ABSTRACT

FUNK-KEENAN, JHONDRA. Genetic and Cellular Effects In Mice Selected For Age-Specific Growth. (Under the direction of William R. Atchley)

The purpose of this research is to better characterize the origin and evolution of mammalian development. This topic is addressed by examining maternal effects, temporal genetic effects, and hepatocyte endopolyploidy and their impact on mouse growth. This dissertation starts with a discussion of several well-characterized maternal effects and each effect's influence on offspring's growth, gene expression, reproduction, behavior, and disease incidence, among other traits. The role of maternal effects in the evolution of quantitative traits is discussed, as well as the interaction between maternal effects and genomic imprinting in mammals.

Growth during ontogeny is characterized by different cellular mechanisms and may be influenced by different sets of genes acting at different ages. To further investigate the differential genetic control of growth during ontogeny, quantitative trait loci (QTL) analysis was performed to search for chromosomal regions influencing growth in two F_2 populations produced from four mouse strains. These four strains are derived from an age-specific restricted index selection project, which has lead to differences in rate of development in body weight, as well as differences in cell number and cell size. Chromosomal regions influencing growth during ontogeny do not overlap between the two populations, suggesting age-specific growth is influenced by different sets of loci, as hypothesized. Epistatic interactions partially overlap between populations, suggesting growth throughout ontogeny shares some aspect of genetic architecture.

In some species, evolution in quantitative traits is associated with variation in endopolyploidy, or the generation of polyploid cells by DNA replication without subsequent

cell division. Given that variation in endopolyploidy affects phenotypic variation, genetic selection for a quantitative trait could alter onset and extent of mammalian endopolyploidy. Flow cytometry is used to characterize hepatic cellular changes as a correlated response to selection for age-specific growth, using the same four mouse strains described above. Polyploid cell frequency within each line increased as ontogeny progressed, as expected from previous research. Selection for divergence in early growth only temporarily changes liver endopolyploidy. However, selection for hypertrophic growth has lead to significant changes in polyploidy frequency, starting at weaning and continuing into adulthood.

GENETIC AND CELLULAR EFFECTS IN MICE SELECTED FOR AGE-SPECIFIC GROWTH

by

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BIOGRAPHY

I was born in Waynesboro, Pennsylvania on July 20, 1978. The things I remember the most about my childhood were taking things apart to put them back together again, counting things, and performing chemistry experiments in my neighbor's driveway (so I wouldn't blow up my own house). I was sure I wanted to be a chemist until my freshman year in college. My career plans changed during my first semester at Pennsylvania State University. I loved my introduction to biology class and decided to study animal biology. After receiving my B.S. in Animal Bioscience, I started work on an M.S. in Animal Science at Penn State. My research focused on the genetics of growth in the chicken, which honed my interest in studying the changes in growth during ontogeny. The following dissertation discusses the work I have been doing for the past 6 years to further understand temporal variation in mammalian growth.

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I would like to thank the members of my committee for their patience, encouragement, and assistance during my time at NC State. I would particularly like to thank my advisor, Bill Atchley, for allowing me to develop as a scientist and make my own mistakes as well as for his mentoring and patience in improving my writing skills. I would like to thank the other members of my committee, Trudy Mackay, Gene Eisen, and Zhao-Bang Zeng, for assistance with analyses and writing, as well as teaching classes that taught me much about the fundamentals of quantitative genetics, both past and future. I would like to thank Stephanie Curtis for her guidance and assistance during my PhD.

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Personally, I would like to thank my mother, Nancy Funk, for encouraging me to start this PhD (Nay, madam, I know not “encourage”). Finally, I want to thank my husband, Scot Barnett, for holding my hand when I got scared, hugging me when I cried, and getting me a margarita when I needed a drink.

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INTRODUCTION

Mammalian growth is a developmental complex trait, comprised of components with different ontogenetic origins and different heritable and non-heritable controlling factors (Atchley, 1987). How components develop individually and integrate to form a quantitative trait is essential to understand, since natural selection will act on each component, as well as the integration of multiple components, to produce evolutionary changes. Each component is influenced by four interacting causal factors: progeny genotype, uterine maternal effects, postnatal maternal effects, and non-maternal environmental effects (see Figure 1.1 on page 11) (Atchley and Hall, 1991). Causal factors do not statically influence phenotypes; rather, each factor's contribution to growth changes over ontogeny. If natural selection occurs at different times during development, the timing of selection will impact different developmental components and causal factors. Since cellular mechanisms also change over the course of development (Atchley *et al*. 2000), natural selection at different times in ontogeny may also elicit different cellular changes via different signaling pathways.

Clearly, we must better understand the dynamic interactions between time, cellular pathways, causal factors, and regulation to understand the origin and evolution of mammalian growth. Unfortunately, many commonly applied evolutionary models frequently ignore temporal factors influencing growth traits. Consequently, such models may be inadequate to describe evolution of traits whose genetic and environmental influences change throughout ontogeny (Cowley and Atchley, 1992). By characterizing temporal aspects of growth, including each causal factor's influence throughout ontogeny, we hope to more accurately model selection's effect on complex traits. This dissertation characterizes three

causal factors whose influence changes over ontogeny and each factor's contribution to phenotypic variance in growth.

The first chapter of this dissertation reviews the influence of uterine and postnatal maternal effects on mouse growth. In mammals, both mother and father make haploid contributions to their progeny. However, mothers may influence their progeny's growth and development beyond her nuclear component, due to the uterine and post-birth relationship between mother and offspring. These additional maternal contributions are termed maternal effects. Maternal effects originate from an individual whose phenotype is not being observed and are perceived as environmental effects by the offspring. However, maternal effects are influenced by genetic variation (Lefebvre *et al.* 1998; Wolf *et al.* 1998) and thus are not true environmental effects. Rather, they are epigenetic effects, a source of heritable variation from outside the offspring's genome (Cowley and Atchley, 1992). Maternal effects for growth traits have a clear ontogenetic dynamic, as maternal variance maximizes near weaning and subsequently decreases, plateauing near puberty (Atchley, 1984; Riska *et al.* 1984; Atchley and Zhu, 1997)

A plethora of maternal effects influence the offsprings' phenotype(s), including maternal nutrition, body size, toxin exposure, parity, age, uterine size, lactation, and post-parturition care, among others. Maternal effects can affect any number of fitness traits in the mother's progeny including growth, fertility, disease onset and maternal care. Since maternal effects impact the next generation's reproduction, they are transgenerational and will clearly impact evolution of quantitative traits. In this review chapter, I examine the role of prenatal and postnatal maternal effects in growth and development of offspring. I also

discuss both the evolutionary origins and consequences of maternal effects. This review chapter was published in the book "The Mouse in Animal Genetics and Breeding Research" (Funk-Keenan and Atchley, 2005).

As discussed above, developmental complex traits are composed of components with different ontogenetic origins. Body size is one such complex trait, comprised of components from cell number (hyperplasia) and cell size (hypertrophy) (Atchley *et al*. 2000). Growth during ontogeny occurs as the relative contribution of these two cellular mechanisms, among others. During prenatal and early postnatal development, growth is primarily by cell number, while growth later in ontogeny is primarily by cell size (Winick and Noble, 1965). Given that cellular attributes of growth vary over ontogeny, the timing of natural selection for growth, or "age-specific selection", may produce different cellular changes, i.e., in hyperplasia and hypertrophy. If these cellular processes are under separate genetic control, selection will act on different genetic loci.

The final two chapters of this dissertation characterize the genetic architecture and cellular consequences of age-specific selection. Chapters 2 and 3 of this dissertation analyze growth and polyploidy in four mouse strains derived from a long-term restricted index selection experiment. Two pairs of mice lines were produced from the selection experiment, each pair subject to different selection criterion. One pair was selected for rate of development in body weight from 0 to 10 days of age (early growth) to model hyperplastic changes. The other pair was selected for rate of development later in ontogeny, from 28 to 56 days of age (late growth) to model hypertrophic changes. This selection experiment was conducted to test the hypothesis that selection for age-specific growth has lead to different

cellular changes and has acted on different sets of genetic loci. Since body weight gain during different phases of ontogeny is correlated with hyperplasia and hypertrophy, age-specific selection for growth is an indirect mechanism to produce cell number and cell size changes (i.e., Falconer *et al.* 1978). Indeed, Atchley and colleagues (2000) verified selection for early growth did produce significant changes in hyperplasia while selection for late growth produced changes in hypertrophy. Selection for age-specific growth has also lead to significant differences in body weight, growth rates, tail length, uterine and maternal effects, reproductive onset, growth curve parameters, and longevity as correlated responses (Ernst *et al.* 1999; Rhees *et al.* 1999; Ernst *et al.* 2000; Miller *et al.* 2000; Rhees and Atchley, 2000).

Chapter 2 discusses the results from a genomewide quantitative trait loci (QTL) mapping to find chromosomal regions influencing two sets of growth phenotypes. By characterizing the genetic architecture of growth in these lines, we can determine if selection for age-specific growth has acted on different sets of loci. In addition, determining the genetic architecture of growth in mice with documented changes in cell number and cell size will also allow us to identify chromosomal regions potentially contributing to differences in hyperplasia and hypertrophy, as well as others such as apoptosis. Ultimately, this will help experimentally determine if cell number and cell size have different genetic architecture, as suggested from earlier experiments (Cheverud *et al* 1996; Vaughn *et al.* 1999). I searched for chromosomal regions influencing growth in two F_2 mapping populations: one population derived from mice selected for changes in early growth to model hyperplastic changes and a second population derived from mice selected for late growth to model hypertrophic changes. Within each population, I mapped QTL influencing two sets of growth phenotypes, growth

rates and growth curve traits, to compare the genetic architecture of different growth traits. Patterns of epistatic interactions are also examined to determine if age-specific selection has lead to different genetic interactions between populations.

I also looked at the genetic architecture of growth regulation in chapter 2. Even with environmental perturbances during early development, individuals can return to normal body size via compensatory growth in order to "catch-up" with animals whose growth was not altered. Compensatory growth narrows the range of final adult size in mammals (so-called targeted growth), as evidenced by a reduction in phenotypic variance later in ontogeny (Riska *et al.* 1984). Mammals use a feedback mechanism via endocrine signals (Hornick *et al.* 2000), which allows the individual to assess their growth versus the target growth and alter growth accordingly. The genetic architecture of compensatory growth has not been studied, primarily because previous age-specific QTL mappings find no evidence of compensatory growth in mapping populations (Cheverud *et al.* 1996; Vaughn *et al.* 1999). Several publications have demonstrated mice from the restricted index selection demonstrate compensatory growth in ontogeny (see Atchley *et al.* 1997; Rhees and Atchley, 1999; Atchley *et al.* 2000). The restricted index selection lines allow me to explore the genetic architecture of compensatory growth, relative to the traits subject to selection criterion.

Chapters 1 and 2 of this dissertation examine the ontogenetic dynamics of effects influencing mouse growth. In contrast, chapter 3 looks at one cellular consequence of age-specific growth. Endopolyploidy involves DNA replication without subsequent cell division and is a normal physiological process by which cells increase the amount of nuclear DNA while maintaining cell number. In the mouse liver, polyploid hepatocytes start to accumulate

at weaning, possibly due to increased metabolic load in the liver resulting from the dietary change to solid food. At approximately the same time, growth begins to shift from hyperplasia (changes in cell number) to hypertrophy (changes in cell size) (Winick and Noble, 1965; Wheatley, 1972). At this point, creation of polyploid cells may allow the organ to increase gene expression by increasing the number of complete genomes per cell. Not surprisingly, hepatocyte polyploidy level is correlated with increases in cell size, a sign of hypertrophic growth (Gupta, 2000; Vinogradov *et al.* 2001).

Variation in endopolyploidy is correlated with phenotypic variation in quantitative traits, such as body size and transcription level in invertebrates (Flemming *et al* 2000; Vidwans and Su, 2001). Given the link between endopolyploidy and phenotypic variation, genetic selection may alter onset and extent of mammalian endopolyploidy. Using the same four mouse strains discussed above and a randombred control mouse line, I examined age-specific selection's effect on onset and extent of hepatocyte endopolyploidy. Flow cytometry was used to examine endopolyploidy changes over ontogeny within each line.

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CHAPTER 3*

MATERNAL EFFECTS, GENOMIC IMPRINTING AND EVOLUTION

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1. Introduction

In mammals, both mother and father contribute a haploid component of their genes to their progeny. However, the mother makes additional contributions through so-called "maternal effects," as well as through the egg cytoplasm, which includes mitochondrial genes and other cytoplasmic effects. Maternal effects are defined as contributions over and above the direct effects of a mother's own genes that she contributes to her progeny. These effects arise from both heritable and non-heritable maternal attributes and can be thought of as "epigenetic effects."[1-3] The developmental consequences of these maternal effects can be substantial during ontogeny and may persist until adulthood. Maternal effects may significantly impact the progeny's growth, development and reproduction. The effect can be transgenerational because the mother is conditioning the expression of her progeny's genes and may influence the action of natural selection acting on the phenotypes in the next generation (Figure 1.1).

Maternal effects originate from an individual whose phenotype is not being observed and are received as environmental effects by the offspring. A plethora of maternal attributes may influence progeny development including maternal age, maternal body size, uterine size, placental exchange of nutrients, quality and quantity of milk, growth factors, hormones, nesting behavior and thermal regulation.

Maternal effects should not be confused with the *Drosophila* maternal genes, such as Bicoid and Nanos. These maternal genes are transcribed by the *Drosophila* mother during oogenesis and are deposited as messenger RNAs in the offspring to start the process of development and body pattern formation. While they do condition the expression of the offspring's genes, maternal genes originate from the mother's direct effects and are not maternal effects as defined above.

Mammalian maternal effects vary in their underlying causes and ramifications. Among maternal effects with significant human impact are the effects of alcohol, drug use and smoking. These non-heritable maternal effects will impact the progeny's development during ontogeny via intrauterine growth retardation, birth defects, and altered behavior. For example, exposure to cigarette smoke either pre- or postnatally decreases weight and growth in rodents.[4] Fetal exposure to alcohol and cocaine can lead to decreased postnatal growth and altered neurological function in progeny.[5]

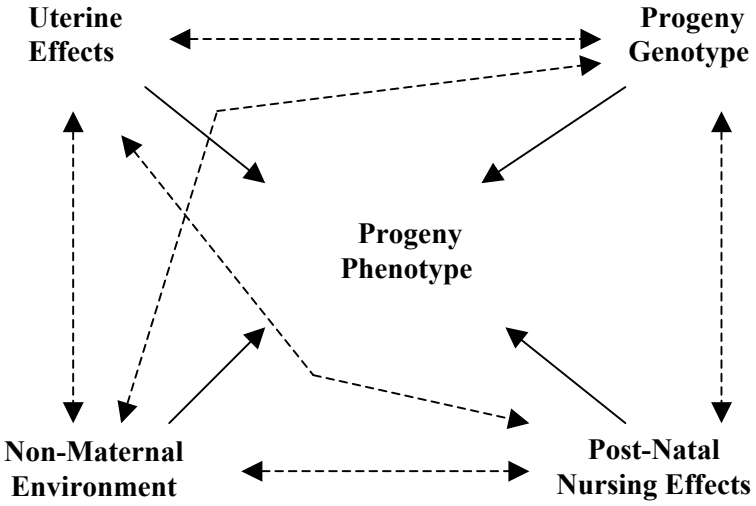

Figure 1.1 Developmental quantitative genetic model of mammalian development. Solid lines denote causal components, while dashed lines denote interactions between components. Modified from references 1, 2, 3, 14.

Maternal effects of greatest interest to evolutionary biologists and animal breeders are those that arise from heritable attributes in the mother, i.e., attributes arising by expression of the mother's own genes. Unlike typical environmental effects, heritable maternal effects can respond to selection and undergo systematic evolutionary changes themselves.[6] In this review, the importance of these heritable maternal effects from a developmental and evolutionary standpoint will be discussed.

