003
BM42_M,_F	Bivariate	-19087.30	-19087.06	0.48	0.9232
BM63	Univariate	-19515.93	-19504.96	21.94	<0.0001
BM63_M,_F	Bivariate	-20849.49	-20846.08	6.82	0.0779
BMM	Univariate	-10663.10	-10663.10	0.00	1.0000
BMM_M,_F	Bivariate	-12127.90	-12127.41	0.98	0.8061

While in the univariate analyses X-chromosomal genetic variability appears to be significant for three of the four body mass traits (BM21, BM42, BM63, Table 3.2) this is clearly not the case in the bivariate models.

According to the RLRT tests estimated genetic parameters for all four traits are provided in two tables. Table 3.3 presents the results from models without X-chromosomal relationships, for results from models including X-chromosomal relationships see the supplement.

Table 3.3 Variances (diagonals), covariances (upper off-diagonals), and genetic correlations (lower off-diagonals) for autosomal additive, common litter environmental and residual effects estimated in a bivariate analysis of sex-specific (M = male; F = female) body weight measurements obtained at day 21 (BM21), 42 (BM42), and 63 (BM63) after birth, and at the day of mating (BMM). The parameter h^2 is the heritability and r_p denotes the phenotypic correlations between sex-specific traits. The standard errors are provided in brackets.

Trait	autosomal additive		common litter environment		residual		h^2		r_p
	M	F	M	F	M	F	M	F	
BM21	M	1.05 (0.09)	1.04 (0.09)	2.47 (0.07)	2.29 (0.06)	0.53 (0.03)		0.26 (0.02)	0.86 (0.01)
	F	0.99 (0.01)	1.06 (0.09)	1.00 (0.00)	2.15 (0.06)		0.50 (0.03)	0.29 (0.02)	
BM42	M	4.48 (0.39)	4.20 (0.28)	3.14 (0.27)	2.94 (0.19)	3.35 (0.16)		0.41 (0.03)	0.65 (0.02)
	F	0.98 (0.02)	4.10 (0.28)	0.78 (0.09)	2.87 (0.20)		1.86 (0.11)	0.54 (0.03)	
BM63	M	7.50 (0.55)	5.83 (0.39)	2.05 (0.17)	1.16 (0.11)	4.02 (0.22)		0.55 (0.03)	0.55 (0.02)
	F	0.90 (0.03)	5.61 (0.45)	0.58 (0.01)	1.95 (0.15)		4.32 (0.19)	0.47 (0.03)	
BMM	M	9.21 (0.77)	7.04 (0.54)	1.83 (0.28)	1.45 (0.17)	4.45 (0.35)		0.59 (0.03)	0.59 (0.03)
	F	0.87 (0.04)	7.16 (0.64)	0.73 (0.11)	2.17 (0.24)		4.24 (0.30)	0.53 (0.03)	

Estimates of autosomal genetic variance components in males and females were almost identical for BM21 and BM42 with almost perfect genetic correlations of 0.99 and 0.98. At later ages, i.e. for BM63 and BMM, the estimated additive-genetic variances became somewhat more divergent and genetic correlations between body mass in males and females declined to lower, yet still high values of 0.90 and 0.87 (Table 3.3). Interestingly for these two traits the male estimates of the genetic variances were higher than females', e.g. 7.50 for BM63_M comparing to 5.61 for BM63_F and 9.21 for BMM_M comparing to 7.16 for BMM_F. In general the estimated autosomal variances increased with higher ages both in

males and females, while there were often only small differences between male and female body mass traits in other variance components. Correlations between common litter environmental effects in males and females, however, changed from positive perfect (1.00) at weaning (day 21) to only moderately high between 0.58 and 0.78 at later ages. Phenotypic correlations between males and females showed a similar trend with age, and declined from .86 for BM21, to .65 for BM42 and to .55 for BM63 and .59 for BMM. The phenotypic correlation of BMM was .59 and close to the phenotypic correlation for BM63.

As a general tendency the estimated heritabilities for body mass increased with age for both sexes, beginning with .26 and .29 for males and for females at day 21, respectively. Further male heritabilities were .41 at day 42 and .55 at day 63. For females heritabilities at later ages were all around 50%, namely .54 at day 42, .47 at day 63, and .53 at mating. Note that for the last trait the average age at first oestrus was 73 days with a range from 56 to 121. Despite this variability the heritability of BMM was close to the heritability of BM63.

In our previous univariate analyses of AIL data the inclusion of X-chromosomal relationships in the model significantly increased the log-likelihood for the three body mass traits BM21, BM42, and BM63. Obviously there is a danger of assigning other sources of variability to X-linked genetic variation, when these factors are not appropriately mirrored by the model, such factors in particular are differences between sexes, sex-specific effects of common litter environment and different residual variation in males and females. Results from bivariate estimations of variance components are in concordance with previous coarse mapping results (chapter one), where no genome-wide significant QTLs were identified on chromosome X.

Minor X-chromosomal genetic variation in body mass traits may nevertheless exist in the AIL, as several weaker signals appeared as chromosome-wide significant QTLs on chromosome X: a QTL for two body weight gain between day 21- 42 (BWG1) and between day 21 - 63 (BWG3), QTLs for maternal nursing traits like litter weight of the first litter at day 10 (LW10st_1) and day 21 (LW21st_1) and QTL for average pup weight per litter at day 10 (AW10st_1_X_1) and day 21 (AW21st_1_X_1).

Bivariate analyses of litter traits in different parities

In order to investigate the genetic relation of litter traits in the first and second parities bivariate analyses were carried out. For litter size and litter weight Table 3.4 presents estimates of variance components and genetic parameters derived from univariate in comparison to bivariate analyses.

Table 3.4 Comparison of litter size (LS0) and litter weight (LW0) obtained in parity 1 and 2 in a bivariate analysis, respectively. Variances (diagonals), covariances (upper off-diagonals), and genetic correlations (lower off-diagonals) are provided for autosomal additive, common litter environmental and residual effects. The parameter h^2 is the heritability and r_p denotes the phenotypic correlations of traits between parities. Variances and heritabilities derived from a univariate analysis of both traits and each parity are provided. The standard errors are given in brackets.

Trait	autosomal additive		common litter environment		residual		h^2		r_p	
	1	2	1	2	1	2	1	2		
Bivariate	LS0	1	3.68 (1.02)	2.43 (0.97)	0.22 (0.26)	0.20 (0.25)	6.94 (0.56)	2.07 (0.53)	0.34 (0.08)	0.35 (0.04)
		2	0.52 (0.16)	5.97 (1.61)	0.96 (0.21)	0.20 (0.42)	0.24 (0.53)	10.37 (0.88)	0.36 (0.08)	
	LW0	1	8.04 (2.26)	9.34 (2.22)	1.55 (0.67)	0.44 (0.58)	15.05 (1.24)	4.19 (1.16)	0.33 (0.08)	
		2	0.84 (0.10)	15.52 (3.36)	0.98 (0.47)	0.13 (0.85)	0.23 (0.11)	20.32 (1.80)	0.43 (0.07)	
Univariate	LS0	1	2.36 (0.46)		0.50 (0.19)		8.55 (0.31)		0.21 (0.04)	
	LS0	2	5.51 (1.46)		0.00 (0.00)		10.76 (0.84)		0.34 (0.07)	
	LW0	1	8.33 (1.17)		1.05 (0.40)		15.68 (0.67)		0.33 (0.04)	
	LW0	2	14.77 (3.17)		0.00 (0.00)		20.84 (1.73)		0.41 (0.07)	

The estimates of the variance components appeared to be largely consistent when results from univariate and bivariate analyses are compared, though the bivariate models picked up some more genetic variability. A notable difference, however, is that estimated genetic variances were higher in bivariate analyses, especially for litter size. Accordingly the heritability for the size of the first litter was .34 in the bivariate analysis, compared to only .21 in the univariate case. For all other traits heritabilities were hardly affected by either univariate or multivariate analysis (Table 3.4). They were remarkably high in the range from 0.33 to 0.43 (Table 3.4). For both traits the genetic variance in the second parity was larger than the estimate for the first parity. For litter size the respective estimated genetic variances were 2.36 and 5.51 in univariate analyses and 3.68 and 5.97 in bivariate analysis. For litter weight the estimated autosomal variances were 8.33 and 14.77 in univariate analyses and 8.04 and 15.52 in bivariate analysis. Univariate models were not able to pick up any common litter environmental variances for size and weight of the second litter. In contrast, bivariate analyses

yielded small common litter environmental variances (with large standard errors) for both characteristics of the second litter, which were 0.20 (0.42) for litter size and 0.13 (0.85) for litter weight. In general, the common litter environmental variances were anyway small for both litter traits. For litter size both the genetic and the phenotypic correlation between the first and second parity remarkably low at .52 and .35, respectively. In contrast, the first and second litters exhibited high genetic correlation for litter weight (.84), which renders litter weight the more repeatable trait over parities in the genetic sense.