Model selection is fundamental for studying evolution of complex traits like growth and development. Unfortunately, many commonly applied models use oversimplifications for modeling complex traits. Direct effects models, for example, include only classical Mendelian inheritance; they ignore real-life factors influencing the variation of traits and consequently may be inadequate to describe the evolution of traits over the course of ontogeny.[7] Use of more complex models such as epigenetic effects models, permit inclusion of temporal and spatial interactions and provide a better description of the evolution of complex traits. The epigenetic effects model[7,8] allows us to look at both intrinsic variation (variation due to additive genetic variation) and heritable extrinsic variation (sources of variation outside the progeny's genome) such as maternal

effects. Further, epigenetic models permit estimation of the covariation between intrinsic and extrinsic or maternal effects and thus represent more accurately how natural selection operates on phenotypic variation.[7]

Research on mammalian maternal effects has focused on the effect on growth and development, especially body weight.[9] Table 1.1 shows a partial list of mammalian traits known to be influenced by maternal effects. While this review discusses only mammalian maternal effects, maternal influences are not restricted to mammalian organisms but rather are wide-spread. They occur for seed dormancy in plants,[10] reproductive traits and diapause in fish and insects,[11] growth in cockroaches,[12] and growth in birds,[13] to name a few.

Table 1.1 A partial list of offspring traits influenced by mammalian maternal effects

Trait	**Species**	**Reference**
Body weight and growth rate	Horse (*Equus*), human, mouse (*Mus*, *Phyllotis darwini*), red squirrel (*Tamiasciurus hudsonicus*)	14, 15, 16, 17, 18, 19, 20, 21
Onset of puberty/vaginal opening	Mouse (*Mus*), rat (*Rattus*), guinea pig (*Cavia porcellus*)	22, 23, 24
Fertility and fecundity	Human, rat (*Rattus*), mouse (*Mus*)	25, 26, 27, 28
Bone strength	Horse (*Equus*), human, rat (*Rattus*)	29, 30, 31
Organ weight	Mouse (*Mus*), rat (*Rattus*), sheep (*Ovis*)	19, 32, 33, 34
Disease state/onset	Human, mouse (*Mus*), rat (*Rattus*)	35, 36, 37, 38
Endocrine function and activity	Human, mouse, (*Mus*), rat (*Rattus*)	22, 39, 40, 41, 42, 43

2. Temporal Aspects of Maternal Effects

Use of a developmental quantitative genetic model for studying the architecture of complex traits requires the recognition that there is a dynamic ontogenetic component. The variance and covariance components for a trait, as well as interactions between traits, change significantly during ontogeny. Only by understanding and documenting these ontogenetic changes can one expect to

accurately model the genetic control of complex traits. There have been a number of studies that document the dynamics of variance and covariance components during ontogeny. [44-46] In each of these, the maternal component of variation has shown a clear temporal dynamic. One of the obvious temporal aspects of mammalian maternal effects is development in different types of maternal environments, e.g., prenatal uterine maternal and postnatal nursing (Figure 1.2).

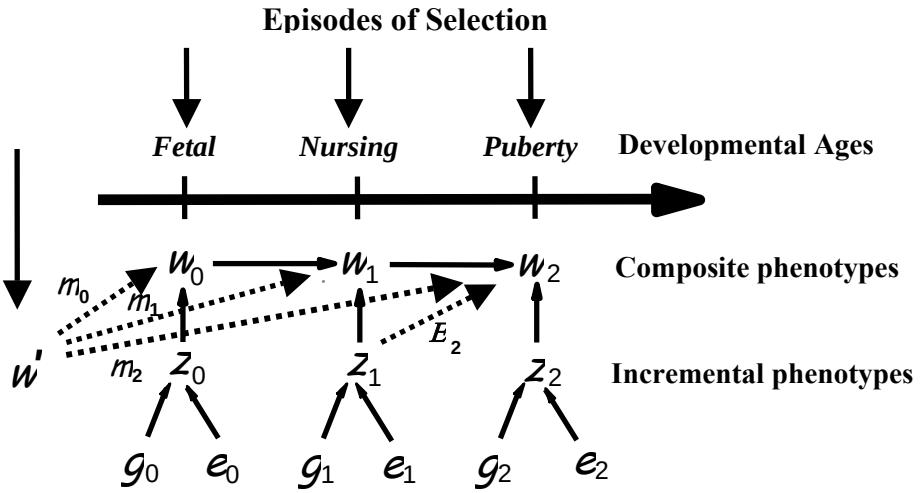

Figure 1.2 Maternal influence during development. For each developmental age (fetal, nursing, and puberty), the incremental phenotype at that age (*z*) is influenced by an additive genetic and environmental component for that age (*g* and *e*). The incremental phenotype combines with a maternal effect (m_0, m_1, or m_2) to produce a unique composite phenotype (*w*) for each age. This maternal effect originates from the mother's composite phenotype, *w*`. In addition, the puberty composite phenotype w_2 has an epigenetic component (E_2), representing the developmental cascade of traits, where early phenotypes will condition development of later phenotypes. Arrows indicate episodes of natural selection influencing both progeny and mother's phenotype. Modified from reference 7.

Thus, in actuality, development of typical mammalian organisms, like the mouse, involves the expression of two genomes (mother and progeny) in three environments (uterine, nursing, and postnatal non-nursing). Maternal effects may directly impact growth in one phase such as prenatal growth or may affect growth in multiple phases. Maternal effects such as maternal age and maternal nutrition are illustrations of multi-phasic maternal effects, affecting both pre-and postnatal growth.

After parturition, the phenotypic variance explained by maternal effects for a quantitative trait typically increases until it is highest near weaning. After weaning, the phenotypic variance due to maternal effects may either plateau or decrease.[47-49] Phenotypic variation explained by maternal effects can be greater than variance from an individual's own genotype, especially right after birth.[50,51] Maternal effects will continue to influence the offspring's phenotypes long after the offspring has left the mother.[47,48] Typically, maternal effects impact adult behaviors such as maternal care performed by female offspring[26,52] and adult size of progeny.[3,14,44,46,47]

As mentioned above, a plethora of maternal attributes may influence progeny development. Consider several of these pre- and postnatal maternal effects and how they influence the progeny's fitness and phenotype

3. Effects of Maternal Age

Both early motherhood (immediately after puberty onset) and late motherhood (near menopause) can negatively impact offspring growth and increase disease incidence. Adult offspring of mice bred in adolescence (soon after puberty onset) or at middle age (near menopause) weighed less than adults born to females bred at early adulthood.[53] These lighter mice also had delayed onset of puberty, relative to offspring from early adulthood females.[53] Eisen[54] found females bred soon after puberty onset showed decreased litter size, littering rate, and pup birth weight with increased pup mortality, compared to females bred at older ages. However, this is not consistent as other research has found females bred soon after puberty showed the same lactational performance as females bred at older ages, suggesting an interaction with genetic background.[55] Increased maternal age is also associated with decreased birth weight in humans and rats.[56-58] The reduced prenatal growth may be due to a decrease in uterine and placental quality affecting nutrients received by the fetus[59] while decreased postnatal growth may be due to decrease in quality and quantity of lactation.

Advanced maternal age is also associated with increased risks for diseases such as diabetes,[60] kidney disease,[61] leukemia,[62] and hypertension in adults.[35] In humans, offspring of young mothers have increased incidence in psychological and physiological disorders such as schizophrenia and cancer.[63-65] Mouse pups born to older mothers may also show diminished mental function relative to mice born to younger mothers.[66] In humans, increased maternal age is correlated with increases in fetal systolic blood pressure.[67]

4. Effects of Maternal Nutrition

Prenatal maternal nutrition influences disease prevalence and onset, growth and fat deposition in the progeny, puberty onset, and gene expression in mammals.[23,35,68,69] We expect caloric restriction during gestation or lactation would lead to decreased fetal growth and maternal weight loss, due to the increased energy requirements on the mother. However, caloric restriction during these two phases may not act via the same mechanism as restricted diet during postnatal development shows greater effects on adult body weight in mice than restricted diet during prenatal development.[70] The progeny of rodent mothers fed calorically-restricted diets during gestation have smaller weights early in ontogeny but show compensatory growth via increases in fat composition after birth.[71,72] Accordingly, mice born to mothers on restricted diets during gestation show increased levels of gene expression in fat synthesis genes.[73] In humans, maternal nutrition during early pregnancy also influences fetal growth.[74] However, it is frequently not possible to estimate the effect of maternal nutrition on human fetal growth, due to geographic and socioeconomic variation in maternal diet.

Some debate exists as to whether maternal nutrition differentially affects the two sexes. Sons of female mice starved during gestation will show decreased adult weight while females have the same weight as control mice.[27, 75] However, the daughters of these food-deprived mothers have fewer pups in their own litters, suggesting a multi-generational effect on fertility.[27] Prenatal malnutrition also differentially affects male and female adult brain function and adult blood pressure in rats.[76, 77]

5. Prenatal Maternal Effects

The uterine environment influences the embryos in any of several ways: uterine size, nutrient exchange, uterine position, and prenatal exposure to toxins. Prenatal maternal effects may be expected to affect only gestational development but research shows this is not correct.[14] In inbred mouse lines, prenatal maternal effects had a greater impact on progeny body weight at some ages than the progeny's own genotype did.[14,15] In mice, the overall uterine environment interacts with the progeny's genotype to influence growth and response to selection.[14-16,49,78] Uterine environment also had a significant and long-lasting impact on skeletal development in inbred mice and mice undergoing artificial selection.[3,14,16,79,80]

Variation in size or weight of the mother (independent of nutritional variation) can also impact progeny development. In both inbred mice and mice undergoing artificial selection, weight of the gestational mother significantly influenced both prenatal and postnatal growth in mice; heavier mothers produced heavier and faster growing pups, regardless of the pup's genotype.[14,16,49] Nonhuman primates and several livestock species also show a similar proportional relationship between maternal weight or size and growth in offspring.[81,82] Weight of the uterine mother also affects skeletal growth in mouse pups.[3,83]

5.1. Effects of Litter Attributes

Litter attributes such as intrauterine position, uterine size, gestational litter size, and placental efficiency are known to significantly influence progeny growth and development. Uterine position impacts several aspects in the offspring: growth, hormone level, hormonal activity, morphology and behavior.[84] Rat and mouse fetuses developing near male fetuses have increased birth weight, compared to fetuses developing near female fetuses.[85,86] However, this pattern in mice is not consistent[87,88] and may not continue after parturition.[89] Rabbit fetuses frequently show intrauterine growth retardation when they develop in the middle of the uterus, being 0.85 times the weight of normal fetuses at birth.[90,91] Pigs also show a uterine position effect on weight of the fetus, with lighter fetuses developing closer to the cervix.[92]

Uterine position may lead to altered reproduction and behavior for both sexes. Male and female rodents developing beside male fetuses show increases in aggression, territoriality and novelty-seeking incidences; this is presumably due to increased exposure to androgens during gestation.[84,86,93] Female rodents developing between two males also show a decrease in "female-like" behaviors such as lordosis (a measure of female receptivity to males) and have later puberty onset and shorter estrus compared to other females.[86,94] Uterine position may also affect fetal response to stimulants such as cocaine.[95,96]

Gestational litter size also affects fetal body weight and morphological traits in mice and other species. Unlike uterine size or uterine position, prenatal litter size can be manipulated via embryo transfer, allowing more experimental access on its effect on progeny phenotypes. Prenatal litter size has a well-documented effect on pre- and postnatal growth of offspring, as well as skeletal growth and organogenesis.[3,14,97-100] Depending on the strain of mouse and the time during ontogeny, litter size explains between 10-50% of the phenotypic variance for

body size.[14,100] Traditionally, mice born to larger litters are smaller and have higher mortality.[3,98,101,102]

This effect of litter size will also impact subsequent generations, as a female's fertility is correlated with her growth. Selection for increased growth has typically increased gestational litter size as a correlated response to selection; selection for decreased growth will decrease litter size.[103-106] However, the increase in fertility is offset by higher pre- and postnatal mortality of fetuses.[102,105] Increased fetal death probably occurs around the time of parturition, since the number of live fetuses during gestation is greater than or equal to that of control mice.[105,107] This positive genetic correlation between litter size and growth is especially interesting because there is a negative environmental correlation between litter size and growth in female mice. Inbred female mice raised in larger litters tend to be smaller and produce smaller litters.[14] Thus, the effect of litter size on growth and growth's subsequent effect on litter size is cyclic and transgenerational.

5.2. Effects of Prenatal Toxin Exposure

Prenatal exposure to toxins typically leads to altered brain development and organogenesis in mammals and birds, but can also affect the immune and endocrine system in chickens.[108] The fetus can be exposed to several different toxins while in utero: alcohol, radiation, environmental toxins such as lead and mercury compounds, and bacterial toxins. Prenatal exposure to bacterial toxins, alcohol and some environmental toxins can alter neuron levels in adult mice and may lead to neurological disease in humans and rats.[109-111] Exposure to bacterial toxins during gestation can also result in altered heart growth.[112]

Fetuses of grazing mammals can be exposed to toxins from plants and forage; these toxins may affect the fetus in a narrow time frame during development or over the whole pregnancy. These toxins typically lead to increases in spontaneous abortion, alterations in fetal growth and morphology, and decreased fertility.[113] Mice fed an endophyte-infected fescue diet during gestation had decreased littering rate, compared to mice fed either control or fescue non-infected diet.[114] The pups exposed to endophytes during prenatal development weighed less and had decreased incidence of vaginal opening at normal puberty onset compared to control or non-infected diet mice.[114] There is evidence for a genotype-by-environment interaction with regard to the mouse physiological response to toxins, as discussed in Chapter 5.

6. Postnatal Maternal Effects

The postnatal nursing environment influences the neonate in a variety of ways, most notably through lactation and maternal behavior, including nest quality, thermoregulation and general care of offspring. Postnatal maternal effects influence offspring body weight and morphology in rodents,[46,47,77,115] as well as other mammals. Postnatal maternal effects have a larger impact on growth in females than males, probably due to the effect of postnatal nutrition on puberty onset in females.[46,116,117] These postnatal maternal effects also impact adult behaviors such as alcohol consumption, stress response, and maternal care.[51,118]

6.1. Effect of Lactation

Milk composition and quantity can vary significantly across mothers and will produce variation in weight gain and growth among pups. Lactation performance of the mother will depend on her growth and parity, as well as the nursing litter size.[49] When nursing larger litters, females produce more milk but offspring with more littermates still get less milk than offspring with few littermates.[119] In addition, the concentration of solids in the milk is decreased in large litters, relative to small litters.[119] Therefore, individuals in large litters get less and poorer quality milk. Milk composition also varies with the number of lactations. Mice pups can have decreased weight gain as the number of lactations increased, but this is strain-specific.[120] In addition, temperature during nursing can also affect lactation quality. Females nursing at cooler temperatures produce more milk with higher energy content, more solids and more fat than females nursing at higher temperatures.[121]

Hormones and proteins involved in milk production and letdown will also influence growth and reproduction in the offspring; for example, the hormone prolactin and its receptor are involved in carbohydrate metabolism. Consequently, prolactin level in milk is correlated with offspring growth, and mice lacking the prolactin receptor have decreased weight gain in offspring.[117,122] The *hub* mutation in mice may also affect the the prolactin pathway.[123] Female *hub* mice have decreased milk yield and lower epidermal growth factor (EGF) levels in milk, with associated increased pup mortality and decreased pup growth compared to normal females.[124,125] Levels of other hormones in milk such as growth hormone and progesterone are also correlated with offspring growth and vaginal opening in mice.[122] Administration of an oxytocin antagonist in pregnant females will lead to decreased growth in offspring starting at 3 days of age, only after lactation is established.[126] Females lacking the *Peg3* gene are deficient in

milk ejection and have a reduced number of oxytocin neurons in the brain; pups nursed by these females have decreased postnatal growth compared to pups nursed by control mice.[127]

6.2. Effect of Maternal Care

Postnatal maternal behavior or care affects pup mortality and growth, especially of the brain development and the associated development of adult behaviors, e.g., stress response and expression of associated genes.[118,128] Maternal care is a transgenerational maternal effect since maternal care influences maternal behavior of female offspring.[26,51] Females lacking the *FosB* and *Dbh* genes showed maternal care defects such as pup cleaning and retrieval, as well as lactation problems.[129] The *hub* mutant mothers have slower pup retrieval and decreased pup grooming compared to control mothers.[125] *Mest1*-deficent females are poor in general maternal care, pup retrieval and nest building, as are *Peg3*-deficent females. [127,130] Female mice lacking one or both copies of the prolactin receptor gene are slow to retrieve pups and defensively crouch over nests, taking longer than control females.[131]

7. Maternal Effects and Role in Selection

The majority of theoretical research on selection response and the evolution of traits have used direct effect genetic models. As mentioned above, direct effect models assume genetic variation and covariation arise only from genes within an individual's genome and ignore other causes of phenotypic variance such as maternal effects and sex effects. Under this model, an individual's phenotypic variance for a single trait will be equal to their additive genetic variance, environmental variance and variance from an interaction between the two.

If we expand this model to one with "n" traits under selection, the relationship becomes

$$\mathbf{P} = \mathbf{G} + \mathbf{E}$$

where **P**, **G**, and **E** are the *n x n* phenotypic, additive genetic, and environmental variance/covariance matrices, respectively. The response to selection with this model is

$$\Delta\mathbf{z} = \mathbf{G}\,\mathbf{P}^{-1}\,\mathbf{s}$$

where $\Delta\mathbf{z}$ is the vector of changes in trait means and **s** is the vector of selection differential for traits under selection.[132]

As noted above, direct effects models are oversimplifications of the underlying causes of genetic variation in mammalian species. Maternal effects influence expression of their offspring's genes, alter phenotypes, possibly changing the correlation between genotype and phenotype, and thus influence the efficacy of selection response.[8] Therefore, response to selection models must include a maternal component to accurately estimate the underlying causes of variation and to accurately predict response to selection.

Under a model with maternal effects, an individual's phenotypic variance for a single trait will now be a function of its additive genetic and environmental effects and a maternal effect coefficient "m" times the maternal phenotype for that trait.[133] This "m" coefficient measures the strength with which a mother's phenotype can alter the progeny's phenotype.[133] These authors point out that this maternal effect coefficient can be negative or positive.

In multi-dimensional biological systems, maternal effects rarely affect only one trait. In the more realistic multivariate case, the phenotypic variance for a single trait in "n" maternally influenced traits is a function of the additive genetic and environmental effects for that trait plus a multivariate maternal effect coefficient, measuring the strength of the maternal effect in trait 2 on trait 1. The response for one generation of selection is modeled by

$$\mathbf{\Delta z}(t)=(\mathbf{C_{az}} + \mathbf{MP})\boldsymbol{\beta}(t) + \mathbf{M\Delta z}(t\text{-}1)\mathbf{-MP}\boldsymbol{\beta}(t\text{-}1)$$

where $\mathbf{C_{az}}$ is a symmetric matrix representing covariances between each additive genetic value and phenotypic value, $\boldsymbol{\beta}$ is a matrix of selection gradients ($= \mathbf{P^{-1}s}$) and **M** is a matrix of maternal effect coefficients.

Thus, response to selection in generation "t" is a function of the force of selection in generation "t", based on the midparent and offspring resemblance,[133]

$$(\mathbf{C_{az}} + \mathbf{MP})\boldsymbol{\beta}(t)$$

However, response in generation "t" also depends on the force of selection and evolutionary change in generation "t-1," which is the key difference between response in maternally influenced traits and response in Mendelian traits.

Direct effects models assume no maternal effects; thus **M** is a matrix where all elements are equal to zero, and $\mathbf{C_{az}} = \mathbf{G}$, where $\mathbf{C_{az}}$ shows how changes in gene frequency lead to changes in phenotype. If a trait is maternally influenced, $\mathbf{C_{az}} \neq \mathbf{G}$ and an individual phenotype may appear "better" or "worse" than its genotype suggests. Thus, maternal effects will alter the genotype/phenotype correlation, impacting the efficiency of selection.[1,8,133]

Maternally influenced traits often show a time-lag in response to selection, since selection response in one generation depends on the force of selection in two generations.[130] Such time lags can lead to continued selection response after

selection has ceased (evolutionary momentum) and a variable selection response across multiple generations even when selection strength is constant.[134] This leads to evolutionary change in traits with no additive genetic variance, since no genetic variance is required; rather, only maternal variance is needed to respond to selection.[6]

Even when using a maternal effects model, estimating response of maternally influenced traits is difficult. The evolutionary change for previous generations ($\mathbf{M}\Delta\mathbf{z}(t-1)$) is difficult to measure since evolutionary change for a given generation can fluctuate around its expectation.[133] In addition, one cannot assume maternal coefficients remain constant as evolution continues, making estimation more difficult.[16, 135]

When maternal effects influence a trait, they create a direct-maternal covariance between direct (effect of genotype) and indirect effects (effect of maternal effect) due to the pleiotropic nature of some genes. Selection for a trait under maternal influence can act on the direct genetic variance, the indirect genetic variance, or the direct-maternal covariance. If this covariance is negative, response to selection will be diminished, since changes in direct effects will be offset by a large (negative) change in maternal effects. This covariance can cause some traits to evolve away from the true fitness optimum ("maladaptive evolution"). As Kirkpatrick and Lande[133] point out, there is a tendency to assume a positive correlation of the same trait between mother and offspring but this is not necessarily true. Negative covariances between direct and maternal effects are common for growth and fitness traits,[136] such as the negative correlation between maternal litter size and subsequently progeny litter size.[137]

This covariance may also change over the course of ontogeny, further complicating selection response modeling. Cheverud[136] found an initially negative covariance between maternal effects and body weight in mice that became positive after weaning. Hanrahan and Eisen[138] also found similar results. Other traits showed negative covariances between maternal effects and offspring traits later in ontogeny, especially near onset of puberty.[136] In pigs, there is a positive covariance between direct and maternal effects for body weight after birth which becomes negative as an animal ages.[139, 140] Other research has found a positive covariance between direct and maternal effects for body weight in mice throughout ontogeny. However, the magnitude of these positive covariances increases later in ontogeny, suggesting direct effects at later ages are more correlated with maternal effects than earlier direct effects.[141]

8. Maternal Effects and Familial Relationships

Maternal effects will also affect two familial relationships, complicating parameter estimation.[142] The first is the resemblance among offspring raised in a common environment; however, the relationship between offspring and mother remains the same.[142] This is related to variation in maternal performance; maternal performance is a composite phenotype, comprised of all the maternal attributes in the mother influencing her offspring.[50] An example of this is variation in an aspect of maternal performance such as pup retrieval leads to an environmental component in sibling covariation for a trait such as body weight.[142] The variation among offspring results from variation in maternal performance. Such variation in maternal performance can be due to genetic variation in behavioral and lactation genes.[143] The second impact is the mother's phenotype for a trait and its conditioning of the offspring's phenotype for the same trait. This will modify the resemblance between full sibs and maternal half sibs, as well as the relationship between offspring and mother.[50,142]

The tendency of sibs to resemble each other due to the common maternal environment is one of the more troublesome environmental aspects to model and assess via experimental design.[142] To separate common maternal effect from other sources of variation, it is necessary to crossfoster full sibs to different nurse mothers Breeding designs to estimate causal components of variance without crossfostering will lead to inaccurate estimation of genetic parameters due to the correlation of genetic covariances between relatives.

Eisen[144] suggested that traditional mating designs used to estimate causal components of variance can lead to doubling the contribution of indirect genetic effects (both maternal effects and the direct effects/maternal covariance). This leads to inflated estimates of additive genetic variance and heritability, and in some cases, can double the heritability estimate.[144,145] Estimating realized heritability via regression of offspring phenotype on midparent phenotype for a trait with maternal effects can also produce a biased estimate, sometimes greater than 1 or less than 0.[133,136] The direction and magnitude of the heritability bias will depend on the magnitude and sign of the direct-maternal covariance.[1]

9. Maternal Effects Across Multiple Generations

Maternal effects can extend across multiple generations, i.e., grandmaternal effects. As expected, they are not as pronounced as typical maternal effects, but so-called "grandmaternal effects" may still condition gene expression and impact the phenotype of the grandchild. Grandmaternal effects influence birth and

weaning weight and may interact with nutrition in certain cattle breeds.[146-149] In humans, grandmaternal effects may influence fetal birth weight, although these results are inconsistent.[150] Some stress-responsive behaviors in adult rats also show grandmaternal effects.[151]

10. Maternal Effects and Interactions with Other Epigenetic Phenomena

Maternal effects are one of several epigenetic phenomena involved in development. These maternal effects can interact with other epigenetic phenomena of DNA modification, such as methylation and acetylation, to impact the progeny's growth and development. One such DNA modification is genomic imprinting, or the differential expression of one parent's allele via methylation for a subset of genes. At an imprinted gene, one parental allele is active (transcribed) and the other allele is silent (not transcribed). Which parental allele is silent or active is determined on a gene-by-gene basis. This differential expression of parental alleles is achieved via differential DNA methylation at the two alleles by the enzyme Dmnt1. The methylation pattern for a gene is established in the germline and is maintained until after implantation.[152]

Previously, it was thought epigenetic patterns were erased during gametogenesis, thereby eliminating transmission from one generation to the next. However, Rakyan et al[153] documented that epigenetic patterns at some genes can be inherited due to incomplete erasure of the epigenetic pattern. This fact, coupled with the fact that DNA replication has a 95-97% fidelity of transferring methylation patterns,[154,155] means there is great potential for introducing epigenetic variation, even within the same individual and certainly to subsequent generations.

Unlike maternal effects, DNA methylation involves only one genome, the progeny's. However, evidence suggests some maternal effects, e.g. maternal nutrition, may interact with genomic imprinting at certain loci to condition their expression. While less than 0.5% of rodent genes are known to be imprinted,[156] a subset of imprinted genes are involved in regulation of fetal growth, placental development and maternal behaviors. This makes imprinted genes a possible source of both quantitative variation for growth and maternal effects.

The link between methylation variation and expression variation at imprinted genes, which will then impact phenotypic variation in a population, has been well documented at several imprinted genes. Methylation levels of both the maternal and paternal *IGF2* gene vary temporally and spatially in prenatal ontogeny.[157] The methylation level of the expressed paternal allele is correlated with *IGF2* expression levels.[157] Deletion of the upstream sequences of the maternal *IGF2*

allele can lead to altered methylation patterns and polymorphic expression of the silent maternal allele in mouse embryos.[158] The methylation patterns at imprinted genes can change due to environmental conditions, and these methylation changes are correlated with changes in expression levels.[159-163] In addition, a small percentage of embryos and adults in natural populations express both alleles of some imprinted genes, confirming the potential for expression variation at imprinted genes.[164-167]

10.1. Maternal Effects and its Effect on Genomic Imprinting

The best-supported link between methylation variation at imprinted genes and maternal effects is through maternal nutrition. Nutrients involved in methyl metabolism such as folate, methionine, and choline are required to maintain DNA methylation,[168] and variations in dietary methyl amount can lead to differential methylation at the *agouti* locus in mice.[169] The imprinted gene *H19* shows differential methylation patterns and expression levels when mouse embryos are raised in different environments.[170] The maternally expressed *H19* allele had increased methylation and an associated decrease in expression when mouse embryos were cultured in fetal calf serum, compared to fetuses cultured in medium without fetal calf serum.[171] These embryos also had decreased expression of the paternal *IGF2* allele, resulting in lower birth weights than control pups.[171]

There is also a postnatal nutritional influence on methylation at imprinted genes. Poor diet during early postnatal development can alter the expression of the *IGF2* gene and other imprinted genes; this expression change and its associated phenotypic effect can persist long after nutrition has improved.[172] Adult rats nursed in small litters showed differential expression at two imprinted genes, compared to rats nursed in litters with more pups, showing the effect of litter size on methylation.[173] Inhibition of DNA methylation in young mice led to changes in *IGF2* and *H19* expression in some tissues.[174] This modified *IGF2* expression was not the same for all tissues; some tissues expressed both alleles, while others expressed only one allele (either maternal or paternal).[174]

10. 2. Genomic Imprinting and its Effect on Maternal Effects

Conversely, genomic imprinting will influence maternal effects such as maternal behavior and placental quality. Loss of the paternally expressed imprinted gene *Mest1* produces female mice deficient in pup retrieval and nest building, leading to increased postnatal pup mortality.[130] *Mest1*-deficent mice also have uterine

growth retardation and had decreased pre- and postnatal growth compared to non-deficient mice.[130] Loss of another paternally expressed gene *Peg3* also leads to growth retardation in pups and abnormal lactation in mothers.[127] Interestingly, the methylation pattern of the imprinted gene *Mest1* does not seem sensitive to nutrient variation as other imprinted genes, such as *IGF2* and *H19*.[171]

Imprinting genes also influence growth of the placenta, impacting nutrient exchange to the fetus. Knockout mice for the paternally expressed genes *IGF2*, *Peg3*, and *Mest1* will have reduced placental size in gestation; knockouts of maternally expressed genes such as *H19* and *IGF2R* will increase placental size.[175] However, this placental growth is dependent on both progeny and maternal IGF2 expression. Both *IGF2*-deficent mothers[176] and normal mothers with embryos lacking a paternal *IGF2* allele have smaller placentas compared to control mothers.[177] The decreased placental size does decrease diffusion of nutrients to the fetus but does not affect the growth of the fetus until late in gestation, possibly due to a compensation mechanism in amino acid transport.[178] Imprinted genes also influence the transfer of nutrients over the placenta: the *Slc22*, *Impt1*, and *Ata3* genes are transporter genes.

11. Evolution of Maternal Effects and Genomic Imprinting

In order for genomic imprinting to evolve, it must convey a selective advantage. In general, paternally expressed genes increase fetal growth, thus requiring more maternal resources. This helps ensure the offspring will survive and reproduce, at the cost of the mother's (and other males') future offspring, to the father's advantage. Conversely, maternally-expressed genes tend to decrease offspring growth, thereby shortening the maternal care period and increasing her chance of bearing subsequent litters.[179] The balance between the best interests of the two parents can be seen in the *IGF2/IGF2R* gene pair. Increased expression of paternally imprinted *IGF2* leads to increased fetal growth, increasing the likelihood of a large offspring. The maternally expressed *IGF2R* gene works to "balance" the expression of the *IGF2* gene and minimize the postnatal care and nursing needed, spreading the mother's resources equally over all her offspring. Classic maternal effects such as postnatal lactation and care can also be explained by a similar hypothesis, the parent-offspring conflict hypothesis. Increased maternal care and lactation are advantageous to the offspring due to increased fitness but deleterious to the mother due to decreases in fertility from nursing. Like the father, it is to the fetus's advantage to obtain more maternal resources at the expense of other (and future) siblings.

Interestingly, genes involved in kin recognition such as genes in the major histocompatability complex are also imprinted.[180] In rodent species that form communal nests, these loci can serve as an advantage to a polygamous male. Without kin recognition, mothers will be willing to nurse any offspring, including ones that are not her own, to the father's advantage.

Both maternally-expressed genomic imprinting and maternal effects serve the same function: to equally distribute maternal resources to all her offspring. Placentally imprinted genes function to serve the same purpose: to decrease the supply of nutrients to the increasingly nutrient-demanding fetuses.[175]

12. Conclusion

Maternal effects arise from both prenatal and postnatal maternal-by-fetal interactions and can significantly influence offspring gene expression patterns, growth, reproduction, behavior and disease incidence. These effects may alter genotype-phenotype correlations in the offspring, thus affecting the efficacy of selection response and influencing evolutionary change.

While some of the well-characterized maternal effects have been discussed, more research is needed on phenomena such as the causal relationship between maternal effects and gene methylation and gene expression. The mouse is well-suited for such research because it is one of the few mammals with well-characterized maternal effects that are known to show temporal methylation variation at imprinted genes. In addition, with the use of mouse microarray technology, the causative relationship between maternal effects and gene expression can be better documented.