The genetic correlation of litter size between the first and second litters was 0.52 in our study. Alfonso *et al.* reported the genetic correlation of pigs born alive (NBA) and total number born (TNB) among first five parities in two Landrace populations (Alfonso *et al.* 1997). In two populations the genetic correlation ranged from 0.47 (between the first and third parities) to 1.00 (between the second and third parities) for NBA and from 0.67 (between the first and fourth parities) to 0.95 (between the first and second parities) for TNB. In contrast, the genetic correlations between these two traits of the first and second parities were around 0.90. Irgang *et al.* reported that the genetic correlations for all breeds applied in their study were high between first and third, and between second and third parities, but were low, in the range of .32 and .48, between first and second parities (Irgang *et al.* 1994). Due to these moderate genetic correlations between two litters it was seen as not appropriate to consider the litter traits of the second litter as the repeated measurement by various other researchers. Instead, a multivariate analysis is recommended when evaluating traits of multiple litters, which was also suggested especially in studies of pig data (Roehe and Kennedy 1995; Noguera *et al.* 2002a, 2002b).

It should be noted that in our study the litter traits of the second parity were only recorded from around 1,400 females. In the intended fine-mapping study litter size of the first and second parity should also be analyzed separately, while for litter weight an average of first and second litter may be the genetically more informative trait compared to separate analyses.

Correlations among litter traits and body mass traits

Four bivariate analyses were conducted for four trait pairs, BM42_M and LS0_1, BM42_M and LW0_1, BM42_F and LS0_1 and BM42_F and LW0_1. All genetic parameters, heritabilities and genetic and phenotypic correlations from these four analyses are given in Table 3.5.

Table 3.5 Comparison of body mass at day 42 (BM42) and litter size (LS0) as well as BM42 and litter weight (LW0) in a bivariate analysis. BM42 was analyzed for males (_M) and females (_F), separately. Variances (diagonals), covariances (upper off-diagonals), and genetic correlations (lower off-diagonals) are provided for autosomal additive, common litter environmental and residual effects of each trait. The parameter h^2 is the heritability and r_p denotes the phenotypic correlations of traits. The standard errors are given in brackets.

Trait	autosomal additive		common litter environment		residual		h^2		r_p
	BM42_M	LS0	BM42_M	LS0	BM42_M	LS0	BM42_M	LS0	
BM42_M	2.97 (0.41)	0.21 (0.32)	2.93 (0.17)	0.84 (0.14)	3.87 (0.16)		0.30 (0.04)		0.10 (0.03)
LS0	0.08 (0.13)	2.14 (0.49)	0.97 (0.23)	0.26 (0.26)		8.90 (0.35)		0.19 (0.04)	
BM42_M	3.03 (0.41)	2.25 (0.52)	2.93 (0.17)	1.10 (0.20)	3.85 (0.16)		0.31 (0.03)		0.21 (0.03)
LW0	0.45 (0.09)	8.20 (1.24)	0.95 (0.29)	0.46 (0.52)		16.25 (0.73)		0.33 (0.04)	
	BM42_F	LS0	BM42_F	LS0	BM42_F	LS0	BM42_F	LS0	
BM42_F	3.80 (0.35)	0.40 (0.31)	1.33 (0.10)	0.43 (0.12)	2.04 (0.13)	0.75 (0.15)	0.53 (0.03)		0.17 (0.02)
LS0	0.14 (0.10)	2.13 (0.49)	0.80 (0.13)	0.21 (0.26)	0.18 (0.01)	8.91 (0.35)		0.19 (0.04)	
BM42_F	3.87 (0.35)	2.73 (0.50)	1.33 (0.10)	0.47 (0.17)	2.03 (0.13)	0.92 (0.22)	0.54 (0.03)		0.31 (0.02)
LW0	0.48 (0.07)	8.56 (1.26)	0.74 (0.11)	0.31 (0.52)	0.16 (0.01)	16.18 (0.73)		0.34 (0.04)	

The estimates of genetic variance components for separate traits as derived from these four bivariate analyses were almost identical with the previous multivariate analyses depicted above. For example, in the bivariate analysis of BM42 and LW0_1 the estimated genetic variance of LW0_1 was 8.56, which matches the autosomal variances estimated by the univariate analysis of LW0_1 (8.33) and the autosomal variances estimated by the univariate analysis of LW0_1 and LW0_2 (8.04). In these four analyses the heritabilities of BM42_M, 0.30 (with LS0_1) and 0.31 (with LW0_1), were smaller than the heritabilities derived in the bivariate analysis of BM42_M and BM42_F (0.41). However, this decline was not observed for BM42_F, e.g., 0.53 (with LS0_1) and 0.54 (with LW0_1) compared to 0.54 in the bivariate analysis. The similar decline was not found for LW0_1, e.g., 0.33 (with BM42_M)

and 0.34 (with BM42_F) compared to .33 derived in the bivariate analysis of litter weight of two litters and the univariate analysis for LW0_1.

Body mass and litter weight of the AIL population showed comparatively higher genetic correlations compared to the genetic correlations between body mass and litter size. The genetic correlations between LS0_1 and BM42 were 0.08 in males and 0.14 in females. In contrast, for litter weight the genetic correlations between LW0_1 and BM42 were 0.45 in males and 0.48 in females. The phenotypic correlations of these four trait pairs agreed with their genetic correlations of them. Notably the male body mass had slightly lower genetic correlations with litter traits, of 0.08 (with LS0_1) and 0.45 (with LW0_1), compared to the genetic correlations of the female body mass and litter traits, of 0.14 (with LS0_1) and 0.48 (with LW0_1).

Our results of the genetic correlations between litter traits and body mass traits were different to correlations reported in other studies. Beniwal *et al.* reported genetic correlation between litter size and lean weight of about 0.25 in a mouse line selected for an index of lean mass traits (Beniwal *et al.* 1992). Hanrahan and Eisen (1974) reported that the correlation between litter size and six-week body weight was 0.36 in a random-bred mouse population (Hanrahan and Eisen 1974). Bakker *et al.* did not find a significant correlated response between body weight and litter size in the mouse line selected for increasing litter size (Bakker *et al.* 1978).

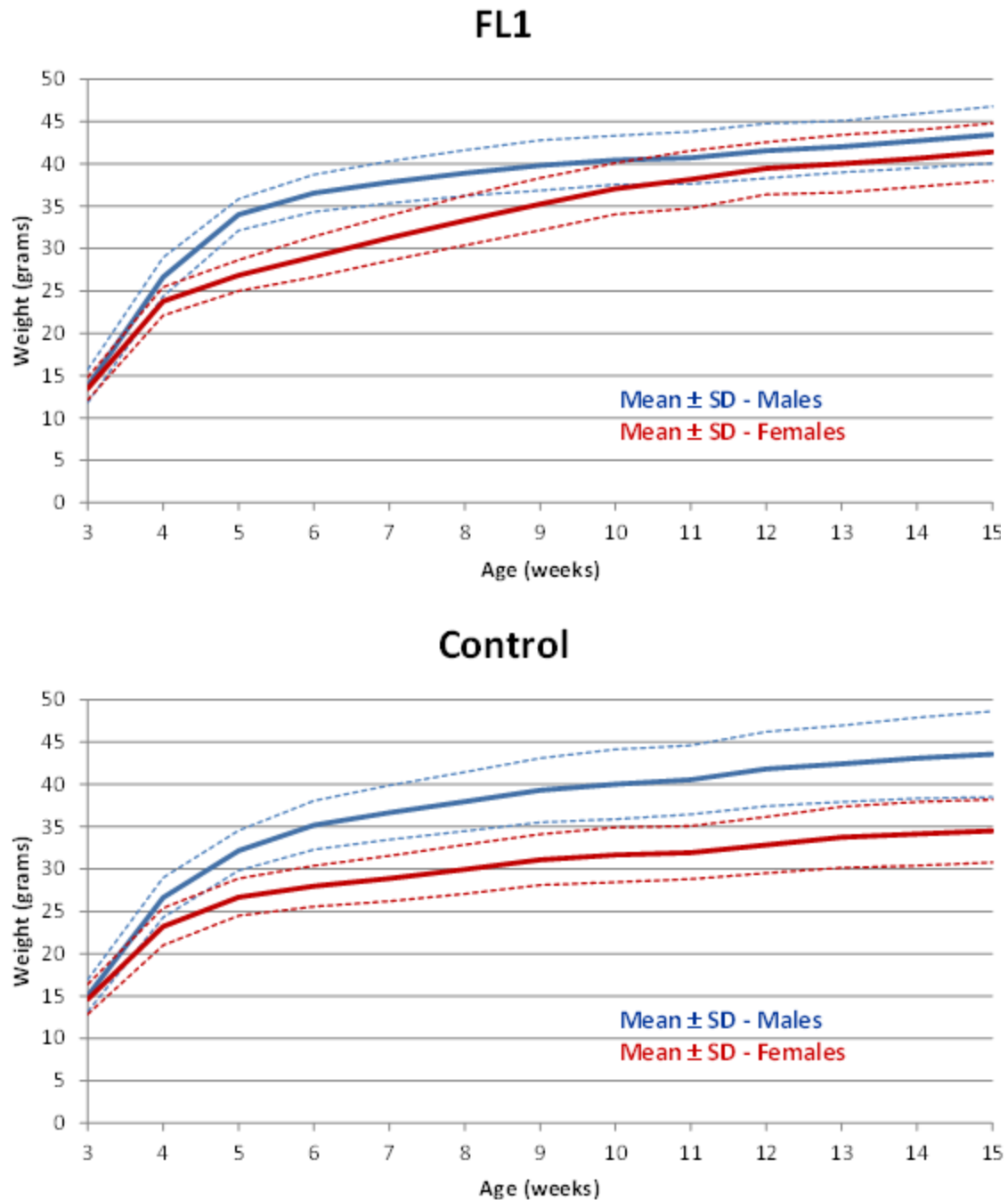
In conclusion the results derived in this study showed that no X-chromosomal QTLs for body weight traits are to be expected in subsequent fine-mapping. Further, there is room for plenty of genetic variation in body mass traits independent from litter-size-related QTLs. If pleiotropic QTLs with effects on both traits exist, they are not expected to explain the majority of genetic variation in both traits, as otherwise one had expected higher respective genetic correlations. Litter weight measured in the first and second parity seems to be controlled by widely the same loci, which can be exploited by averaging phenotypes in mapping studies. This is somewhat in contrast to litter size, where genetic correlations between parities were lower and independent genetic variation seems to exist for the size of the second litter.