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CHAPTER 2

LITERATURE REVIEW:

THE GENETICS OF MOUSE GROWTH

INTRODUCTION

Individuals within and between populations vary in a plethora of growth attributes, such as weight, height, limb length and muscle: fat ratio, among others. Such traits are quantitative traits, varying in degree of phenotype rather than kind of phenotype and are influenced by multiple genetic loci and the environment. Phenotypic variation (V_P) in quantitative traits can be explained by genetic and environmental variation (V_G, V_E), as well as their interaction (V_{GE}). In order to understand how selection (both artificial and natural) leads to changes in quantitative traits, we must first understand the genetic variation influencing a trait, i.e., the genetic architecture of growth. We use the term genetic architecture to refer to the number, location, frequency, and effects of quantitative trait loci (QTL) influencing growth, epistatic interactions between QTL, pleiotropic effects at each QTL, and the timing of QTL expression. The end goal in quantitative genetic is to characterize the genetic variation (s) underlying these QTL and understand what biological change the genetic variation produces, i.e., a change in protein folding leading to reduced activity, increased gene expression due to genetic variation in the promoter region, etc. Indeed, one of the primary objectives of quantitative genetics for the past few decades has been to identify the casual genetic variation leading to phenotypic variation in mammalian growth.

Understanding the genetic architecture of mammalian growth is relevant for several reasons. First, mammalian growth is a key agricultural and biomedical trait. Knowing the genetic architecture of growth can assist in artificial selection (marker-assisted selection), aid in understanding evolution's role in growth and morphology, and help explain the

development of growth-related diseases, such as cancer and obesity. In addition, growth is correlated with other traits that natural selection acts upon: fertility and longevity, among others. By studying genes influencing growth, we may also shed light on the genetic architecture of other fitness traits.

Among mammals, the mouse has been used extensively to study the genetics of growth, due to its short generational time, relative ease of inbreeding, and large number of inbred strains. The mouse also lends itself to the study of growth due to its ease of uterine manipulation, such as embryo transfer, to assess maternal influences on growth. Finally, the release of the mouse genome, including chromosomal maps of DNA sequence variation between and among strains, has greatly facilitated mapping QTL influencing growth.

MUTATIONS IN GROWTH GENES

Some of the first genes influencing mouse growth were identified as single gene mutations, discovered due to their large phenotypic effect. A partial list of single-gene mutants affecting body size are in Table 2.1 (Corva and Medrano, 2001). Of the seven mutants in Table 2.1, *hg* is the only mutation that increases growth. The *hg* gene was first identified in mice selected for increased 3- to 6-week gain (Bradford and Famula, 1984). Homozygous *hg* mice are not obese; rather, they are proportionately larger in skeleton and organ size. Homozygous *hg* mice have decreased growth hormone (GH) levels and increased insulin-like growth factor-1 (IGF-1) levels (Medrano *et al.* 1991). Increased growth in *hg* mice is caused by a mutation in the *Socs2* gene, whose protein binds to GH receptor. Lack of the SOCS2 protein leads to deregulation of GH and IGF-1 expression and increased growth (Horvat and Medrano, 2001).

Three mutations that decrease growth, snell (*dw*), ames (*df*), and little (*li*), are also in the GH pathway (Corva and Medrano, 2001). *Dw* mice have a missense mutation in the *Pit1* gene on chromosome 16, affecting pituitary growth hormone (Camper *et al.* 1990). The PIT1 protein activates transcription of the *GH* and prolactin (*Prl*) genes, among others (Efstratiadis, 1998). *Dw* mutants do express the *Pit1* gene early in embryonic development but stop expression before parturition. Ames (*df*) mutants also have low *Pit1* expression (Camper *et al.* 1990). Later research showed *df* mice have a mutation in the *Prop1* gene, an early enhancer of *Pit1*. Both mutations decrease GH protein levels, which then decrease activity in the IGF-1 pathway (Sornson *et al.* 1996). *Li* mice have a mutation in the Growth Hormone Releasing Hormone receptor (*Ghrhr*) gene, reducing GH secretion (Godfrey *et al.* 1993). Mutants for these three genes have normal growth until approximately 14 days of age, after which growth is retarded (Efstratiadis, 1998). This timing approximately agrees with the activity of GH, which becomes a mitogen near 21 days of age (Hyatt *et al.* 2004).

Pygmy (*pg*) mice were first described as being extremely small in a line selected for small size. *Pg* mice have normally growth until day 16 of embryonic development when growth starts to become retarded (Efstratiadis, 1998). A mutation in the *Hmgi-c* gene is responsible for the decreased growth in *pg* mice, likely due to alteration of the cell cycle (Zhou *et al.* 1995). Mutations in the remaining two genes, miniature (*mn*) and diminutive (*dm*), decrease both growth and viability but no functional or protein-encoding genes have been identified for the mutations (Stevens and Mackensen, 1958; Bennett, 1961). Some researchers suggest the *Ghr* (Growth Hormone receptor) and the *Tgm2* (Transglutaminase)

genes are candidate genes for the *mn* and *dm* mutations, respectively (Barton *et al.* 1989; Nanda *et al.* 1999). However, these hypotheses have never been confirmed.

STATISTICAL ADVANCES IN QTL MAPPING

Apart from mutations, mapping genes influencing quantitative traits was limited to those genes in linkage with easily genotyped morphological markers (i.e., Sax, 1923). These early mapping experiments typically compared the phenotypic means for the two or three genotypic classes for these morphological markers. If means between classes were significantly different via a t-test, this suggested the marker was associated with a QTL. QTL effects, such as additive and dominance values, were then calculated from differences between genotypic classes. With the advent of molecular genetic markers, our chance of finding a QTL increased due to increased coverage throughout the genome. Indeed, single-marker analyses can successfully find markers associated with QTL and, with sufficient marker coverage and progeny, QTL regions can be relatively small in size. However, single marker analyses have several downfalls. First, QTL effects are typically underestimated since the effects are confounded with recombination frequency between the marker and QTL. In addition, single marker analyses do not estimate QTL position, can't distinguish between one or multiple QTL in a region, and have a higher false-positive rate due to multiple testing.

The development of interval mapping by Lander and Botstein (1989) and others (Haley and Knott, 1992; Jansen, 1993) allows us to avoid most of the problems associated with single marker analyses. Interval mapping is a genomewide search for QTL and is statistically similar to single marker analysis. However, QTL genotypes are treated as

missing data in a mixture model. For example, if we are testing for QTL between markers i and (i+1) with a backcross design, we can decompose the phenotype:

$$Y_j=\mu + b^* x_j^* + e_j$$

where Y_j is individual *j*'s phenotype, b^* is the effect of the QTL, and x_j^* is the conditional probability of *j*'s QTL genotype, given *j*'s marker genotypes and QTL testing location (θ). Calculating the conditional probability requires three recombination fractions: recombination between the two markers (which we estimate via recombination events in the mapping population), and recombination between each marker and the QTL. The unknown parameters (μ, b^*, σ, and θ) are estimated via maximum likelihood using the likelihood function of the mixture model above. The test statistic from interval mapping is the likelihood ratio

$$-2 \ln (L_0)/L(_1)$$

where L_0 is the likelihood of the null hypothesis ($b^*=0$ in the backcross example discussed here) and L_1 is the likelihood of the alternative hypothesis ($b^* \neq 0$) at the specific QTL location (θ).

The likelihood ratio for a given position in the genome is chi-square distributed with one or two degrees of freedom for a backcross or F_2 population, respectively (Lander and Botstein, 1989). However, due to multiple testing throughout the genome, we cannot compare test statistics to a simple chi-square distribution. The development of permutation testing for interval mapping (Churchill and Doerge, 1994) allows us to produce population-specific significance criterion while accounting for multiple testing. Permutation tests randomly pairs an individual's phenotype with another individual's genotypes and performs

interval mapping on the permuted samples. This is repeated, generating a distribution of likelihood ratio values that may be obtained under the null hypothesis. By pairing individual *i*'s genotype with individual *j*'s phenotype, we simulate the null hypothesis of no relationship between genetic and phenotypic variation while maintaining the statistical properties of the population.

However, one downfall of simple interval mapping is an increased bias of detection. While searching for QTL in a given marker interval, QTL located in other intervals may artificially increase the likelihood ratio, increasing our risk of false positive QTL. This is especially an issue when searching marker intervals on the same chromosome as a significant QTL (Zeng, 1994). The development of composite interval mapping (CIM) by Zeng (1994) allows us to fit background molecular markers to account for background QTL. CIM expands the mixture model for interval mapping above to include background markers. As with interval mapping, CIM tests the null hypothesis that an interval between two markers has no QTL ($b^* = 0$) while controlling for genetic background effects with molecular marker co-factors. As a result of conditioning on background markers, variance of the test statistic will decrease, increasing the power for QTL detection (Zeng, 1994). CIM also uses permutation tests to determine a significance threshold, as long as the user is careful that permutations use the same number of molecular cofactors as the QTL analysis.

QUANTITATIVE TRAIT LOCI INFLUENCING GROWTH IN MICE

There are over 200 QTL that influence some aspect of mouse growth (Pomp, 2005). A partial list of mouse QTL influencing body weight or weight gain during ontogeny are outlined in Table 2.2. A variety of strains have been used in mapping the QTL in Table 2.2.

Thus, some portion of these growth QTL may represent strain-specific genetic variation. However, we do see certain genomic regions are consistently identified in genomewide searches across multiple strains. These include the 70-80 cM region of chromosome 2, the 50-65 cM region of chromosome 4, the 25-50 cM region of chromosome 8, the 30-60 cM region of chromosome 10 and the 30-45 cM region of chromosome 11.

Until recently, little attention was paid to the fact that different growth traits were analyzed to find growth genes/QTL. However, there is clear evidence that weights at different times in ontogeny are not equivalent. Rather, mammalian body size clearly changes over the course of ontogeny, in terms of causal influences, such as prenatal and postnatal maternal effects. In addition, cellular aspects of growth change over ontogeny, including hyperplasia (cell number), hypertrophy (cell size), and programmed cell death (apoptosis), among others (Winick and Noble, 1965; Atchley *et al.* 2000). Since cellular attributes of growth vary over ontogeny, body weight at different times will have different cellular aspects, i.e., different proportions of cell number and size. If cellular differences are under different genetic control, body weights in different phases of ontogeny may be influenced by different QTL (age-specific growth QTL). These age-specific growth loci would only be detected by analyzing multiple growth traits within the same experiment.

Cheverud and colleagues (1996) were among the first to search for age-specific growth QTL in mice by analyzing multiple body weights (also listed in Table 2.2). They found three sets of growth QTL: one set acting from birth to between 3 and 6 weeks of age, one set acting from 6 to 10 weeks of age, and one set influencing growth throughout ontogeny (Cheverud *et al.* 1996). Researchers hypothesize the first two sets of QTL

influence hyperplasia and hypertrophy, respectively (Cheverud *et al.* 1996). However, this has not been verified and we do not know if the mice used in this mapping experiment have altered cellular components of growth. Subsequent experiments in other strains found similar results, although significant genomic regions vary across experiments (Morris *et al.* 1999; Vaughn *et al.* 1999; Brockmann *et al.* 2004; Rocha *et al.* 2004a). Several genomic regions influencing age-specific growth overlap with genomic regions previously described as influencing a single body weight (Table 2.2).

All documented age-specific growth QTL in table 2.2 are positively pleiotropic, where one QTL allele increases all age-specific weights (Cheverud *et al.* 1996; Vaughn *et al.* 1999; Rocha *et al.* 2004; Brockmann *et al.* 2004). This is somewhat surprising, given previous statistical analyses of growth during development. Phenotypic variance for age-specific body weights typically reaches a maximum near weaning and subsequently decreases, eventually plateauing near puberty (Eisen, 1975; Riska *et al.* 1984). Riska and colleagues (1984) outlined that variance in a trait at time "t" is a function of variance of previous growth ("t-1") plus covariance(s) among growth rates. Thus, since phenotypic variance for body weight decreases as ontogeny continues, it is likely due to negative covariances or correlations between growth rates. We therefore expect a proportion of age-specific growth QTL would reflect the negative genetic covariation in ontogeny, i.e., negatively pleiotropic. However, this is not the case.

One downfall to previous age-specific QTL mappings is that multiple weights have been treated as single traits. This ignores the correlational structure between age-specific weights and may decrease the power of QTL detection (Jiang and Zeng, 1995). In addition,

analyzing age-specific weights as single traits makes it difficult to test if QTL effects change over ontogeny. The extension of CIM analysis to analyze multiple traits (multi-trait composite interval mapping, or MCIM) from Jiang and Zeng (1995) allows us to treat age-specific weights as multiple, correlated traits and to determine if a QTL pleiotropically influences multiple weights. MCIM uses similar models to that of CIM, combining interval mapping and multiple regression. As with CIM, fitting multiple markers in the regression model decreases residual variance and increases power (Jiang and Zeng, 1995). The test statistic produced from MCIM is a likelihood ratio for all mapped traits, i.e., does the potential QTL have a significant additive or dominant effect for at least one of "n" traits? Once a QTL is significant at the genome-wide level, trait-specific likelihood ratios are examined to determine if a QTL influences a given trait. If the likelihood ratio for trait "m" at the suggested QTL location is greater than a threshold value "X" (chi-square distribution with 'n' degrees of freedom equal to the number of genetic parameters), the QTL significantly influences "m". If a QTL influences multiple traits, genotype-by-environments tests can test if genetic effects are significantly different between a pair of traits influenced by the same QTL. This is especially relevant in mapping QTL influencing age-specific weights, since it allows us to test if the QTL has an ontogenetic dynamic, i.e., if QTL effects change as development continues.

GROWTH IN MICE SELECTED FOR AGE-SPECIFIC GROWTH

Despite the clear biological interest, no age-specific growth QTL mappings have been performed in mice selected for age-specific growth. Indeed, QTL in table 2.2 have been mapped in mice selected for altered growth in one phase of ontogeny, such as

increased 6-week body weight or gain between 3 and 6 weeks of age. However, there are several advantages to characterizing the genetic architecture of growth in mice selected for age-specific growth. Body weight gain during different phases of ontogeny is correlated with cell number and cell size (Falconer *et al.* 1978). Thus, selection for age-specific growth provides an indirect mechanism to produce changes in cell number and cell size. By characterizing the genetic architecture of growth in mice subject to age-specific selection, we can determine if 1. selection for altered growth at different stages in ontogeny has acted on different sets of loci; and 2. which loci influence hyperplasia and/or hypertrophy. This first experimental goal is especially relevant since QTL influencing growth in inbred strains can differ from QTL influencing growth in strains subject to selection (Keightley *et al.* 1996; Morris *et al.* 1999). Thus, age-specific QTL in inbred strains may not reflect QTL on which age-specific selection would act.

The next chapter of this dissertation characterizes the genetic architecture of growth in four mice strains produced from a restricted index selection experiment (Atchley *et al.* 1997). Mice were selected for changes in either body weight gain between birth and ten days of age (early gain, or EG) or body weight gain between 28 and 56 days of age (late gain, or LG). Four replicated selection lines and a randombred control line were produced, each subjected to a different selection treatment. The four selection treatments were as follows: $E^{+}L^{0}$ mice were selected for increased early gain, EG, while holding late gain, LG, constant. Its reciprocal line, $E^{-}L^{0}$, was selected for decreased EG while holding LG constant. $E^{0}L^{+}$ mice were selected for increased LG while holding EG constant; $E^{0}L^{-}$ mice

were selected for decreased LG while holding EG constant. Each selection treatment was replicated three times to assess for genetic drift, producing a total of 15 selection lines.

After 14 generation of restricted index selection, the two early-selected lines ($E^{+}L^{0}$ and $E^{-}L^{0}$) were significantly different in early gain (EG) and body weights throughout ontogeny, starting at 10 days of age and continuing into adulthood (Atchley *et al.* 1997). Early-selected mice also had significant differences in late gain (LG) as a result of selection, but on a lower magnitude than early gain changes (Atchley *et al.* 1997). The two late selected lines ($E^{0}L^{+}$ and $E^{0}L^{-}$) were significantly different for LG and body weights after 28 days of age but showed no differences in early gain or early body weights from control mice as anticipated with restricted index selection (Atchley *et al.* 1997). As hypothesized, selection for divergence in EG has lead to significant differences in cell number; selection for divergence in late growth has lead to differences in cell size (Atchley *et al.* 2000). After 35 generations of genetic selection, the best performing replicate from each of the four selection lines were brother-sister mated to create inbred strains. These resultant inbred strains were analyzed in Chapters 3 and 4 of this dissertation.