Supplementary Information

Table S3.1 Variances (diagonals), covariances (upper off-diagonals), and genetic correlations (lower off-diagonals) for autosomal additive, common litter environmental and residual effects estimated in a bivariate analysis of sex-specific (M = male; F = female) body weight measurements obtained at day 21 (BM21), 42 (BM42), and 63 (BM63) after birth, and at the day of mating (BMM). The parameter h^2 is the heritability, r_g and r_p denote the genetic and phenotypic correlations between sex-specific traits. The standard errors are provided in brackets.

Trait		autosomal additive		X-chromosomal additive		common litter environment		residual		h^2		r_g	r_p
		M	F	M	F	M	F	M	F	M	F		
BM21	M	1.02 (0.09)	1.03 (0.09)	0.10 (0.05)	0.02 (0.02)	2.45 (0.07)	2.29 (0.06)	0.51 (0.03)		0.27 (0.02)		0.96 (0.02)	0.86 (0.01)
	F	1.00 (0.01)	1.04 (0.09)	0.64 (0.43)	0.01 (0.02)	1.00 (0.00)	2.15 (0.06)		0.51 (0.03)		0.28 (0.02)		
BM42	M	4.40 (0.41)	4.13 (0.32)	0.16 (0.29)	0.06 (0.13)	3.07 (0.17)	1.73 (0.09)	3.34 (0.16)		0.42 (0.03)		0.97 (0.03)	0.65 (0.02)
	F	0.98 (0.02)	4.04 (0.35)	0.93 (0.75)	0.03 (0.11)	0.78 (0.11)	1.59 (0.09)		1.86 (0.12)		0.54 (0.03)		
BM63	M	7.06 (0.57)	5.76 (0.46)	1.10 (0.45)	0.11 (0.21)	1.93 (0.17)	1.16 (0.11)	3.90 (0.22)		0.58 (0.03)		0.87 (0.03)	0.55 (0.02)
	F	0.90 (0.03)	5.58 (0.58)	0.90 (0.81)	0.02 (0.20)	0.60 (0.02)	1.95 (0.15)		4.33 (0.20)		0.47 (0.03)		
BMM	M	9.10 (0.81)	6.91 (0.61)	0.23 (0.60)	0.10 (0.32)	1.81 (0.29)	1.46 (0.17)	4.42 (0.36)		0.60 (0.03)		0.86 (0.04)	0.58 (0.03)
	F	0.87 (0.04)	7.03 (0.87)	0.85 (0.65)	0.07 (0.34)	0.74 (0.13)	2.17 (0.24)		4.26 (0.31)		0.52 (0.03)		

Figure S3.1 Average growth rate curves of the FL1 line and its control line. The solid and dotted lines indicate the mean and \pm SD, respectively. Males and females were colored in blue and red, respectively.



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GENERAL DISCUSSION

In the coarse QTL-mapping study for the AIL population a large number of genome-wide and chromosome-wide significant QTLs was identified for litter size related traits (e.g. litter size including and excluding the number of stillborn pups), litter weight related traits (e.g. birth litter weight and average pup weight) and body mass at different ages. Generally the chromosome-wide significant QTLs (including all genome-wide significant QTL) identified for these three trait groups were mainly located on MMU2, MMU4, MMU9 and MMU13, MMU2, MMU4, MMU15 and MMU16 and MMU2, MMU4, MMU7 and MMU8 respectively.

Many QTL regions we detected agreed with results of other mouse QTL studies, e.g. litter-size-related QTL on MMU2 (Kirkpatrick *et al.* 1998; Spearow *et al.* 1999; Rocha *et al.* 2004a), MMU4 (Kirkpatrick *et al.* 1998; Spearow *et al.* 1999), MMU9 (Liljander *et al.* 2009) and MMU13 (Kirkpatrick *et al.* 1998; Spearow *et al.* 1999; SUTO 2015) and the body-mass-related QTL on MMU2 (Brockmann *et al.* 1998, 2004; Reed *et al.* 2003), MMU4 (Brockmann *et al.* 2000; Zhang and Gershenfeld 2003; Parker *et al.* 2011; Yuan *et al.* 2012), MMU7 (Vaughn *et al.* 1999; Itoi-Babaya *et al.* 2007; Reed *et al.* 2008; Parker *et al.* 2011) and MMU8 (Rocha *et al.*, 2004; Brockmann *et al.*, 2004; Vitarius *et al.*, 2006). The potential existence of causative genes for large litter size has been increasingly strengthened through all these studies. However the understanding of the molecular mechanisms that affect litter size remained sparse.

The identification of the causative genes/mutations for growth traits was comparatively successful. Several candidate genes were identified for body mass and other growth traits in multiple species. With the identifications of their causative mutations growth-related mechanisms have gradually gained clear contour. For example the IGF family was identified to have a close relation with growth traits (Liu *et al.* 1993) and the identification of their causative mutations (Abuzzahab *et al.* 2003; Raile *et al.* 2006; Solomon-Zemler *et al.* 2017) gave clear explanation for the relation.

In terms of the reproductive traits the identification of causative genes/mutations was an outstandingly success in uniparous species like sheep. A series of QTL studies were carried out for reproduction in sheep (Crawford 2001). Several important mutations were discovered for genes *BMPRI1B* (Wilson *et al.* 2001; Mulsant *et al.* 2001; Souza *et al.* 2001), *BMP15*

(Galloway *et al.* 2000) and *GDF9* (Hanrahan *et al.* 2004). Various mutations and causative alleles were found (Galloway *et al.* 2000; Hanrahan *et al.* 2004; Nicol *et al.* 2009; Silva *et al.* 2011) and directly linked to the phenotypic performance, e.g. *FecB^B/FecB^B* and *FecB⁺/FecB⁺* had two lambs per litter to one lamb per litter and three lambs per litter in average (Davis *et al.* 2006).

In multiparous species the identification of the litter-size-related genes/mutations has been unsatisfactory so far. There was almost no clear and complete chain that connected QTL, candidate gene, its mutation and (abnormal) phenotypic performance, which was similar as the *Fec* mutation studies in sheep. QTL-mapping successively identified a large number of QTL for litter size and linked them with the phenotypic variance they accounted for. For example, Kirkpatrick *et al.* detected a QTL on MMU2 for litter size which accounted for 13.2% of the phenotypic variance (Kirkpatrick *et al.* 1998). From another direction of the chain several genes were verified to be associated with litter size through gene edited mouse models, e.g. genes *Lin28a* (Zhu *et al.* 2010) and *Mcp* (or CD46; Inoue *et al.* 2003).

The mystery of litter size, however, still drives people to explore candidate genes for reproductive traits. The large quantity of studies on litter size in different aspects provided a long list of the potential candidate genes. A study indicated that the mouse with gain-of-function mutations of gene follicle stimulating hormone receptor (*Fshr*) presented premature follicle depletion and estrogen excess and consequently resulted in larger litter size for knock-in females than the wildtype females (Peltoketo *et al.* 2010). The gene follicle stimulating hormone (*Fsh*) and the genes related to it (e.g. its receptor *Fshr*) particularly drew our attentions. In pigs a study demonstrated the gene follicle stimulating hormone beta (*FSHB*) to have association with high litter size with a candidate gene approach (Li *et al.* 1998). In mice gene *Fshr* is located at 58.76 cM on MMU17, which overlaps with the QTL region we identified for the number of stillborns. Interestingly, a study in humans identified two mutations for dizygotic twinning, which were near *FSHB* gene and in *SMAD3* gene, respectively (Mbarek *et al.* 2016). In mice gene *Fshb* is located at 56.02 cM on MMU2 and gene *Smad3* is located at 34.22 cM on MMU9. In our coarse QTL-mapping QTLs for litter size were identified exactly at these two regions (e.g. the genome-wide significant QTL LS0_1_02_1 and LS0_2_02_1 and the chromosome-significant QTL ALS0_09_1). All these results highlight the *Fshb* gene, its “relatives” and the respective chromosomes as e.g. MMU2, which should be played more attentions in the subsequent fine-mapping studies.

Interestingly most potential causal genes as previously mentioned in the general introduction can be classified in two clusters according to the proximity of their positions in the mouse genetic map to the QTLs we identified for the number of stillborn pups and birth litter size excluding stillborn pups, respectively (Table 4.1).

Table 4.1 Potential causal genes in two clusters which were close to the QTL for number of stillborn pups and birth litter size excluding stillborn pups respectively.

Chr.	Gene	Position	Gene name	Reference
Close to QTL for number of stillborn pups				
1	<i>Cd46</i>	98.41	CD46 antigen	Inoue <i>et al.</i> 2003
3	<i>Sox2</i>	16.93	SRY (sex determining region Y)-box 2	du Puy <i>et al.</i> 2011
	<i>Bmpr1b</i> *	66.11	bone morphogenetic protein receptor, type 1B	Wilson <i>et al.</i> 2001; Mulsant <i>et al.</i> 2001; Souza <i>et al.</i> 2001
9	<i>Pgr</i>	2.46	progesterone receptor	Lydon <i>et al.</i> , 1995
	<i>Dnmt1</i>	7.66	DNA methyltransferase 1	Huan <i>et al.</i> , 2015
	<i>Abhd5</i>	73.03	abhydrolase domain containing 5	Metodiev <i>et al.</i> , 2018
11	<i>Lif</i>	2.94	leukemia inhibitory factor	Spötter <i>et al.</i> 2009
	<i>Igfbp1</i>	4.75	insulin-like growth factor binding protein 1	
	<i>Gdf9</i> *	31.94	growth differentiation factor 9	Hanrahan <i>et al.</i> 2004
	<i>Sco1</i>	40.59	Sco1 cytochrome c oxidase assembly protein	Metodiev <i>et al.</i> 2018
15	<i>Lifr</i>	3.46	LIF receptor alpha	Modrić <i>et al.</i> , 2000
	<i>Sema5a</i>	13.02	semaphoring 5A	Metodiev <i>et al.</i> 2018
	<i>Ago2</i>	33.92	argonaute RISC catalytic subunit 2	Krawczynski <i>et al.</i> 2015
	<i>Hsf1</i>	35.95	heat shock factor 1	Kohata <i>et al.</i> 2013
Close to QTL for birth litter size excluding stillborn pups				
4	<i>Hdac1</i>	63.26	histone deacetylase 1	Zhou <i>et al.</i> , 2014
	<i>Lin28a</i>	66.50	lin-28 homolog A	Zhu <i>et al.</i> 2010
9	<i>Pgr</i>	2.46	progesterone receptor	Lydon <i>et al.</i> , 1995
	<i>Dnmt1</i>	7.66	DNA methyltransferase 1	Huan <i>et al.</i> , 2015
	<i>Abhd5</i>	73.03	abhydrolase domain containing 5	Metodiev <i>et al.</i> , 2018

* Fecundity genes in sheep. Gene positions were in cM. Bolded chromosomes harbored the genome-wide significant QTL for the corresponding trait.

There was, however, no extra evidence in our study to corroborate the potential association between the QTLs and the mentioned candidate genes.

In the second chapter two new types of genotype-pedigree-derived relationship matrices were presented together with rules for their construction. In this study the genotyped individuals came from the founder generation, a situation which is different for livestock breeds established decades ago. In these breeds it is impossible to trace their origins so the relations among their founders or ancient ancestors usually were assumed to be zero. In contrast, the two founder lines of the AIL population were highly inbred and genetically divergent after long term breeding and were expected to have potential relations because they were bred from the same initial gene pool (Langhammer *et al.* 2014). The observed genomic relationship matrix, relative to a hypothetical F2-population with average allele frequencies, was constructed for the eight founders jointly by adapting VanRaden's method (VanRaden 2008). The self-relationships of founders were around 1.4, indicating increased homozygosity, and animals from the two founder lines were negatively related, indicating their divergence. The negative relationships occurred in AIL were mainly due to the existence of line-specific SNPs. When all eight founders are considered around 45% of all informative SNPs were line-specific, i.e. only genotype AA occurred in one and only genotype aa occurred in the other line. A close-to-zero relationship between two lines was obtained if all line-specific SNPs were omitted.

Marker genotypes of founders not only provided the genomic relationships among founders but also determined the allele frequencies of all loci for each founder line. We constructed a modified pedigree-derived relationship matrices $\tilde{\mathbf{A}}_a$ from allele frequencies of autosomal loci and $\tilde{\mathbf{A}}_x$ from allele frequencies of X-linked loci. Interestingly we observed a fluctuation of X-chromosomal self-relationships in the first generations due to the oscillation of allele frequencies of X-chromosomal loci before a genetic equilibrium is reached. This phenomenon cannot be detected by the tabular method for computing the pedigree-derived relationships or by genomic relationship method if the genotyped individuals were not drawn from those early generations after the formation of the population. Normally, if the pedigree is long enough the influence due to the fluctuation of the X-chromosomal self-relationships is negligible for the estimations of the genetic parameters. This influence, however, should be concerned for populations that have short pedigrees. Besides in this chapter we offered an opportunity to include the X-chromosomal effects in the genetic analysis by applying the genotyped-derived X-chromosomal relationships. It is constructive for exploring the genetics of the sex-related traits like litter size.

There is room for our method to be adapted to other situations or research needs. In terms of sampling allele frequencies may be questioned for their representativeness of the original population. Here the eight founders were randomly drawn from two founder lines. It is possible to accidentally sample minor alleles for non-line-specific loci and consequently cause a large gap between the population- and the sampled allele frequencies, although two founder lines were proved to be highly inbred according to pedigrees. This sampling error did not affect the genetic analyses for the traits recorded from the AIL population, as its genetic make-up is fully determined by the eight founders. However it may result in misunderstanding for the genetic causes of increased litter size in FL1 line if we don't have the genotypes of other FL1 individuals as the reference genotype data. Genotyping more FL1 individuals (and more control individuals) is necessary to provide deeper information for the fine-mapping experiment (see more discussion in the perspectives for fine-mapping section) and understanding of line differences. Second, differences between various kinds of SNP arrays as well as between SNP array genotyping and whole genome sequencing should be concerned. Genotyping with SNP arrays cannot detect all informative SNPs for a certain population compared to whole genome sequencing. The probe lists of various kinds of SNP arrays also are different due to variable preferences and objectives of their designers. In order to choose an appropriate genotyping approach more efforts may be helpful, e.g. sequencing several more individuals from each founder line in order to calculate precise founder relationships (see more discussion in the perspectives for fine-mapping section). At the moment our method only fits to the population which was generated as a cross of two founder lines. Several modifications can be implemented to adapt this method to the breeds with more complex pedigree structures and genotyping situations. For instance, it can be adapted to breeds which have more than two founder lines by simply defining the average allele frequencies among all founder lines appropriately and to breeds with genotyped individuals only in later generations by defining the genotyped generation as the "founder generation" on condition of having reliable allele frequencies.

In the third chapter we applied our modified genotype-pedigree-combined relationship matrices \mathbf{H}_a and \mathbf{H}_x , analyzed several traits jointly by multivariate models and evaluated correlations among traits. The analyses in this chapter mainly answered the questions raised by ourselves with the aim of giving advices to the design of the fine-mapping experiment. The suggestions are discussed in detail in perspective for fine-mapping section.

Litter size

It is not easy to understand the genetics underlying litter size due to its complexity in several aspects. Litter size depended on fertilities of parents but the maternal fertility was believed to be predominant in the genetics of litter size (Falconer 1960). Paternal genetics mainly results in the decreased litter size in two ways, failure of the fertilization due to male's infertility (Dix *et al.* 1996), or high embryo mortality due to male's genetic defects (Ouyang *et al.* 2002). An increased litter size purely due to avoidance of male's genetic defects may be a possible yet rare situation. Thus for livestock breeders, who mainly focused on how to increase litter size or improve reproductive traits, the genetics of females was a more attractive starting point.

In females there are many factors related to litter size, e.g., meiosis, ovulation, fertilization, implantation, embryogenesis, nutrition through placenta, age, metabolisms and hormone level of mother, and so on (see general introduction). The difficulty of quantifying these component traits was the first impediment to understand litter size. For example the trait ovulation rate was hard to obtain in some breeds, e.g. the AIL population. In our study the number of corpora lutea was measured as an indirect index of the ovulation rate. However these two traits can show a large difference. For the AIL the pup numbers of the first two litters were recorded, but normally the number of corpora lutea could only be obtained when the animal was slaughtered. In this case the number of corpora lutea cannot reflect the ovulation rates of the first and second litters. The embryo mortality was also hard to measure. For the AIL we counted the numbers of the stillborn pups for two litters. It probably also cannot reflect the embryo mortality even ignoring the difference between these two traits. One difficulty for measuring the number of the stillborn pups was that parturition might happen in the evening and the dam might swallow few stillborn pups after parturition. Then it was difficult to ensure the numbers of the stillborn pups counted in the morning included all stillborn pups. Consequently "apparent" litter size itself was the only trait that can be measured relatively accurately.