CORRELATED RESPONSES IN MICE SELECTED FOR AGE-SPECIFIC GROWTH

Selection for age-specific growth has also lead to significant differences in a variety of correlated traits. Rhees and Atchley (2000) demonstrated selection for altered growth has produced correlated changes in growth curve parameters. Rhees and Atchley (2000) examined the effect of selection treatment and replicate on five growth curve parameters (mature body weight [MBW], shape parameter, intrinsic growth rate [IGR], maximum growth rate [MGR], and age of maximum growth rate/inflection point of the growth curve

[IP]) in mice from the 19th generation of selection. Selection treatment was a significant source of variation ($p<0.0001$) for MBW, IGR, MGR, and IP. Four of the five growth curve parameters were also influenced by selection replicate, illustrating drift's role in correlated responses. In general, selection for increased growth (E^+ and L^+) increased mature body weight (MBW) and maximum growth rate (MGR), relative to control, E^- and L^- mice. Interestingly, selection for increased early growth (E^+) did not alter the inflection point of the growth curve, relative to control mice. Rather, E^- and L^+ mice delayed the inflection point, relative to E^+, L^-, and control mice. Selection for altered growth has also lead to correlated responses in tail lengths, with selection for increased growth (E^+ and L^+) leading to increased tail length.

Miller and colleagues (2000) demonstrated selection for altered growth had also lead to differences in longevity between selection treatments. In general, L^- mice increased longevity, relative to L^+ mice; however, replicates of the same selection treatment varied in longevity, again illustrating drift's role in correlated responses. Interestingly, drift also played a role in the cause of death in one selection treatment. One replicate of the E^+ selection treatment (E^+, replicate 2) had an increased incidence of pituitary adenoma (84%; 16 out of 19 mice with determinable causes of death), relative to E^+, replicates 1 and 3 mice (Miller *et al.* 2000).

Selection for altered growth has also impacted reproduction and fertility. Ernst and colleagues (1999) demonstrated early down-selected females from the 23rd generation of selection had delayed vaginal opening, likely due to a later achievement of the body weight or body size required for puberty onset. In contrast, late down-selected females had vaginal

opening at the same age as control females, but weighed less at vaginal opening (Ernst *et al.* 1999). Selection for early growth has also affected ovulation rate. Early up-selected females from the 28th and 29th generation of selection had increased ovulation rate, relative to control females, when induced by hormones (Ernst *et al.* 2000). Embryo transfers between early up-selected, early down-selected, and control females illustrated clear maternal effects for body weight and tail lengths of gestated progeny. Body weight and tail length of progeny were largest if progeny were gestated in E^+ females and smallest if gestated in control females (Ernst *et al.* 2000). Later research by Rhees and colleagues (1999) showed uterine and nursing effects changed as a correlated response to selection for altered early growth. More importantly, uterine components interacted with the fetus's genotype to significantly influence growth from birth to adulthood. Thus, the specific uterine change in mothers was conditioned by their progeny's developmental requirements, illustrating maternal effects responded to selection for altered early growth.

FURTHER PROSPECTS

Considerable effort has been put forth determining initial genomic regions influencing growth, as evidenced in Table 2.2. However, too little effort has been put forth to following-up initial results to narrow initial QTL regions and ultimately determine the nucleotide-level variation. Part of the difficulty lies in where to start fine mapping after initial genomewide screens. Initial screens can find QTL regions up to 20 cM in length, with up to thousands of putative candidate genes. Several new developments in molecular technology can help improve the likelihood of success and shorten the time necessary to fine map QTL regions. Single nucleotide polymorphism arrays (SNP chips) can be used to

decrease the size of these initial regions; the first commercially available pre-printed mouse 5K SNP chip was released from ParAllele in 2005. Alternatively, we can explore gene expression differences in candidate genes with a combined QTL mapping/mRNA microarray analysis (e.g., Wayne and McIntyre, 2002).

In addition, more conventional mapping procedures can be used to better characterize QTL and narrow initial QTL regions. These genetic strategies involve developing new strains or offspring and include the use of overlapping congenic and interval-specific congenic strains, advanced intercross lines (AIL), recombinant progeny testing, recombinant inbred strains (RIS), and selective phenotyping of recombinant progeny. Congenic strains are developed by introgressing QTL alleles from a "donor" strain into the genetic background of a "recipient" strain via a series of backcrosses. Congenic strains, in addition to progeny testing, have been used to fine map a growth QTL on chromosome 2 and to better explore the role of diet and age on expression of the QTL (Jerez-Timaure *et al.* 2004; Jerez-Timaure *et al.* 2005). Interval-specific congenic strains (ISCS) are strains with recombination points in specific intervals, where multiple strains are used to cover regions of a chromosome (Darvasi, 1997). With defined recombination points, QTL can be resolved to a 1-cM window, decreasing the number of candidate genes for follow up. ISCS have also been used to isolated modifiers of growth QTL, such as those modifying the *hg* gene (Farber *et al.* 2006).

RIS are produced by creating an F_1 population from two inbred strains, then brother-sister mating randomly selected pairs from the F_2 population for at least 20 generations. Since RIS are inbred strains, mice within a strain are genetically identical so each strain can

be genotyped once but phenotypic measurements on multiple individual will decrease environmental variation. In addition, there is greater recombination (i.e., more breakpoints), leading to greater mapping resolution, such as that needed to fine-map QTL. In addition, RIS have been used to map novel growth QTL in mouse strains, such as the SM/J and A/J (Anunciado *et al.* 2001) and LXS strains (Bennett *et al.* 2005).

Despite these genetic and molecular biology resources, there are only a few examples of fine-mapped regions where the causal genetic variation has been identified and described. The QTL ObwX (Table 2.2; Rance *et al.* 1997) was initially fine mapped to a 2-cM region on the X chromosome (Liu *et al.* 2001). Subsequent work via progeny testing determined the causal genetic variation was regulatory variation in the glypican-3 (*Gcp-3*) gene (Oliver *et al.* 2005). Mice with the high-growth ObwX allele have lower expression of the *Gcp-3* gene but the downstream effects of the altered expression were not determined (Oliver *et al.* 2005). Interestingly, loss-of-function mutations in *Gcp-3* lead to the Mendelian disease Simpson-Golabi-Behmel syndrome, showing a gene underlying a Mendelian disease can also impact phenotypic variation.

Christians and colleagues (2006) recently narrowed a QTL influencing 6-week body weight on chromosome 1 (QTL Bw6a; see Table 2.2) to a region containing only four genes. Of the four genes, there was one clear candidate gene (*Pappa2*) with genetic variation between QTL alleles. The *Pappa2* gene encodes an enzyme that cleaves insulin-like growth factor binding protein 5 (IGFBP-5) that then stimulates bone formation (Christians *et al.* 2006). Interestingly, Christians and colleagues (2006) found no altered expression of *Pappa2* but did find altered expression of genes downstream in the bone formation pathway.

This suggests a combined QTL mapping/mRNA microarray analysis may not find altered expression in the genes with causal genetic variation, but rather genes downstream in a biological pathway. In addition, what appears to be one initial QTL on chromosome 1 fractionated into four closely-linked QTL (Christians and Keightley, 2004; Christians *et al.* 2006), suggesting even one identified QTL may represent several causal genes.

No follow-up work has been conducted on QTL influencing age-specific growth. Such work is necessary to determine what biological signal triggers age-specific QTL expression. For example, the 50-70 cM region of chromosome 7 contains age-specific growth QTL in several mouse strains (see table 2.2). This genomic region also contains the genomically imprinted *IGF2* gene, among other genomically imprinted genes. Genomically imprinted genes are characterized by one active parental allele that is transcribed and one silent parental allele. The differential expression of parental alleles is achieved via differential DNA methylation at the two alleles. Methylation levels of both the maternal and paternal *IGF2* gene vary temporally and spatially in prenatal ontogeny, where methylation and *IGF2* levels are correlated (Weber *et al.* 2001). In addition, recent QTL experiments have shown genetic variance and/or epigenetic variation at genomically imprinted genes is associated with phenotypic variation, denoting a potential role in selection response (Mantey *et al.* 2005 and references therein). Methylation variation may play a role in age-specific growth, where methylation level varies over ontogeny and alters expression at genomically imprinted genes. However, more follow-up research is necessary to better understand how age-specific growth QTL lead to different cellular and physiological aspects of growth in ontogeny.

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Table 2.1 Summary of single gene mutations influencing growth in the mouse.
[a] denotes the QTL name as assigned by Mouse Genome Informatics (http:// informatics.jax.org) or publication.
[b] denotes publication in which genetic mutation was described at the molecular level. If mutation not known, publication is first description of mutation.
[c] denotes traits(s) a given QTL influences. BW denotes body weight at a specific time point in development.
[d] denotes chromosome and peak chromosomal cM location via Mouse Genome Informatics.

Name[a]	Reference[b]	Trait[c]	Chr, cM[d]	Comments
diminutive (*dm*)	Stevens and Mackensen, 1958	Decreased body size, skeletal abnormalities, decreased viability	2, 80 cM	Gene unknown
little (*lit*)	Godfrey et al. 1993	Decreased bone mass, decreased BW after 2-weeks of age	6, 26 cM	Mutation in *Ghrhr* gene
high growth (*hg*)	Horvat and Medrano, 2001	Increased 8 and 10 week BW	10, 52 cM	Mutation in *Soc2/Cish2* gene
pygmy (*pg*)	Zhou et al. 1995	Decreased body weight, altered expression of IGF-2 binding proteins	10, 68 cM	Mutation in *Hmgic* gene
ames (*df*)	Sornson et al. 1996	Decreased growth hormone, prolactin, and thyroid hormone production	11, 25 cM	Mutation in *Prop1* gene
miniature (mn)	Bennett, 1961	Severe growth retardation; early death	15, 24 cM	Gene unknown
snell (*dw*)	Camper et al. 1990	Decreased growth hormone, prolactin, and thyroid hormone production	16, 44 cM	Mutation in *Pit1* gene

Table 2.2 Summary of QTL influencing growth in the mouse

[a] denotes QTL name as assigned by Mouse Genome Informatics (http:// informatics.jax.org) or publication.
[b] denotes publication in which QTL was described
[c] denotes traits(s) a given QTL influences. BW denotes body weight at a specific time point in development.
[d] denotes chromosome and peak chromosomal cM location
[e] denotes mapping strains. Females' strain is listed first. Unique strains: NMRI8 mice were selected for high BW at 8-weeks; DU6 mice was selected for high BW at 6-weeks; DU6i mice are inbred mice derived from DU6 mice; M16i mice are inbred mice derived from a strain selected for increased 3- to 6-week growth (M16); L6 mice were selected for low 6-week BW; C57Bl/6J x M. m. castaneus reflects an intersubspecies cross of Mus musculus domesticus x M. m. castaneus. C57Bl/6J x MH reflects a cross between inbred strain C57Bl/6J and strain MH, a strain selected for high heat loss.
[f] The term "Cluster of QTL influencing multiple traits" denotes multiple QTL within the same marker interval influencing multiple growth traits. The term "Same marker interval influences multiple body weights" reflects that the authors suggest a single QTL in the marker interval influences multiple body weights.

Name[a]	Reference[b]	Trait[c]	Chr, cM[d]	Strain pair[e]	Comments[f]
Wt10q1	Moody et al. 1999	10-week BW	1, 25 cM	C57Bl/6J x MH	
Wt6q1	Moody et al. 1999	6-week BW	1, 27 cM	C57Bl/6J x MH	
W3q13	Rocha et al. 2004	3-week BW	1, 33 cM	M16i x L6	
Bw5	Brockmann et al. 1998[1]; 2000[2]	6-week BW	1, 36 cM	[1]DUK X DU6 [2]DU6i x DBA/2	
Bwnd4wk1 Bwnd5wk1	Brockmann et al. 2004	4-week BW 5-week BW	1, 44 cM 1, 45 cM	NMRI8 x DBA/2	Cluster of QTL influencing multiple traits
-------	Morris et al. 1999	3-week BW	1, 49 cM	DBA/2J x C57Bl/6J	
W3q12 Lgq5 W10q6 W6q14 W10q7	Rocha et al. 2004	3-week BW 6-10 week gain 10-week BW 6-week BW 10-week BW	1, 51 cM 1, 54 cM 1, 56 cM 1, 61 cM 1, 72 cM	M16i x L6	Cluster of QTL influencing multiple traits
Bqlq1	Cheverud et al. 1996	6-10 week gain	1, 53 cM	LG/J x SM/J	
-------	Morris et al. 1999	10-week BW	1, 54 cM	DBA/2J x C57Bl/6J	
-------	Morris et al. 1999	6-week BW	1, 56 cM	DBA/2J x C57Bl/6J	
Bwnd6wk1	Brockmann et al. 2004	6-week BW	1, 72 cM	NMRI8 x DBA/2	
Wt3q1	Moody et al. 1999	3-week BW	1, 72 cM	C57Bl/6J x MH	
Bw6a	Keightley et al. 1996	6-week BW	1, 76 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
---------	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	1, 84 cM	LG/J x SM/J	
Lgq4	Rocha et al. 2004	6-10 week gain	1, 93 cM	M16i x L6	
Bqeq1	Cheverud et al. 1996	1-3 week gain	1, 102 cM	LG/J x SM/J	
Wt6q2	Moody et al. 1999	6-week BW	1, 108 cM	C57Bl/6J x MH	
Wt3q2	Moody et al. 1999	3-week BW	1, 108 cM	C57Bl/6J x MH	

Table 2.2 (continued)

Pbwg1	Ishikawa et al. 2000; Ishikawa and Namikawa, 2004; Ishikawa et al. 2005	Weekly BW from 4-weeks of age to 10-weeks of age; Principal component for weekly BW	2, 33 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights
Bwnd2wk1 Bwnd4wk2 Bwnd5wk2 Bwnd6wk2	Brockmann et al. 2004	2-week BW 4-week BW 5-week BW 6-week BW	2, 47-50 cM	NMRI8 x DBA/2	Cluster of QTL influencing multiple BW
Egq1 W6q1 W10q9 W3q1	Rocha et al. 2004	3-6 week gain 6-week BW 10-week BW 3-week BW	2, 49 cM 2, 51 cM 2, 51 cM 2, 54 cM	M16i x L6	Cluster of QTL influencing multiple BW
Bw6	Brockmann et al. 1998	6-week BW	2, 56 cM	DUK X DU6	
Q2Ucd2-wg29	Corva et al. 2001	2-9 week gain	2, 61 cM	C57BL/6J-*hg/hg* x Cast/EiJ	Likely modifier of *hg* locus
Bqeq2	Cheverud et al. 1996	1-3 week gain	2, 69 cM	LG/J x SM/J	
W3q2 W6q2 Egq2 W10q1 Lgq2	Rocha et al. 2004	3-week BW 6-week BW 3-6 week gain 10-week BW 6-10 week gain	2, 78 cM 2, 78 cM 2, 78 cM 2, 80 cM 2, 82 cM	M16i x L6	Cluster of QTL influencing multiple BW
---------	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	2, 80 cM	LG/J x SM/J	
Bqlq2	Cheverud et al. 1996	6-10 week gain	2, 86 cM	LG/J x SM/J	
Bwnd2wk2 Bwnd5wk3	Brockmann et al. 2004	2-week BW 5-week BW	2, 109 cM	NMRI8 x DBA/2	
Lgq3	Rocha et al. 2004	6-10 week gain	3, 16 cM	M16i x L6	
W10q3	Rocha et al. 2004	10-week BW	3, 30 cM	M16i x L6	
Egq3	Rocha et al. 2004	3-6 week gain	3, 40 cM	M16i x L6	
W6q7	Rocha et al. 2004	6-week BW	3, 47 cM	M16i x L6	

Table 2.2 (continued)