Selection for increased litter size was basically successful in laboratory animal models (Holt *et al.* 2005; Langhammer *et al.* 2014) or in livestock breeding (e.g. Meishan pig breed; Haley and Lee 1993), although the genetics of litter size was hard to understand. Too high litter sizes sometimes create difficulties. In a laboratory environment birth litter size is often standardized to 8-12 pups (Eisen 1978; Langhammer *et al.* 2014) in order to get rid of its negative impact on pup growth before weaning. In the livestock industry similar negative impact of a too-high litter size is also to be avoided. Several changes, e.g. adding extra trait in the selecting index,

could improve a breeding strategy that simply focuses on increasing litter size. For example, due to inbreeding depression increasing litter size by selection and inbreeding led to smaller average pup weight or larger intra litter standard deviation. So the variability of pups' weights can be involved in the selection index to avoid such inbreeding depression. Accordingly the breeding goal can be altered to have a breed with moderate litter size but with the pups that have similar and heavy birth weights. Our study and others (Alfonso *et al.* 1997; Fernández *et al.* 2008) indicated that litter traits of different parities exhibit a moderate genetic correlations and thus should be considered as independent traits and analyzed by multivariate models. This experience could be referenced according to the specific breeding purposes of the breeds. For instance, in the modern pork industry the great grandparent line (GGP line) and the grandparent line (GP line) normally needed to generate offspring litters many times, in order to constantly provide founders for the market hog populations. They should present high and stable performance on key traits like litter size. For these kinds of lines the average performance of litter traits in multiple parities is more important than litter traits in the first parity only. Correspondingly their selection index should consider traits from multiple parities.

Perspectives for fine-mapping

The aim of constructing the AIL population was to identify candidate genes and their causal mutations for increased litter size by fine-mapping. Prior to a fine-mapping study, we aimed at an in-depth understanding of the genetics of litter traits in the AIL population through a series of studies, e.g. coarse QTL-mapping and genetic analyses for all traits. The suggestions to the fine-mapping study can be summarized in three aspects, the suggestions for genotyping, phenotyping and data-analyzing.

In the previous studies two kinds of marker were applied for genotyping, the microsatellite markers and the SNP markers. They have merits and demerits. Microsatellite marker scan have more than two alleles because its polymorphism depends on the polymorphic repeats of a short DNA sequence. This feature can be utilized to trace the alleles inherited from mother or father. However they are rare on the whole genome and consequently can only compose a relatively coarse genetic map. In contrast SNPs are distributed evenly and densely on the whole genome. For example in our study we genotyped the founders of the AIL with the Mouse Diversity Genotyping Array (Yang *et al.* 2009) and identified 142,541 SNPs on the whole genome, which in average was around 7,000 SNPs per chromosome, around 51 SNPs per Mbp (see supplement materials of chapter two). If cutting the whole genome into 50,000 bp long segments (which were roughly 0.025 to 0.05 cM long in average in the genetic map),

less than 10% of them didn't harbor a SNP in our case. This density ensures all genes can have at least one linked marker nearby. However no commercialized SNP array can detect all SNPs of any populations. So whole genome sequencing can be an optimal alternative. In our case genotyping more individuals of two founder lines, especially by whole genome sequencing, is necessary in order to correct the allele frequencies of two founder lines and avoid missing any SNPs. It should be noted that genotyping by SNP array or whole genome sequencing is, to some extent, wasteful if we only need genotype data for QTL mapping. In all SNP loci included in the SNP array we applied only around 23% of them were informative. Sequencing the whole genome can provide a complete SNP list but also meaningless sequences, especially when genotyping a large amount of the AIL individuals. In order to efficiently utilize available funds designing our own customized SNP array might be alternative. The complete SNP list can be derived by sequencing more individuals of two founder lines (say, 10 from each line) and then applied to customize the SNP probes of the SNP array. The genotype data derived from this kind of the customized SNP array can also be applied to analyze other genetic features without any complexities, e.g., the runs of homozygosity.

In the subsequent fine-mapping study litter size and litter weight are the core. Other traits can also be measured and included in the multivariate analyses in order to explain the cause of high litter size, e.g. ovulation rate. Unlike litter size and litter weight, most of their component traits cannot be measured directly, e.g., ovulation rate. For ovulation rate the litter average could be one option on condition that the variability of the ovulation rate in the same litter is small. If this condition is true, the female siblings in the same litter can be divided into two groups before their first estrus, one for mating and generating litter traits, and another for measuring the average ovulation rate after their first estrus. However this precondition should be checked by a pretest, for instance, generating multiple litters and counting the ovum number for each pup of each litter after their first estrus (or other estruses if necessary). Predictably this variability exists. Its impact on the estimation of genetic parameters should be evaluated. In the third chapter correlations among traits were estimated. It is notable that the males' and females' body masses have a high genetic correlation and both have high genetic correlations with litter weight but significantly lower genetic correlations with litter size. However during phenotyping litter traits were only considered as the traits of dam. In order to evaluate the correlations between litter weight and parents' body mass litter weight is also suggested to be recorded as the trait of sire.

When detecting the QTL effects with dense genetic map the choice of the response variable is important. EBVs and de-regressed EBVs were frequently applied as the response variable in genomic prediction (Ostersen *et al.* 2011) and also in the fine-mapping (Duijvesteijn *et al.* 2014; Höglund *et al.* 2014). The reason for applying de-regressed EBVs was due to the shrinkage property of the BLUP method (Henderson 1975). The shrinkage caused by BLUP resulted in the underestimated BV for the offspring (Garrick *et al.* 2009). Hence de-regression for EBVs was suggested with extra treatments, like removing the parent average and computing the weighting values for each EBV (Garrick *et al.* 2009; Ostersen *et al.* 2011). In the third chapter EBVs of body mass at day 42 were derived from a multivariate analysis were de-regressed with removing the parent average according to Garrick's method (Garrick *et al.* 2009). Negative reliabilities of the de-regressed EBVs occurred for several individuals, too large to be explained by numerical inaccuracies. A possible reason to explain this error was the discrepancy of the assumptions of sire and dam. In Garrick's method the sire and dam are assumed to be unrelated and not inbred. So the additive genetic covariance matrix for parent average and offspring was summarized to $\mathbf{G} = \begin{bmatrix} 0.5 & 0.5 \\ 0.5 & 1 \end{bmatrix} \sigma_g^2$. However in our method the initial assumption for the relations among founders was they were negatively correlated and highly inbred. This problem remains to be solved if de-regressed EBVs are to be applied in fine-mapping.

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SUMMARY

Two high fecundity mouse lines, FL1 and FL2, were established after a long-term selection for increased litter size. In order to identify potential candidate genes for litter size an advanced intercross mouse population (AIL) was established as a crossbred between FL1 and a particular control line.

A coarse QTL-mapping analysis was conducted in the F₂-generation of this population. In total 47 genome-wide significant (5%) QTL were identified for 31 different traits. QTL for litter size, litter weight and body mass traits were mainly identified on chromosomes 2, 4, 9 and 13, on chromosomes 2, 4, 7 and 8 and on chromosomes 2, 4 and 13 respectively. This study verified the existence of the potential candidate genes with larger effect for litter size and emphasized several chromosomes to which more attentions should be paid in subsequent fine-mapping.

A new method was developed to construct a modified relationship matrix for AIL-crossbreds that makes use of founder allele frequencies for markers and the pedigree. Thereby founder relationships were deduced from about 140k biallelic marker loci. This relationship matrix was derived both for autosomal and X-chromosomal loci and may be useful in the context of analyses of selection experiments. Real application was demonstrated with data from 30 generations of the AIL population, comprising about 19000 pedigreed animals.

By applying the newly relationship matrices litter size-related traits and body mass related traits from the AIL were more deeply analyzed. Thereby X-chromosomal genetic variation for body mass traits was not found to be significant, in agreement with no significant X-chromosomal QTL in the coarse mapping study. Genetic correlation between the size of the first and the second litter was found to be only 0.52, in contrast to the respective correlation of 0.84 for litter weight. Genetic correlations between body weight traits and litter size were in the range between 0.08 and 0.14, which is not a strong indicator for the existence of pleiotropic QTLs. The picture is somewhat different for litter weight with respective correlations from 0.45 to 0.48.

Both the results from coarse mapping and quantitative analyses suggest that it is worthwhile to further fine-map the already coarsely mapped QTL, especially for litter size and litter weight.

ZUSAMMENFASSUNG

Zwei Mauslinien mit hoher Fruchtbarkeit, FL1 und FL2, wurden mittels Langzeits Selektion auf eine größere Wurfgröße etabliert. Um potenzielle Kandidatengene für die Wurfgröße zu identifizieren, wurde eine fortgeschrittene Intercross-Linie (AIL) als Kreuzung zwischen FL1 und einer eigenen Kontrolllinie erstellt.

Eine grobe QTL-Kartierung wurde in der F₂-Generation dieser Population durchgeführt. Insgesamt wurden 47 genomweit signifikante (Signifikanzniveau 5%) QTL für 31 verschiedene Merkmale identifiziert. QTL für Wurfgröße, Wurfgewicht und Körpermassenmerkmale wurden hauptsächlich auf den Chromosomen 2, 4, 9 und 13, auf den Chromosomen 2, 4, 7 und 8 sowie auf den Chromosomen 2, 4 und 13 identifiziert. Diese Studie bestätigte die Existenz potenzieller Kandidatengene mit größerem Effekt auf die Wurfgröße. Den entsprechenden Chromosomen sollte bei einer anschließenden Feinkartierung mehr Aufmerksamkeit geschenkt werden.

Eine neue Methode wurde entwickelt, um eine modifizierte Verwandtschaftsmatrix für AIL-Kreuzungen aufzustellen, die die Markerallelfrequenzen der Gründertiere und das Pedigree aller Nachkommen berücksichtigt. Die Verwandtschaft zwischen Gründertieren wurde aus ca. 140k biallelen Marker-Loci abgeleitet. Diese Verwandtschaftsmatrix wurde sowohl für autosomale als auch für X-chromosomale Loci aufgestellt und ist allgemein für die Auswertung von Selektionsexperimenten von Nutzen. Als Anwendungsbeispiel wurden Daten von 30 Generationen der AIL-Population ausgewertet, wobei rund 19000 Tiere im Pedigree enthalten waren.

Merkmale der Wurfgröße und der Körpermasse in der AIL wurden anschließend eingehender analysiert, wobei die neuartigen Verwandtschaftsmatrizen eingesetzt wurden. Im Ergebnis war X-chromosomale genetische Variation für Merkmale der Körpermasse nicht signifikant, in Übereinstimmung mit fehlenden X-chromosomalen QTL in der Grobkartierungsstudie. Die genetische Korrelation zwischen der Größe des ersten und des zweiten Wurfes war niedrig bei 0,52, im Gegensatz zu derentsprechenden Korrelation von 0,84 für das Wurfgewicht. Genetische Korrelationen zwischen Körpergewichtsmerkmalen und Wurfgröße lagen im Bereich zwischen 0,08 und 0,14, was kein starker Indikator für die Existenz pleiotroper QTLs ist. Etwas anders sieht es beim Wurfgewicht mit entsprechenden Korrelationen von 0,45 bis 0,48 aus.

Sowohl die Ergebnisse der Grobkartierung als auch die quantitativen Analysen deuten darauf hin, dass es sich lohnt, die Positionen der bereits grob kartierten QTL weiter zu verfeinern, insbesondere für Wurfgröße und Wurfgewicht.

APPENDIX

ASReml command files for the univariate analyses

1 the univariate analysis for BM42 with the relationship matrix H_a

```
Analysis of mice data BM42 H matrix autosomes

# data field definition
generation      !I          # generation
sex             !I          # sex
autosome        !P          # autosomal effect
cle             !I 3133     # common litter environmental
effect
BM42            !M-99       # body mass at day 42
BMM_M           !M-99       # body mass of dam at mating day
pe             !M-99       # permanent environmental effect

# pedigree file and qualifiers
pedigree.txt    !ALPHA      !SKIP 1

# inverse of the relationship matrix
H_inverse.giv   # modified H matrix of autosomes

# data file and qualifiers
BM42.txt        !MAXIT 30    !AISING      !MVREMOVE    !FCON

# linear mixed model
BM42 ~ generation sex BMM_M !r autosome ide(cle) ide(pe)

# R and G structures
1 1 1

0 0 I

# assign the input relationship matrix to genetic variance
autosome 1
0 0 GIV1 10. !GP

# calculations based on the variances
VPREDICT !DEFINE
# F varcle          # 1 cle variance
# F varpe           # 2 pe variance
# F varre           # 3 residual variance
# F vara            # 4 autosomal genetic variance
F phenvar 1 + 2 + 3 + 4 # 5 phenotypic variance
F cvara 4*1.4284 # 6 genetic variance corrected by
Dk
F cphenvar 1 + 2 + 3 + 6 # 7 corrected phenotypic variance
F crevar 6 + 2 # 8 variance for repeatability
H herit 4 5 # heritability
H cherit 6 7 # corrected heritability
```

H repeat 8 7 # corrected repeatability

2 the univariate analysis for BM42 with the relationship matrices H_a and H_x

Analysis of mice data BM42 H matrix autosomes + X

```

generation      !I      # generation
sex             !I      # sex
autosome        !P      # autosomal effect
X_chromosome    !P      # X_chromosomal effect
cle             !I 3133  # common litter environmental
effect
  BM42          !M-99   # body mass at day 42
  BMM_M        !M-99   # body mass of dam at mating day
  pe           !M-99   # permanent environmental effect

pedigree.txt    !ALPHA    !SKIP 1
H_inverse.giv   # inverse of autosomal matrix
H_inverse_X.giv # inverse of X chromosome matrix
BM42.txt        !MAXIT 30  !AISING    !MVREMOVE    !FCON

```

BM42 ~ generation sex BMM_M !r autosome X_chromosome ide(cle) ide(pe)

1 1 2

0 0 I

```

autosome 1
0 0 GIV1 10. !GP
X_chromosome 1
0 0 GIV2 10. !GP

```

```

VPREDICT !DEFINE
# F varcle      # 1 cle variance
# F varpe      # 2 pe variance
# F varre      # 3 residual variance
# F vara       # 4 autosomal genetic variance
# F varx       # 5 X chromosomal genetic variance
F phenvar 1 + 2 + 3 + 4 + 5 # 6 phenotypic variance
F genvar 4 + 5 # 7 genetic variances
F cvara 4*1.4284 # 8 corrected autosomal variance
F cvarx 5*0.8186 # 9 corrected X chromosomal
variance
F cphenvar 1 + 2 + 3 + 8 + 9 # 10 corrected phenotypic variance
F cgenvar 8 + 9 # 11 corrected genetic variance
F crevar 11 + 2 # 12 variance for repeatability
H herit 7 6 heritability
H herit 11 10 corrected heritability
H repeat 12 10 corrected repeatability

```

3 the univariate analysis for LS0 with the relationship matrix H_a

Analysis of mice data LS0 H matrix autosomes

```

generation      !I      #
autosome        !P      # autosomal effect
cle             !I 2404  # common litter environmental
effect
a_lp           !M-99   # age at first parturition
sq_a_lp        !M-99   # quadratic a_lp
LS0            !M-99   # litter size
SBP            !M-99   # stillborn number
LW0           !M-99   # litter weight
pe            !M-99   # permanent environmental effect
litter        !I 2     # either the first litter or second

pedigree.txt    !ALPHA    !SKIP 1
H_inverse.giv  # inverse of autosomal matrix
litter_traits.txt !MAXIT 20 !AISING !MVREMOVE !FCON

LS0 ~ generation litter litter.a_lp litter.sq_a_lp !r autosome
ide(cle) ide(pe)

1 1 1

0 0 I

tierid_T_founder 1
0 0 GIV1 10. !GP

VPREDICT !DEFINE
# F varcle      # 1 cle variance
# F varpe      # 2 pe variance
# F varre      # 3 residual variance
# F vara      # 4 autosomal genetic variance
F phenvar 1 + 2 + 3 + 4 # 5 phenotypic variance
F cvara 4*1.4284 # 6 genetic variance corrected by
Dk
F cphenvar 1 + 2 + 3 + 6 # 7 corrected phenotypic variance
F crevar 6 + 2 # 8 variance for repeatability
H herit 4 5 # heritability
H cherit 6 7 # corrected heritability
H repeat 8 7 # corrected repeatability

```


4 the univariate analysis for LS0 with the relationship matrices H_a and H_x

Analysis of mice data LS0 H matrix autosomes

```

generation      !I      #
autosome        !P      # autosomal effect
X_chromosome    !P      # X_chromosomal effect
cle             !I 2404  # common litter environmental
effect
a_lp            !M-99   # age at first parturition
sq_a_lp        !M-99   # quadratic a_lp
LS0            !M-99   # litter size
SBP            !M-99   # stillborn number
LW0           !M-99   # litter weight
pe             !M-99   # permanent environmental effect
litter         !I 2    # either the first litter or second

pedigree.txt    !ALPHA    !SKIP 1
H_inverse.giv   # inverse of autosomal matrix
H_inverse_X.giv # inverse of X chromosome matrix
litter_traits.txt !MAXIT 20 !AISING !MVREMOVE !FCON

LS0 ~ generation litter litter.a_lp litter.sq_a_lp !r autosome
X_chromosome ide(cle) ide(pe)

1 1 2

0 0 I

autosome 1
0 0 GIV1 10. !GP
X_chromosome 1
0 0 GIV2 10. !GP

VPREDICT !DEFINE
# F varcle # 1 cle variance
# F varpe # 2 pe variance
# F varre # 3 residual variance
# F vara # 4 autosomal genetic variance
# F varx # 5 X chromosomal genetic variance
F phenvar 1 + 2 + 3 + 4 + 5 # 6 phenotypic variance
F genvar 4 + 5 # 7 genetic variances
F cvara 4*1.4284 # 8 corrected autosomal variance
F cvarx 5*0.8186 # 9 corrected X chromosomal
variance
F cphenvar 1 + 2 + 3 + 8 + 9 # 10 corrected phenotypic variance
F cgenvar 8 + 9 # 11 corrected genetic variance
F crevar 11 + 2 # 12 variance for repeatability
H herit 7 6 heritability
H herit 11 10 corrected heritability
H repeat 12 10 corrected repeatability