W3q9	Rocha et al. 2004	3-week BW	3, 53 cM	M16i x L6	
Wt10q2	Moody et al. 1999	10-week BW	3, 61 cM	C57Bl/6J x MH	
Bwnd2wk3	Brockmann et al. 2004	2-week BW	3, 66 cM	NMRI8 x DBA/2	
Bqlq3	Cheverud et al. 1996	6-10 week gain	3, 93 cM		
Bw6b	Keightley et al. 1996	6-week BW	4, 26 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
W6q15	Rocha et al. 2004	6-week BW	4, 36 cM	M16i x L6	
---------	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	4, 50 cM	LG/J x SM/J	
Bqeq3	Cheverud et al. 1996	1-3 week gain	4, 54 cM	LG/J x SM/J	
-------	Morris et al. 1999	3-week BW	4, 55 cM	DBA/2J x C57Bl/6J	
W6q13 W10q10	Rocha et al. 2004	6-week BW 10-week BW	4, 55 cM 4, 55 cM	M16i x L6	Cluster of QTL influencing multiple BW
Bw7	Brockmann et al. 1998[1]; 2000[2]	6-week BW	4, 57 cM	[1]DUK X DU6 [2]DU6i x DBA/2	
Pbwg2	Ishikawa et al. 2000; Ishikawa and Namikawa, 2004; Ishikawa et al. 2005	9-week BW; Principal component for weekly BW	4, 62 cM	C57Bl/6J x M. m. castaneus	
W3q17	Rocha et al. 2004	3-week BW	4, 64 cM	M16i x L6	
Pbwg14	Ishikawa et al. 2005	8-week BW	5, 1 cM	C57Bl/6J x M. m. castaneus	Female-specific
Lgq6	Rocha et al. 2004	6-10 week gain	5, 26 cM	M16i x L6	
Bw6d	Keightley et al. 1996	6-week BW	5, 35 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
W10q16	Rocha et al. 2004	10-week BW	5, 42 cM	M16i x L6	

Table 2.2 (continued)

Bw8	Brockmann et al. 1998	6-week BW	5, 42 cM	DUK X DU6	
Bw6c	Keightley et al. 1996	6-week BW	5, 60 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
Bw13	Brockmann et al. 2000	6-week BW	5, 81 cM	DU6i x DBA/2	
---	Vaughn et al. 1999	3-6 week gain	5, 130 cM	LG/J x SM/J	
---	Morris et al. 1999	10-week BW	6, 4 cM	DBA/2J x C57Bl/6J	
Bw6e	Keightley et al. 1996	6-week BW	6, 22 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
Egq8 W6q4 W3q3 W10q11	Rocha et al. 2004	3-6 week gain 6-week BW 3-week BW 10-week BW	6, 26 cM 6, 27 cM 6, 29 cM 6, 29 cM	M16i x L6	Cluster of QTL influencing multiple BW
Pbwg8	Ishikawa and Namikawa, 2004;	Principal component for weekly BW	6, 32 cM	C57Bl/6J x M. m. castaneus	
Bqeq4	Cheverud et al. 1996	1-3 week gain	6, 45 cM	LG/J x SM/J	
W6q5 Egq11	Rocha et al. 2004	6-week BW 3-6 week gain	6, 50 cM 6, 51 cM	M16i x L6	Cluster of QTL influencing multiple BW
Bqlq5	Cheverud et al. 1996	6-10 week gain	6, 62 cM	LG/J x SM/J	
---	Morris et al. 1999	6-week BW	6, 71 cM	DBA/2J x C57Bl/6J	
---	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	6, 88 cM	LG/J x SM/J	
W6q17 W10q18 W3q10	Rocha et al. 2004	6-week BW 10-week BW 3-week BW	7, 18 cM 7, 18 cM 7, 24 cM	M16i x L6	Cluster of QTL influencing multiple BW

Table 2.2 (continued)

Bwnd2wk4 Bwnd4wk3 Bwnd5wk4 Bwnd6wk3	Brockmann et al. 2004	2- week BW 4- week BW 5- week BW 6-week BW	7, 20-30 cM	NMRI8 x DBA/2	Cluster of QTL influencing multiple BW
Bw6f	Keightley et al. 1996	6-week BW	7, 25 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
Bw14	Brockmann et al. 2000	6-week BW	7, 28 cM	DU6i x DBA/2	
W3q8	Rocha et al. 2004	3-week BW	7, 34 cM	M16i x L6	
Bqeq5	Cheverud et al. 1996	1-3 week gain	7, 37 cM	LG/J x SM/J	
---------	Vaughn et al. 1999	1-3 week gain	7, 50 cM	LG/J x SM/J	
Bqlq6	Cheverud et al. 1996	6-10 week gain	7, 51 cM	LG/J x SM/J	
Bqeq6	Cheverud et al. 1996	1-3 week gain	7, 60 cM	LG/J x SM/J	
Pbwg3	Ishikawa et al. 2000; Ishikawa and Namikawa, 2004; Ishikawa et al. 2005	Weekly BW from 5-weeks of age to 9-weeks of age; Principal component for weekly BW	7, 72 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights
---------	Vaughn et al. 1999	1-3 week gain	8, 24 cM	LG/J x SM/J	
Egq12 W10q14 W6q9	Rocha et al. 2004	3-6 week gain 10-week BW 6-week BW	8, 24 cM 8, 26 cM 8, 29 cM	M16i x L6	Cluster of QTL influencing multiple BW
Bqeq7	Cheverud et al. 1996	1-3 week gain	8, 32 cM	LG/J x SM/J	
W3q5	Rocha et al. 2004	3-week BW	8, 37 cM	M16i x L6	
Q8Ucd1-wg29	Corva et al. 2001	2-9 week gain	8, 45 cM	C57BL/6J-*hg/hg* x Cast/EiJ	Likely modifier of *hg* locus
---------	Vaughn et al. 1999	3-6 week gain	8, 56 cM	LG/J x SM/J	
Bwnd2wk5 Bwnd3wk1 Bwnd4wk4	Brockmann et al. 2004	2- week BW 3- week BW 4-week BW	8, 57-66 cM	NMRI8 x DBA/2	Cluster of QTL influencing multiple BW

Table 2.2 (continued)

-------	Morris et al. 1999	3-week BW	9, 8 cM	DBA/2J x C57Bl/6J	
Bwnd4wk5 Bwnd5wk5 Bwnd6wk4	Brockmann et al. 2004	4- week BW 5- week BW 6-week BW	9, 27-35 cM	NMRI8 x DBA/2	Cluster of QTL influencing multiple BW
Bw6g	Keightley et al. 1996	6-week BW	9, 32 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
Bqlq7	Cheverud et al. 1996	6-10 week gain	9, 35 cM	LG/J x SM/J	
---------	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	9, 42 cM	LG/J x SM/J	
Pbwg15	Ishikawa et al. 2005	Weekly BW from 3-weeks of age to 4-weeks of age	9, 43 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights
-------	Morris et al. 1999	6-week BW	9, 50 cM	DBA/2J x C57Bl/6J	
W10q13	Rocha et al. 2004	10-week BW	9, 54 cM	M16i x L6	
Pbwg4	Ishikawa et al. 2000; Ishikawa and Namikawa, 2004; Ishikawa et al. 2005	5-week BW; Principal component for weekly BW	9, 69 cM	C57Bl/6J x M. m. castaneus	
Pbwg9	Ishikawa et al. 2000; Ishikawa and Namikawa, 2004; Ishikawa et al. 2005	3-6 week gain; Principal component for weekly BW	10, 14 cM	C57Bl/6J x M. m. castaneus	
W3q11 Egq7 W6q6 W10q5	Rocha et al. 2004	3-week BW 3-6 week gain 6-week BW 10-week BW	10, 26 cM 10, 28 cM 10, 30 cM 10, 32 cM	M16i x L6	Cluster of QTL influencing multiple BW
Pbwg16	Ishikawa et al. 2005	3-week BW	10, 42 cM	C57Bl/6J x M. m. castaneus	

Table 2.2 (continued)

W6q8 Egq9	Rocha et al. 2004	6-week BW 3-6 week gain	10, 53 cM 10, 53 cM	M16i x L6	Cluster of QTL influencing multiple BW
Pbwg5	Ishikawa et al. 2000; Ishikawa and Namikawa, 2004; Ishikawa et al. 2005	7-week BW; Principal component for weekly BW	10, 68 cM	C57Bl/6J x M. m. castaneus	
---------	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	10, 84 cM	LG/J x SM/J	
Bqlq8	Cheverud et al. 1996	6-10 week gain	11, 2 cM	LG/J x SM/J	
W3q4 W10q2 Lgq1	Rocha et al. 2004	3-week BW 10-week BW 6-10 week gain	11, 13 cM 11, 13 cM 11, 17 cM	M16i x L6	Cluster of QTL influencing multiple BW
Bw16	Brockmann et al. 2000	6-week BW	11, 14 cM	DU6i x DBA/2	
W6q3 Egq4	Rocha et al. 2004	6-week BW 3-6 week gain	11, 20 cM 11, 23 cM	M16i x L6	Cluster of QTL influencing multiple BW
--------	Kirkpatrick et al. 1998	6-week BW	11, 25 cM		Position estimated from SSR location
-------	Morris et al. 1999	3-week BW	11, 28 cM	DBA/2J x C57Bl/6J	
Wt10q3	Moody et al. 1999	10-week BW	11, 32 cM	C57Bl/6J x MH	
Wt6q3	Moody et al. 1999	6-week BW	11, 36 cM	C57Bl/6J x MH	
---------	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	11, 36 cM	LG/J x SM/J	
Bw4	Brockmann et al. 1998	6-week BW	11, 42 cM	DUK X DU6	
Bqeq9	Cheverud et al. 1996	1-3 week gain	11, 45 cM	LG/J x SM/J	
Bw6h	Keightley et al. 1996	6-week BW	11, 45 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines

Table 2.2 (continued)

Q11Ucd1-wg29	Corva et al. 2001	2-9 week gain	11, 46 cM	C57BL/6J-*hg/hg* x Cast/EiJ	Likely modifier of *hg* locus
Bw4	Brockmann et al. 2000	6-week BW	11, 55 cM	DU6i x DBA/2	
Bqeq10	Cheverud et al. 1996	1-3 week gain	11, 77 cM	LG/J x SM/J	
Bqlq9	Cheverud et al. 1996	6-10 week gain	12, 11 cM	LG/J x SM/J	
---------	Vaughn et al. 1999	3-6 week gain	12, 24 cM	LG/J x SM/J	
Bw9	Brockmann et al. 1998[1]; 2000[2]	6-week BW	12, [1]17 cM; 12, [2]49 cM	[1]DUK X DU6 [2]DU6i x DBA/2	
Bqeq11	Cheverud et al. 1996	1-3 week gain	12, 41 cM	LG/J x SM/J	
W6q10 Egq10	Rocha et al. 2004	6-week BW 3- to 6-week gain	12, 53 cM 12, 55 cM	M16i x L6	Cluster of QTL influencing multiple BW
---------	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	12, 74 cM	LG/J x SM/J	
Bw15	Brockmann et al. 2000	6-week BW	13, 10 cM		
---------	Vaughn et al. 1999	1-3 week gain	13, 14 cM	LG/J x SM/J	
---------	Vaughn et al. 1999	3-6 week gain	13, 14 cM	LG/J x SM/J	
Bqlq10	Cheverud et al. 1996	6-10 week gain	13, 21 cM	LG/J x SM/J	
Bw10	Brockmann et al. 1998[1]; 2000[2]	6-week BW	12, [1]34 cM; 13, [2]47 cM		
Pbwg17	Ishikawa et al. 2005	Weekly BW from 6-weeks of age to 10-weeks of age	13, 46 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights
Eqaq4 W6q16 W10q17	Rocha et al. 2004	3- to 6-week gain (Adjusted for 3-week BW) 6-week BW 10-week BW	13, 35 cM 13, 50 cM 13, 55 cM	M16i x L6	Cluster of QTL influencing multiple BW

Table 2.2 (continued)

Pbwg6	Ishikawa et al. 2000; Ishikawa and Namikawa, 2004; Ishikawa et al. 2005	Weekly BW from 5-weeks of age to 10-weeks of age; Principal component for weekly BW	13, 53 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights
Bw6i	Keightley et al. 1996	6-week BW	13, 60 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
Bw6j	Keightley et al. 1996	6-week BW	14, 0 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
Bwnd4wk6 Bwnd5wk6 Bwnd6wk6	Brockmann et al. 2004	4-week BW 5-week BW 6-week BW	14, 12-15 cM	NMRI8 x DBA/2	Cluster of QTL influencing multiple BW
Bwnd3wk2	Brockmann et al. 2004	3-week BW	14, 23 cM	NMRI8 x DBA/2	
Pbwg18	Ishikawa et al. 2005	6-10 week gain	14, 30 cM	C57Bl/6J x M. m. castaneus	Female-specific
Bqlq11	Cheverud et al. 1996	6-10 week gain	14, 41 cM	LG/J x SM/J	
Bwnd4wk7	Brockmann et al. 2004	4-week BW	14, 49 cM	NMRI8 x DBA/2	
---------	Vaughn et al. 1999	3-6 week gain	14, 58 cM	LG/J x SM/J	
Bw11	Brockmann et al. 1998	6-week BW	15, 6 cM	DUK X DU6	
W3q7	Rocha et al. 2004	3-week BW	15, 21 cM	M16i x L6	
W3q6	Rocha et al. 2004	3-week BW	15, 35 cM	M16i x L6	
-------	Morris et al. 1999	10-week BW	15, 41 cM	DBA/2J x C57Bl/6J	
---------	Vaughn et al. 1999	1-3 week gain and 3-6 week gain	15, 46 cM	LG/J x SM/J	
Pbwg19	Ishikawa et al. 2005	5-week BW	16, 2 cM	C57Bl/6J x M. m. castaneus	Male-specific

Table 2.2 (continued)

---------	Vaughn et al. 1999	1-3 week gain	16, 14 cM	LG/J x SM/J	Male-specific
---------	Vaughn et al. 1999	1-3 week gain	16, 44 cM	LG/J x SM/J	Female-specific
Wt3q3	Moody et al. 1999	3-week BW	17, 14 cM	C57Bl/6J x MH	
Bw6k	Keightley et al. 1996	6-week BW	17, 14 cM	C57Bl/6J X DBA/2J (selected for 6-week BW)	Detected via marker allele frequency difference between selected lines
---------	Vaughn et al. 1999	3-6 week gain	17, 18 cM	LG/J x SM/J	
W6q11 W10q12 Egq5	Rocha et al. 2004	6-week BW 10-week BW 3- to 6-week gain	17, 19 cM 17, 19 cM 17, 19 cM	M16i x L6	Cluster of QTL influencing multiple BW
W10q15 W6q12 Egq6	Rocha et al. 2004	10-week BW 6-week BW 3- to 6-week gain	17, 30 cM 17, 35 cM 17, 35 cM	M16i x L6	Cluster of QTL influencing multiple BW
W3q15	Rocha et al. 2004	3-week BW	18, 30 cM	M16i x L6	
---------	Vaughn et al. 1999	3-6 week gain	18, 38 cM	LG/J x SM/J	
W3q16	Rocha et al. 2004	3-week BW	18, 40 cM	M16i x L6	
Bqlq12	Cheverud et al. 1996	6-10 week gain	18, 46 cM	LG/J x SM/J	
Pbwg20	Ishikawa et al. 2005	Weekly BW from 3-weeks of age to 4-weeks of age	19, 4 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights
W3q14	Rocha et al. 2004	3-week BW	19, 36 cM	M16i x L6	
Bqlq13	Cheverud et al. 1996	6-10 week gain	19, 51 cM	LG/J x SM/J	
Pbwg7	Ishikawa et al. 2000; Ishikawa and Namikawa, 2004; Ishikawa et al. 2005	Weekly BW from 5-weeks of age to 8-weeks of age; Principal component for weekly BW	X, 17 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights; male-specific
Pbwg21	Ishikawa et al. 2005	3-week BW; 3 to 6 week gain	X, 27 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights; male-specific

Table 2.2 (continued)

QbwX	Rance et al. 1997	3-week BW 6-week BW 10-week BW	X, 23 cM	?	
Pbwg22	Ishikawa et al. 2005	Weekly BW from 9-weeks of age to 10-weeks of age	X, 35 cM	C57Bl/6J x M. m. castaneus	Same marker interval influences multiple body weights; female-specific
Bw12	Brockmann et al. 1998	6-week BW	X, 42 cM	DUK X DU6	

Quantitative Trait Loci influencing Growth in Mice Selected for Rate of Development

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ABSTRACT

Growth during ontogeny, or age-specific growth, is characterized by different cellular attributes, i.e., increases in hyperplasia or hypertrophy, and may be influenced by different genes. To further investigate the differential genetic control of growth, quantitative trait loci (QTL) analysis was performed to search for chromosomal regions influencing growth. QTL were mapped in two F_2 populations derived from an age-specific growth selection experiment. These two F_2 populations differ in terms of selection for rate of development in early or late ontogeny, which has lead to correlated responses in hyperplasia and hypertrophy, respectively. Most chromosomal regions influencing growth do not overlap between populations, suggesting selection for age-specific growth has acted on different sets of loci, as hypothesized. However, epistatic interactions partially overlap between populations. Several loci in both populations were negatively pleiotropic, with genetic effects significantly changing over ontogeny, likely due to compensatory growth from selection. A high proportion of sex-specific genetic effects were detected in the hyperplastic F_2 population, suggesting early growth has some sex-specific pathways. Results in this report suggest age-specific growth is influenced by different direct effect QTL but may share genetic architecture via epistasis.

INTRODUCTION

Control of mammalian growth is a dynamic process involving the interaction of numerous genetic, cellular, and environmental variables. These interactions have been the subject of numerous analyses (Atchley, 1984; Riska *et al.* 1984; Cheverud *et al.* 1996; Atchley *et al.* 1997; Morris *et al.* 1999; Vaughn *et al.* 1999; Atchley *et al.* 2000; Cheverud *et al.* 2001; Brockmann *et al.* 2004; Rocha *et al.* 2004a; Yi *et al.* 2006 and references therein). However, despite abundant sources of heritable and nonheritable variation, final adult size in mammals typically falls within a narrow range. Even with environmental perturbances early in ontogeny, individuals can return to normal body size via compensatory growth in order to "catch-up" with animals whose growth was not altered. This limitation in adult phenotypes has been termed targeted or convergent growth (Tanner, 1963) and it plays an essential evolutionarily role. For example, body size in mice is correlated with a variety of physiological and fitness traits, including puberty onset and life span. Thus, an individual has a "target" in order to be reproductively competitive with the population (Calder, 1984). Individuals must assess their body size, relative to the target growth, and regulate growth accordingly.

Mammals use a feedback mechanism to accomplish this regulation, where the organism receives hormonal cues such as proteins in the growth hormone and insulin-like growth factor pathways, among others (Tanner, 1963; Yambayamba *et al.* 1996; Sonntag *et al.* 1999; Hornick *et al.* 2000). In response to these endocrine signals, an organism alters its growth rate through changes in metabolic activity, feed intake, and digestibility of food (Hornick *et al.* 2000). These physiological changes then lead to changes in cell number, cell

size, RNA expression, and protein concentrations, among other phenomena (Atchley, 1984; Riska *et al.* 1984; Hornick *et al.* 2000). Hormones including Insulin-like Growth Factors I and II (IGF-1, IGF-2), Growth Hormone (GH), thyroid and steroid hormones, and associated receptors are differentially expressed during compensatory growth in mammals and fish, illustrating they play key roles in induction and maintenance of altered growth (Hornick *et al.* 2000; Chauvigne *et al.* 2003; Picha *et al.* 2006).

In mice, targeted growth reduces variance in post-weaning growth, despite heterogeneity in pre-weaning growth. Phenotypic variance for log-transformed growth traits typically reaches a maximum near weaning and subsequently decreases, eventually plateauing near puberty (Eisen, 1975; Atchley, 1984; Riska *et al.* 1984). Additive genetic variance follows a similar pattern, increasing after birth to peak at weaning and subsequently decreasing. However, after weaning, there is often a secondary peak in additive genetic variance, between 4 and 8 weeks of age in the mouse (Atchley, 1984; Riska *et al.* 1984). This second peak in variance is independent of the first peak, suggesting novel gene expression at puberty (Rutledge *et al.* 1972; Riska *et al.* 1984). At the same time in ontogeny, a cellular shift in growth also occurs. During prenatal and early postnatal development, growth is primarily by hyperplastic growth (cell number), while growth later in ontogeny is primarily by hypertrophy, or cell size (Winick and Noble, 1965). This hyperplastic-to-hypertrophic cellular shift occurs between 3 and 6 weeks of age in the mouse, near the timing of the second peak in additive genetic variance. These two phenomena may be related; for example, this novel gene expression may reflect genes responsible for targeted

growth, repression of cell number, or activation of cell enlargement, leading to the cellular shift.

Developmentally complex traits such as growth are composite traits, comprised of components with different ontogenetic origins and influenced by different genetic and environmental factors (Atchley, 1987). How individual components develop and integrate to form a quantitative phenotype is essential to understand, as natural selection acts at both the individual and joint level of the component hierarchy. Hence, each component must be studied both individually and in concert, in terms of heritable and non-heritable factors. Body size is one such complex trait with components from cell number and cell size; growth during ontogeny occurs as the relative contribution of these two cellular mechanisms, among others. Thus, in order to understand how natural selection changes body size, we must understand how natural selection changes cell number and cell size, as well as their interaction via compensatory growth. Given that cellular attributes of growth vary over ontogeny, the timing of natural selection for growth (or, age-specific selection) may produce different changes in hyperplasia and hypertrophy. If these cellular processes are under separate genetic control, selection will act on different loci.

Previous molecular attempts to assess the differential genetic control of growth find three sets of growth loci: one set acting from birth to 3 weeks of age, one set acting from 6 to 10 weeks of age, and one set growth throughout ontogeny (Cheverud *et al.* 1996; Morris *et al.* 1999; Vaughn *et al.* 1999; Brockmann *et al.* 2004; Rocha *et al.* 2004a). Researchers suggest the first two sets of loci reflect hyperplastic and hypertrophic genes, respectively, but this has not been verified. Over 200 growth quantitative trait loci (QTL) have been identified

in the mouse, with the majority identified in inbred strains or lines subject to genetic selection for post-weaning growth (Corva and Medrano, 2001; Pomp, 2005). Such experiments use mice selected for phenotypic changes in growth, but such mice may not have altered developmental processes. Thus, previously mapped growth QTL do not necessarily represent genes leading to cellular aspects of growth, i.e., hyperplasia and hypertrophy. In addition, mapping experiments using inbred strains may not find selection-response genes, as Keightley and colleagues demonstrated (Keightley *et al.* 1996; Morris *et al.* 1999). In order to understand evolutionary forces' influence on the genetic architecture of growth, we must look at genes upon which natural selection will most likely act, i.e., selection response genes.

Herein, we describe results of a QTL mapping experiment using four mouse strains derived from a restricted index selection experiment, where each pair of mice was selected for changes in early growth (0 to 10 days of age) or late growth (28 to 56 days of age). These mouse lines were created to explore the cellular and genetic responses to selection for age-specific growth. It is well documented that body weight gain during different phases of ontogeny is correlated with cell number and cell size (e.g., Falconer *et al.* 1978). Thus, selection for age-specific growth provides an indirect mechanism to produce changes in cell number and cell size. Indeed, Atchley and colleagues (2000) verified selection for early and late growth did produce changes in hyperplasia and hypertrophy. By characterizing the genetic architecture of growth in these lines, we can experimentally determine if selection for altered growth at different stages in ontogeny has acted on different sets of loci and which loci influence hyperplasia and/or hypertrophy.

In this report, we discuss the results from a genomewide QTL mapping to find chromosomal regions influencing two sets of growth phenotypes. We map growth QTL in two populations: one population derived from mice selected for changes in early growth to model hyperplastic changes and a second population derived from mice selected for late growth to model hypertrophic changes. We evaluate the hypothesis that age-specific selection during two phases in ontogeny has acted upon two different sets of genes and ask the following questions:

1. What are the number, location, and effects of QTL influencing early and late growth in mice? Do genomic regions mapped here agree with previously-mapped growth loci in terms of location and genetic effect?
2. Are early and late growth under similar genetic control within and between populations?
3. Are different sets of growth-related phenotypes under similar genetic control within a population?
4. How do populations compare in terms of epistatic influences on growth during ontogeny?
5. Do covariances between phases of growth provide information on compensatory growth?

MATERIAL AND METHODS

Generation of Selection lines

Fifteen restricted index selection lines were created from an ICR Harlan Sprague-Dawley randombred mouse population (Atchley *et al.* 1997). The selection criteria were

body weight gain between birth and ten days of age (early gain, or EG) and body weight gain between 28 and 56 days of age (late gain, or LG). EG is growth primarily via changes in cell number while LG is growth via changes in cell size (Atchley *et al.* 2000). Four replicated selection lines and a randombred control line were produced, each subjected to a different selection treatment. Lines were each replicated three times to assess for genetic drift, producing a total of 15 selection lines. Each replicate was maintained as a separate entity and no crosses were made between replicates. Within family selection was performed to reduce maternal effects.

The four selection treatments were as follows: $E^{+}L^{0}$ mice were selected for increased early gain, EG, while holding late gain, LG, constant. Its reciprocal line, $E^{-}L^{0}$, was selected for decreased EG while holding LG constant. $E^{0}L^{+}$ mice were selected for increased LG while holding EG constant; $E^{0}L^{-}$ mice were selected for decreased LG while holding EG constant.

After 14 generation of restricted index selection, the two early-selected lines ($E^{+}L^{0}$ and $E^{-}L^{0}$) were significantly different in early gain (EG) and body weights throughout ontogeny, starting at 10 days of age and continuing into adulthood (Atchley *et al.* 1997). Early-selected mice also had significant differences in late gain (LG) as a result of selection, but on a lower magnitude than early gain changes (Atchley *et al.* 1997). The two late selected lines ($E^{0}L^{+}$ and $E^{0}L^{-}$) were significantly different for LG and body weights after 28 days of age but showed no differences in early gain or early body weights from control mice as anticipated with restricted index selection (Atchley *et al.* 1997). Statistically significant differences have accumulated by correlated response to selection in tail length, uterine and

maternal effects, reproductive onset, growth curve parameters, longevity, and hepatic endopolyploidy (Ernst *et al.* 1999; Rhees *et al.* 1999; Ernst *et al.* 2000; Miller *et al.* 2000; Rhees and Atchley, 2000; Funk-Keenan *et al.* 2006).

After 35 generations of genetic selection, the best performing replicate from each of the four selection lines were brother-sister mated to create inbred strains. Systematic inbreeding permits fixation of genetic variation for QTL mapping. The resultant inbred strains are designated in this report as E^+, E^-, L^+, and L^-.

Mapping Populations

Two F_2 mapping populations were produced: a hyperplastic F_2 population derived from the early-selected inbred strains (E^+ and E^-) and a hypertrophic F_2 population derived from the late-selected inbred strains (L^+ and L^-). The early population consisted of 551 F_2 mice originating from 23 E^+ females and 23 E^- males in two separate sets of parental matings. Similarly, the late population consisted of 519 F_2 mice originating from 20 L^+ females and 16 L^- males in two sets of matings. Parents from the first and second sets of matings were from the 8^{th} and 12^{th} generation of brother-sister breeding, respectively. All litters were standardized at birth to 8 pups per litter and, where possible, a balanced sex ratio between males and females. Pups were forcibly weaned at 21 days of age. Same sex adults were housed 3-4 per cage.

Growth Measurements

Eleven individual body weights and tail lengths were measured on each F_2 mouse, starting at 7 days of age and continuing weekly until 70 days of age. Measurements were also taken at 10 days of age as part of the selection criterion. Weights were recorded at 0.01g

accuracy using a digital balance. Birth weight per pup was calculated as the total litter weight divided by the number of pups in the litter. All mice were sacrificed between 70 and 73 days of age and bodies were saved for later morphological study. Mice with complete body weights from 7 to through 56 days of age were included in the analysis.

Several growth rates were calculated from body weights. Early gain (EG) and late gain (LG) are the selection criterion of 0- to 10-day gain and 28- to 56-day gain, respectively. A weaning growth rate (WG; weight gain between 7 and 21 days of age) and a middle growth rate (MG; weight gain between 21 and 35 days of age) were also calculated.

Growth curve traits were estimated for each individual mouse using the logistic growth curve equation (Laird and Howard, 1967). Calculations were done using nonlinear regression via PROC NLIN in SAS (SAS Institute, 2003). The equation was of the form

$$Y_i(t) = \frac{A_i}{1 + e_i^{b_i - kit}}$$

where $Y_i(t)$ is the body weight at day *t* for individual *i*. For each individual *i*, we estimated three growth curve parameters: A (the asymptotic or mature body weight; MBW), k (the intrinsic growth rate), and b (the shape parameter). We then calculated the maximum growth rate (MGR; calculated as *Ak*/4) which is achieved at time t' (inferred as the inflection point of the growth curve; calculated as *b/k*) for each individual.

Genotypes

Genomic DNA was isolated from F_2 tail samples. PCR amplification of polymorphic microsatellite loci was performed according to a protocol modified from Dietrich *et al*. (1992). Due to low microsatellite variation in certain genomic regions, single nucleotide polymorphisms (SNPs) were also genotyped. Polymorphic SNPs were discovered based on

variable regions from either the Broad Institute Mouse SNP map or from NCBI Mouse dbSNP. Polymorphic markers were genotyped using 10 or 12% nondenaturing polyacrylamide gels on the Mega-Gel High Throughout Vertical Electrophoresis Unit (CBS Scientific, Del Mar, CA) and visualized using ethidium bromide. A total of 88 and 85 polymorphic loci were genotyped in the early and late F_2 mice, respectively. A subset of markers was not informative for families derived from parents in the 8^{th} generation of inbreeding and were dropped from the analysis.

Linkage maps for the 19 autosomes were produced for both populations in MapMaker (Lander *et al.* 1987). The X chromosome was not included in the QTL analysis due to low genetic variation. Transmission ratio distortion of unknown origins was seen in both populations at a few isolated markers and is denoted in tables 3.1 and 3.2.

Single Trait Analyses

Seven growth traits were used as phenotypes for QTL mapping: four growth rates and three growth curve attributes. Growth rates analyzed were EG, WG, MG, and LG. EG and LG were included as selection criterion. WG was included to replicate the early growth trait used by Cheverud and colleagues (Cheverud *et al.* 1996; Vaughn *et al.* 1999). WG, as well as MG, were also included to genetic effects for times in ontogeny not under genetic selection (assessing compensatory growth). Three growth curve attributes were analyzed: mature body weight (MBW), maximum growth rate (MGR), and time of maximum growth rate, inferred as the inflection point of the growth curve (IP).

All seven traits were adjusted to remove fixed effects from F_2 mating set, litter (nested within line and set) and uterine litter size. The resultant residual values were used for

analysis. Residual values were found to be normally distributed by the Shapiro-Wilk test (Shapiro and Wilk, 1965).

QTL affecting variation for the 7 single traits were mapped in each F_2 population using composite interval mapping (CIM) (Zeng, 1994) in QTL Cartographer, version 1.17 (Basten *et al.* 2002). CIM tests the null hypothesis that an interval between two markers has no QTL while controlling for genetic background via multiple regression with molecular marker co-factors. Co-factors were chosen by a forward-backward stepwise regression with $p < 0.005$ to enter or leave the model. Initially, the two sexes were analyzed separately via CIM. The likelihood ratio (LR) statistic for each sex was calculated as

$$-2 \ln (L_0)/L(_1)$$

where L_0 is the likelihood of the null hypothesis (no QTL in the marker interval) and L_1 is the likelihood of the alternative hypothesis (presence of QTL in the marker interval). We denote test statistics for sex-specific analyses as LR_M and LR_F. For each trait, experiment-wide sex-specific significance levels were determined by 1000 permutations of trait and genotypes (Churchill and Doerge, 1994). LR test statistics that exceeded the 95th percentile LR threshold were considered significant for sex-specific analysis at the α=0.05 level. The 95th percentile LR threshold was between 13 and 15, depending on the trait, sex, and population.