```

ASReml command files for multivariate analyses

1 the bivariate analysis for BM42_M and BM42_F with the relationship matrix H_a

Analysis of mice data BM42 H matrix autosomes

```

generation      !I          # generation
sex              !I          # sex
autosomes        !P          # autosomal effect
Xchromosome     !P          # X chromosomal effect
cle              !I 4362     # common litter environmental
effect
  BMM_M          !M-99      # body mass of dam at mating day
  BM42           !M-99      # body mass at day 42
  BM42M          !M-99      # body mass at day 42 for males, -
99 value for females
  BM42F          !M-99      # body mass at day 42 for females,
-99 value for males

pedigree.txt     !SKIP 1      !ALPHA
H_inverse.giv
BM42.txt         !SKIP 1      !MAXIT
2000  !AISING    !MVREMOVE   !EXTRA 50    !FCON      !CONTINUE

```

```

BM42M BM42F ~ Trait Trait.generation !r Trait.autosomes
Trait.ide(cle)

```

```

1 2 2

```

```

0
2 0 US !GPFP
9.
0. 5.

```

```

Trait.autosomes 2
2 0 US !GPUP
5.
5. 5.
autosomes 0 GIV1

```

```

Trait.ide(cle) 2
2 0 US !GPUP
5.
5. 5.
ide(cle)

```

```

VPREDICT !DEFINE
# F res11          # 1 residual variance of 1st trait
# F res22          # 2 residual variance of 2nd trait
# F aut011         # 3 autosomal variance of 1st trait
# F aut012         # 4 covariance of autosomal
variance
# F aut022         # 5 autosomal variance of 2nd trait
# F cle11          # 6 cle variance of 1st trait
# F cle12          # 7 covariance of cle variance

```

```

# F cle22                # 8  cle variance of 2nd trait
F cauto11                3*1.4284      # 9  corrected auto11
F cauto12                4*1.4284      # 10 corrected auto12
F cauto22                5*1.4284      # 11 corrected auto22
F pheno11                1 + 3 + 6     # 12 phenotypic variance of 1st
trait
F pheno22                2 + 5 + 8     # 13 phenotypic variance of 2nd
trait
F cpheno11              1 + 9 + 6     # 14 corrected pheno11
F cpheno22              2 + 11 + 8    # 15 corrected pheno22
F var12                 4 + 7         # 16
F cvar12                10 + 7        # 17
H herit11               3 12         heritability of 1st trait
H herit22               5 13         heritability of 2nd trait
H cherit11              9 14         corrected herit11
H cherit22             11 15        corrected herit22
R rgeno                 3 4 5         genetic correlation
R rcgeno                9 10 11      corrected rgeno
R rpheno                12 16 13     phenotypic correlation
R rcpheno               14 17 15     corrected rpheno

```

2 the bivariate analysis for BM42 with the relationship matrix H_a and H_x

Analysis of mice data BM42 H matrix autosomes

```

generation              !I           # generation
sex                     !I           # sex
autosomes                !P           # autosomal effect
Xchromosome             !P           # X chromosomal effect
cle                      !I 4362      # common litter environmental
effect
BMM_M                   !M-99       # body mass of dam at mating day
BM42                    !M-99       # body mass at day 42
BM42M                   !M-99       # body mass at day 42 for males, -
99 value for females
BM42F                   !M-99       # body mass at day 42 for females,
-99 value for males

pedigree.txt            !SKIP 1     !ALPHA
H_inverse.giv
BM42.txt                !SKIP 1     !MAXIT
2000  !AISING           !MVREMOVE   !EXTRA 50   !FCON       !CONTINUE

```

```

BM42M BM42F ~ Trait Trait.generation !r Trait.autosomes
Trait.Xchromosome Trait.ide(cle)

```

```
1 2 3
```

```
0
```

```
2 0 US !GFPF
```

```
3.35374
```

```
0. 1.86075
```

```
Trait.autosomes 2
```

```

2 0 US !GPUP
3.13698
2.94105 2.86696
autosomes 0 GIV1

```

```

Trait.Xchromosome 2
2 0 US !GPUP
0.1
0.1 0.1
Xchromosome 0 GIV2

```

```

Trait.ide(cle) 2
2 0 US !GPUP
3.09454
1.72482 1.58986
ide(cle)

```

```
VPREDICT !DEFINE
```

```

# F res11 # 1 residual variance of 1st trait
# F res22 # 2 residual variance of 2nd trait
# F autol1 # 3 autosomal variance of 1st trait
# F autol2 # 4 covariance of autosomal
variance
# F auto22 # 5 autosomal variance of 2nd trait
# F Xchr11 # 6 X chr variance of 1st trait
# F Xchr12 # 7 covariance of X chr variance
# F Xchr22 # 8 X chr variance of 2nd trait
# F cle11 # 9 cle variance of 1st trait
# F cle12 # 10 covariance of cle variance
# F cle22 # 11 cle variance of 2nd trait
F cauto11 3*1.4284 # 12 corrected autol1
F cauto12 4*1.4284 # 13 corrected autol2
F cauto22 5*1.4284 # 14 corrected auto22
F cXchr11 6*0.8186 # 15 corrected Xchr11
F cXchr12 7*0.8186 # 16 corrected Xchr12
F cXchr22 8*0.8186 # 17 corrected Xchr22
F geno11 3 + 6 # 18 genetic variances of 1st trait
F geno12 4 + 7 # 19 covariance of genetic
variances
F geno22 5 + 8 # 20 genetic variances of 2nd trait
F cgeno11 12 + 15 # 21 corrected geno11
F cgeno12 13 + 16 # 22 corrected geno12
F cgeno22 14 + 17 # 23 corrected geno22
F phenol1 1 + 3 + 6 + 9 # 24 phenotypic variance 1st trait
F phenol2 4 + 7 + 10 # 25 covariance of phenotypic
variance
F pheno22 2 + 5 + 8 + 11 # 26 phenotypic variance 2nd trait
F cphenol1 1 + 12 + 15 + 9 # 27 corrected phenol1
F cphenol2 13 + 16 + 10 # 28 corrected phenol2
F cpheno22 2 + 14 + 17 + 11 # 29 corrected pheno22
H herit11 18 24 heritability of 1st trait
H herit22 20 26 heritability of 2nd trait
H cherit11 21 27 corrected herit11
H cherit22 23 29 corrected herit22
R rgeno 18 19 20 genetic correlation
R rcgeno 21 22 23 corrected rgeno

```

```
R rpheno      24 25 26      phenotypic correlation
R rcpheno     27 28 29      corrected rpheno
```

3 the multivariate analysis for BM21, 42 and 63 with the relationship matrix H_a

Analysis of mice data BM21_42_63 H : autosomes

```
generation      !I      # generation
Sex             !I      # sex
autosomes       !P      # autosomal effect
Xchromosome     !P      # X chromosomal effect
cle             !I 4362  # common litter environmental
effect
BMM_M           !M-99  # body mass of dam at mating day
BM21            !M-99  # body mass at day 21
BM42            !M-99  # body mass at day 42
BM63            !M-99  # body mass at day 63
BMM             !M-99  # body mass at mating

pedigree.txt    !SKIP 1      !ALPHA
H_inverse.giv
BM21_42_63.txt  !SKIP 1      !MAXIT 2000
!AISING        !MVREMOVE   !EXTRA 50     !FCON        !CONTINUE
```

```
BM21 BM42 BM63 ~ Trait Trait.generation Trait.sex !r Trait.autosomes
Trait.ide(cle)
```

```
1 2 2
0
3 0 US !GPUPUUP
1.
0.1 1.
0.1 0.1 1.
```

```
Trait.autosomes 2
3 0 US !GPUPUUP
1.
1. 1.
1. 1. 1.
autosomes 0 GIV1
```

```
Trait.ide(cle) 2
3 0 US !GPUPUUP
2.
1. 2.
1. 1. 2.
ide(cle)
```

```
VPREDICT !DEFINE
# F res11      # 1 residual variance of 1st trait
# F res21      # 2
# F res22      # 3 residual variance of 2nd trait
# F res31      # 4
# F res32      # 5
```

# F res33		# 6	residual variance of 3 rd trait
# F auto11		# 7	autosomal variance of 1 st trait
# F auto21		# 8	
# F auto22		# 9	autosomal variance of 2 nd trait
# F auto31		# 10	
# F auto32		# 11	
# F auto33		# 12	autosomal variance of 3 rd trait
# F cle11		# 13	cle variance of 1 st trait
# F cle21		# 14	
# F cle22		# 15	cle variance of 2 nd trait
# F cle31		# 16	
# F cle32		# 17	
# F cle33		# 18	cle variance of 3 rd trait
F cauto11	7*1.4284	# 19	corrected auto11
F cauto21	8*1.4284	# 20	
F cauto22	9*1.4284	# 21	corrected auto22
F cauto31	10*1.4284	# 22	
F cauto32	11*1.4284	# 23	
F cauto33	12*1.4284	# 24	corrected auto33
F pheno11	1 + 7 + 13	# 25	phenotypic variance of 1 st trait
F pheno21	2 + 8 + 14	# 26	
F pheno22	3 + 9 + 15	# 27	phenotypic variance of 2 nd trait
F pheno31	4 + 10 + 16	# 28	
F pheno32	5 + 11 + 17	# 29	
F pheno33	6 + 12 + 18	# 30	phenotypic variance of 3 rd trait
F cpheno11	1 + 19 + 13	# 31	corrected pheno11
F cpheno21	2 + 20 + 14	# 32	
F cpheno22	3 + 21 + 15	# 33	corrected pheno22
F cpheno31	4 + 22 + 16	# 34	
F cpheno32	5 + 23 + 17	# 35	
F cpheno33	6 + 24 + 18	# 36	corrected pheno33
H herit11	7 25		heritability of 1 st trait
H herit22	9 27		heritability of 2 nd trait
H herit33	12 30		heritability of 3 rd trait
H cherit11	19 31		corrected herit11
H cherit12	21 33		corrected herit12
H cherit22	24 36		corrected herit22
R rgeno12	7 8 9		genetic correlation 12
R rgeno13	7 10 12		genetic correlation 13
R rgeno23	9 11 12		genetic correlation 23
R rcgeno12	19 20 21		corrected rgeno12
R rcgeno13	19 22 24		corrected rgeno13
R rcgeno23	21 23 24		corrected rgeno23
R rpheno12	25 26 27		phenotypic correlation 12
R rpheno13	25 28 30		phenotypic correlation 13
R rpheno23	27 29 30		phenotypic correlation 23
R rcpheno12	31 32 33		corrected rpheno12
R rcpheno13	31 34 36		corrected rpheno13
R rcpheno23	33 35 36		corrected rpheno23

4 the bivariate analysis for LS0_1 and LS0_2 with the relationship matrix H_a

Analysis of mice data LS0_1_2 H : autosomes

```

generation      !I          # generation
autosomes       !P          # autosomal effect
Xchromosome     !P          # X chromosomal effect
cle             !I 4362     # common litter environmental
effect
  BMM           !M-99     # body mass at mating day
  a_m           !M-99     # age at mating for the first mate
  a_p_1         !M-99     # age at the first parturition
  LS0_1         !M-99     # litter size of the first litter
  LW0_1         !M-99     # litter weight of the first litter
  a_p_2         !M-99     # age at the second parturition
  LS0_2         !M-99     # litter size of the second litter
  LW0_2         !M-99     # litter weight of the second
litter

pedigree.txt    !SKIP 1      !ALPHA
H_inverse.giv
LitterTrait_1_2.txt !SKIP 1      !MAXIT 2000
!AISING        !MVREMOVE    !EXTRA 50      !FCON        !CONTINUE

```

```

LS0_1 LS0_2 ~ Trait Trait.generation at(Trait,1).ide(a_p_1)
at(Trait,2).ide(a_p_2) !r Trait.autosomes Trait.ide(cle)

```

```

1 2 2
0
2 0 US !GPUP
5.88987
0.1 8.62786

```

```

Trait.autosomes 2
2 0 US !GPUP
3.94252
4.07117 6.52940
autosomes 0 GIV1

```

```

Trait.ide(cle) 2
2 0 US !GPUP
0.387425
0.375919 0.397381
ide(cle)

```

```

VPREDICT !DEFINE
# F res11          # 1 residual variance of 1st trait
# F res22          # 2 residual variance of 2nd trait
# F aut011         # 3 autosomal variance of 1st trait
# F aut012         # 4 covariance of autosomal
variance
# F aut022         # 5 autosomal variance of 2nd trait
# F cle11          # 6 cle variance of 1st trait

```

```

# F cle12          # 7  covariance of cle variance
# F cle22          # 8  cle variance of 2nd trait
F cauto11         3*1.4284    # 9  corrected autol1
F cauto12         4*1.4284    # 10 corrected autol2
F cauto22         5*1.4284    # 11 corrected auto22
F phenol1         1 + 3 + 6    # 12 phenotypic variance of 1st
trait
F pheno22         2 + 5 + 8    # 13 phenotypic variance of 2nd
trait
F cphenol1        1 + 9 + 6    # 14 corrected phenol1
F cpheno22        2 + 11 + 8   # 15 corrected pheno22
F var12           4 + 7        # 16
F cvar12          10 + 7       # 17
H herit11         3 12        heritability of 1st trait
H herit22         5 13        heritability of 2nd trait
H cherit11        9 14        corrected herit11
H cherit22        11 15       corrected herit22
R rgeno           3 4 5       genetic correlation
R rcgeno          9 10 11     corrected rgeno
R rpheno          12 16 13    phenotypic correlation
R rcpheno         14 17 15    corrected rpheno

```

5 the bivariate analysis for LS0_1 and BM42F with the relationship matrix H_a

Analysis of mice data LS0_1 BM42F H : autosomes

```

generation        !I          # generation
sex               !I          # sex
autosomes         !P          # autosomal effect
Xchromosome       !P          # X chromosomal effect
cle               !I 4362     # common litter environmental
effect
  BMM_M           !M-99     # body mass of dam at mating day
  BM42            !M-99     # body weight at day 42
  BM42M          !M-99     # body weight at day 63 for males,
-99 value for females
  BM42F          !M-99     # body weight at day 63 for females,
-99 value for males
  LS0_1          !M-99     # birth litter size of the first
litter
  LW0_1         !M-99     # birth litter weight of the first
litter

pedigree.txt      !SKIP 1      !ALPHA
H_inverse.giv
BM42_LT.txt       !SKIP 1      !MAXIT 2000
!AISING          !MVREMOVE    !EXTRA 50    !FCON       !CONTINUE

LS0_1 BM42F ~ Trait Trait.generation Trait.BMM_M !r Trait.autosomes
Trait.ide(cle)

```

1 2 2


```

0
2 0 US !GPUP
2.
0.1 2.

Trait.autosomes 2
2 0 US !GPUP
4.
3. 3.
autosomes 0 GIV1

Trait.ide(cle) 2
2 0 US !GPUP
1.
1. 1.
ide(cle)

VPREDICT !DEFINE
# F res11 # 1 residual variance of 1st trait
# F res22 # 2 residual variance of 2nd trait
# F autol1 # 3 autosomal variance of 1st trait
# F autol2 # 4 covariance of autosomal
variance
# F auto22 # 5 autosomal variance of 2nd trait
# F cle11 # 6 cle variance of 1st trait
# F cle12 # 7 covariance of cle variance
# F cle22 # 8 cle variance of 2nd trait
F cauto11 3*1.4284 # 9 corrected autol1
F cauto12 4*1.4284 # 10 corrected autol2
F cauto22 5*1.4284 # 11 corrected auto22
F phenol1 1 + 3 + 6 # 12 phenotypic variance of 1st
trait
F pheno22 2 + 5 + 8 # 13 phenotypic variance of 2nd
trait
F cphenol1 1 + 9 + 6 # 14 corrected phenol1
F cpheno22 2 + 11 + 8 # 15 corrected pheno22
F var12 4 + 7 # 16
F cvar12 10 + 7 # 17
H herit11 3 12 heritability of 1st trait
H herit22 5 13 heritability of 2nd trait
H cherit11 9 14 corrected herit11
H cherit22 11 15 corrected herit22
R rgeno 3 4 5 genetic correlation
R rcgeno 9 10 11 corrected rgeno
R rpheno 12 16 13 phenotypic correlation
R rcpheno 14 17 15 corrected rpheno

```

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M.Sc. Jilun MENG

Hiermit erkläre ich an Eides statt, dass ich die vorgelegte Dissertation mit dem Titel „INVESTIGATIONS ON GENETIC VARIABILITY IN A MOUSE ADVANCED INTERCROSS“ selbständig und ohne unerlaubte Hilfe angefertigt habe und dass ich die Arbeit noch keinem anderen Fachbereich bzw. noch keiner anderen Fakultät vorgelegt habe.

Dummerstorf, 26.11.2018

M.Sc. Jilun MENG

Hiermit erkläre ich, dass die Dissertation nach den Regeln guter wissenschaftlicher Praxis (Standard wissenschaftlichen Arbeitens nach den Empfehlungen der DFG) abgefasst wurde.

Dummerstorf, 26.11.2018

M.Sc. Jilun MENG

Hiermit erkläre ich, dass gegen mich kein strafrechtliches Ermittlungsverfahren schwebt.

Dummerstorf, 26.11.2018