Trait-specific combined-sex analysis was also performed to increase power of QTL detection. The test statistic for each marker interval in the combined-sex analysis was generated by summing male- and female-specific LR test statistics from CIM for the nth trait in the mth marker interval ($LR_{CSnm} = LR_{Mnm} + LR_{Fnm}$). The combined-sex significance threshold was calculated by adding male- and female-specific LR test statistics from the pth

sex-specific permutation for m^{th} marker interval (LR_{Mnmp} + LR_{Fnmp}) and selecting the maximum test statistic across all intervals (LR_{CSnp}). This was performed 1000 times for each trait (Churchill and Doerge, 1994). Combined-sex LR test statistics that exceeded the 95^{th} percentile combined-sex LR threshold (LR ≈ 20, depending on the trait and population) were considered significant for combined-sex analysis at the α=0.05 level.

A QTL-by-sex effect was also calculated (LR_{QS}) for each marker interval to test if genetic effects were significantly different between the two sexes. The null hypothesis of the QTL-by-sex interaction is that the two sexes have equivalent QTL effects. Thus, the test statistic of the null hypothesis is LR_{BS}, the test statistic obtained by analyzing both sexes via CIM simultaneously. CIM analysis to produce LR_{BS} was performed using residuals with fixed effects from sex, F_2 mating set, litter (nested within line and set) and uterine litter size removed. The likelihood ratio of the QTL-by-sex interaction was calculated as $LR_{QSnm} = LR_{CSnm} - LR_{BSnm.}$ Significance threshold for the QTL-by-sex interaction effect was calculated by adding male- and female-specific LR test statistics and subtracting the joint-sex LR test statistic from the p^{th} sex-specific permutation for m^{th} marker interval (LR_{Mnmp} + $LR_{Fnmp} - LR_{BSnmp}$). This was repeated 1000 times, with QTL-by-sex LR test statistics exceeding the 95^{th} percentile LR threshold (LR ≈ 13) considered significant.

Multiple Trait Analyses

Due to significance of the same marker interval in several single trait analyses, joint trait QTL mapping was performed for each of the two datasets (Set 1: 4 growth rates; Set 2: 3 growth curve traits) using Multitrait CIM (MCIM; Jiang and Zeng, 1995) in QTL Cartographer, version 1.17 (Basten *et al.* 2002). As indicated by Jiang and Zeng (1995),

MCIM can increase the power of detection by utilizing the correlational structure between variables while using molecular marker cofactors to control for genetic background. The joint mapping hypothesis test for the two-trait model is

$$H_o : a_1^* = 0, d_1^* = 0, a_2^* = 0, d_2^* = 0$$

H_1 : At least one of the parameters is not zero

where a_n^* and d_n^* are the respective additive and dominance estimates of the putative QTL on the n^{th} trait. MCIM calculates the LR statistic as

$$-2 \ln (L_0)/L(_1)$$

where L_0 is the likelihood of the null hypothesis and L_1 is the likelihood of the alternative hypothesis. Sex-specific, combined-sex, and QTL-by-sex analyses were performed as outlined above. Model parameters for MCIM were the same as CIM. Experiment-wide significance LR threshold were comparable across sexes and populations.

Once a multi-trait QTL was significant at the experiment-wide level, LR test statistics for each trait were examined for evidence of pleiotropy. Pleiotropy was indicated if the likelihood ratio for a single trait at the suggested QTL location is greater than threshold value of 5.99 for sex-specific analysis ($\chi^2_{0.05, 2}$) or 9.58 for combined-sex analysis ($\chi^2_{0.05, 4}$) (Jiang and Zeng, 1995). If a pleiotropic QTL significantly influenced multiple traits, genotype-by-environment tests were conducted in each sex for each QTL to test if genetic effects were different for traits (Jiang and Zeng, 1995). Effects were significantly different if the LR from the genotype-by-environment test for a pair of traits was greater than threshold value of 5.99 ($\chi^2_{0.05, 2}$) (Jiang and Zeng, 1995). Neither pleiotropy nor genotype-by-environment tests

require correction for multiple tests since testing position is fixed in the genome (Jiang and Zeng, 1995).

Epistasis

Significant interactions between marker loci were tested using the Epistacy program (Holland, 1998) for each of the seven single traits. For each marker pair, the Epistacy program fits the model

$$Y_{ijkl} = M_j + M_k + M_j*M_k + E_{ijkl}$$

where individual *l*'s phenotype for trait *i* is modeled by *l*'s genotypes at markers *j* and *k* and interaction between the genotypes (M_j*M_k). Least-square means of the nine genotypic classes were calculated for significant marker-by-marker interactions.

All marker loci were included in epistasis analysis to detect regulatory interactions not significant in the single or multi-trait analysis. To decrease the likelihood of finding significant epistatic effects due to low sample size, marker-by-marker interactions were considered only if 500 F_2 individuals were genotyped for both markers. No epistatic interactions were significant when Bonferroni correction for the number of tests was applied (> 2000 in both population). However, this correction assumes all loci are independent, which may not be correct for markers on the same chromosome. A more liberal Bonferroni-correction for the number of chromosomes was used [19*(18/2), or 171 tests], yielding $p=0.0002$ as the $\alpha=0.05$ Bonferroni-corrected significance value for both populations.

RESULTS

Figure 3.1 shows the periods in ontogeny subject to genetic selection (EG and LG), as well as the other two growth rates analyzed (WG and MG) and the timing of the three growth

curve traits [mature body weight (MBW); maximum growth rate (MGR); inflection point (IP)], relative to a fixed time point (weaning; 21 days of age). Informative molecular markers for the two F_2 populations are listed in tables 3.1 and 3.2, along with the number of genotyped F_2 individuals. With one exception, molecular markers map to the approximate predicted chromosomal location as suggested by the Mouse Genome Informatics (MGI) (http://www.informatics.jax.org). The exception is microsatellite marker D7Mit252 in the late F_2 mice, which should localize to chromosome 7 per the MGI but linkage analysis assigns it to chromosome 1. BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) analysis of the microsatellite's primers and amplicon sequence confirm its placement on chromosome 1. Markers with significant segregation ratio distortion ($p<0.01$; $X^2 > 9.21$) are noted in Tables 3.1 and 3.2. We detected significant distortion at three of the four molecular markers on chromosome 3 in the early mapping population. This region may illustrate a large genomic section influencing viability in the early-selected lines. Regions with significant marker distortion differ between the two populations, suggesting genetic selection or drift plays a role in determining such viability regions.

The first four statistical moments, as well as the coefficient of variation, for traits under genetic selection (EG and LG) for both parental generations (8^{th} and 12^{th}), as well as both F_1 generations, are listed in Tables 3.3 and 3.4 for early and late-selected mice, respectively. Means for early gain (EG) differ between up-selected and down-selected inbred lines (table 3.3), as do means for late gain (LG) in late-selected inbred lines (table 3.4). Variance for traits is lower in parents from the 12^{th} generation of inbreeding, as we may expect from continued inbreeding. Variance in late gain is higher than variance in early gain

in all samples (tables 3.3 and 3.4), likely reflecting increasing variance as ontogeny continues. However, coefficients of variation suggest three of the four parental lines (E^+, E^-, L^-) are more variable for the trait under restricted gain than the trait under selection. Early up-selected individuals from both generations have high kurtosis values for early gain (EG), suggesting a wide distribution of EG phenotypes. In contrast, late-selected mice from the 8th inbred generation have a platykurtic distribution for both EG and LG. However, mice from the 12th generation have normal kurtosis, with the exception of LG in the late up-selected males. Corrected skewness values range between 1 and –1.6 for all traits, suggesting distributions are symmetric.

Statistics from the 12th generation of inbreeding (tables 3.3 and 3.4) were used to calculate the minimum number of effective loci for traits under selection since some parents from the 8th generation were not fixed for all marker loci. The minimum number of effective loci was calculated as $n=R^2_T/8\sigma^2_A$, where R_T is the difference in means of the up-selected and down-selected lines and σ^2_A is the estimate of the additive genetic variation (Falconer and Mackay, 1996). Under a strictly additive model, $\sigma^2_A = \sigma^2_{P\,(F2)} - \sigma^2_{P\,(F1)} = (\sigma^2_A + \sigma^2_E) - (\sigma^2_E)$ where the phenotypic variance of the F_1 population is an estimate of the environmental variance since the F_1 population has no genotypic variation. Calculations suggest more effective loci influence EG in early-selected males than females (2.66 in males versus 1.2 in females). This pattern agrees with the coefficient of variation for EG in table 3.3, where males from both lines have a higher CV than females. Calculations also suggests late-selected males have more effective loci influencing LG than late-selected females (4.4 versus 7.4).

The first four statistical moments, as well as the coefficient of variation, for the seven traits analyzed are given in Tables 3.5 and 3.6 for the early and late F_2 populations, respectively. Sexual dimorphism is evident in all traits for both populations. Means for males are significant greater than those of females ($p<0.0001$), except for EG in the early mice. Inflection point means in Table 3.3 suggest the hyperplastic-to-hypertrophic growth shift occurs immediately after weaning, i.e., between 23 and 25 days of age. The third and fourth moments for both populations fluctuate around zero, in contrast to parental data (tables 3.3 and 3.4). The only exceptions are males' inflection points, which have moderate skewness and kurtosis in both populations, suggesting a threshold value for inflection point but only in males. Coefficients of variation for growth rate in both populations decrease as ontogeny continues, indicative of decreasing phenotypic variation and potentially targeted growth (Riska *et al.* 1984).

To facilitate discussion, the following terminology is used to characterize quantitative trait loci (QTL). The terms locus or loci are used to discuss chromosomal region or regions that significantly influence traits analyzed in this report. Pre-weaning growth loci influence only pre-weaning growth rates (EG and/or WG) while post-weaning loci influence only post-weaning growth rates (MG and/or LG). The term "pleiotropic QTL" is used to denote influence on both pre-weaning and post-weaning growth rates. Single-trait loci represent marker intervals significant only in single trait analysis (tables 3.7 and 3.9, footnote a). The term "sex-specific QTL" denotes significance in only one sex-specific test statistics and/or a significant QTL-by-sex interaction from combined-sex analysis. We include loci that are significant at the $p=0.1$ level and refer to loci with $0.05 < p < 0.1$ as suggestive QTL. For

each QTL, one LOD-drop support intervals are presented and are considered appropriate 90-95% confidence intervals (Lander and Botstein, 1989).

Loci Influencing Growth Rate--Early Population

The first question addressed is if early and late growth are under similar genetic control in the same population. That is, do the same chromosomal regions influence growth throughout ontogeny in early population? Genomewide LR test statistics for multi-trait CIM for the four growth rates (EG, WG, MG, and LG) are given in Figure 3.2. Significant loci are listed in Table 3.7 and denoted as GainXE, with "X" representing the sequential QTL number and "E" denoting detection in the early population.

Our results suggest three chromosomal regions influence multiple growth rates in the hyperplastic population. Chromosomes 5 and 7 contain QTL significant in both female-specific and combined-sex analyses (Gain3E and Gain4E) while chromosome 15 contains a male-specific locus (Gain8E). Gain3E on chromosome 5 also has a significant QTL-by-sex interaction. We also found six QTL influencing single traits. Two loci influence middle gain in both sexes: MGain1E and MGain2E on chromosomes 1 and 5, respectively. Neither MGain1E nor MGain2E had significant genotype-by-sex interaction, suggesting similar genetic effects between the sexes. The remaining single-trait QTL are sex-specific. Male mice have two single-trait loci in the same marker interval, influencing EG and WG, on chromosome 8 (EGain5E, WGain6E). Males also have a QTL influencing late gain (LG) on chromosome 17 (54 cM, LGain9E). Female mice have a single-trait locus influencing late gain (LGain7E) on chromosome 11 (57 cM).

Table 3.8 indicates each locus's influence on growth rates. MCIM denotes which traits a given QTL influences by pleiotropy test. Thus, QTL effects for a specific trait are only listed in table 3.8 if the trait had a significant pleiotropy test (Jiang and Zeng, 1995). The three multi-trait QTL (Gain3E, Gain4E, and Gain8E) do not influence all four growth rates; rather, loci influences only a subset of the four growth rates. Two QTL significantly influence early gain (EG): Gain3E and sex-specific EGain5E (males). Gain3E also influences 1 to 3-week gain (WG) and is the only multi-trait locus influencing both pre-weaning growth rates. The remaining loci influence post-weaning growth, with most loci in table 3.8 influencing MG and/or LG. While the number of QTL influencing EG is low (two in males, one in females), this agree with the predicted number of effective loci. Previous QTL studies found approximately equal numbers of loci (between 10 and 15, depending on genetic background) influencing both pre- and post-weaning growth rates or body weights (Cheverud *et al.* 1996; Vaughn *et al.* 1999). The low number of loci in this report may be due to restricted index selection. Typically, mapping experiments use crosses from mice selected for hypertrophic growth. Individuals may have altered a variety of developmental components and trajectories in response to genetic selection. Thus, previous mapping experiments may find genetic loci from various ontogenetic origins influencing different developmental trajectories. Restricted selection for age-specific growth seems to have acted upon fewer loci than selection for multi-phase growth, i.e., selection allowing hyperplastic and hypertrophic changes. In addition, selection for gain in a trait, i.e. early gain in the early population, seems influenced by fewer loci than restricted gain in a trait, i.e, late gain in the early population.

Genetic effects of loci are in Table 3.8. Positive estimates of additive values indicate the E^+ allele increases the trait, while negative estimates indicate the E^- allele increases the trait. As expected, the E^+ allele increases EG and/or WG at most loci. The E^+ allele has a mixture of negative and positive genetic effects for post-weaning growth rates (MG and LG), as expected from genetic selection for no change in post-weaning growth. The E^+ allele is partially dominant for most traits. Dominance values for early gain (EG) are completely additive (i.e., EGain5E/Gain3E) or partially dominant (i.e., Gain9E). There is no evidence of under- or overdominant QTL.

Two of the three QTL influencing multiple growth rates are negatively pleiotropic (Gain3E, Gain4E), but only Gain4E contrast pre- and post-weaning growth. The E^+ allele at Gain4E increases 7-to 21-day growth rate (WG) and decreases post-weaning growth rates (MG and LG). Interestingly, Gain3E shows a rapid shift in allelic effect for pre-weaning growth. At Gain3E, the E^+ allele increases 0-to 10-day growth (EG) while the E^- allele increases 7-to 21-day growth (WG). Both sexes have this allelic pattern; however, females have higher additive effects than males, which likely explains the QTL-by-sex interaction.

All three multi-trait QTL had significant genotype-by-environment tests, illustrating loci-specific genetic effects change during ontogeny. For example, genetic effects for MG (3- to 5-week gain) were significantly different from EG effects at Gain3E. This is likely due to compensatory growth in the E^+ mice, with genetic effects for MG being higher to increase growth during the non-selected period between 21 to 28 days of age. Similarly, genetic effects for MG were significantly different from effects for LG at Gain8E in males.

However, caution should be used in interpreting differences between trait-specific genetic effects, since estimates have large standard errors.

Chromosomal regions listed in Table 3.8 have been reported in previous age-specific growth QTL mappings (table 2.2; Cheverud *et al.* 1996; Vaughn *et al.* 1999; Ishikawa *et al.* 2000; Brockmann *et al.* 2000; Brockmann *et al.* 2004; Rocha *et al.* 2004a; Rance *et al.* 2005). However, loci described here differ from previously mapped QTL in terms of nature of influence, i.e., positively versus negatively pleiotropic, and the ages a specific locus influenced. Interestingly, none of the loci on chromosome 5 (table 3.7) were reported in previous studies seeking age-specific growth genes. Experiments have described a growth QTL in the same region as Gain3E, near 64 cM (Keightley *et al.* 1996; Rance *et al.* 2005), but these experiments did not assess for age-specific growth as early in ontogeny as we have here. The age-specific growth effect seen on chromosome 5 may be unique to either the ICR background or to restricted index, i.e., selection to alter a unique developmental trajectory.

Loci Influencing Growth Rate--Late Population

The next question addressed is if early and late growth rates are under similar genetic control in the late population. In addition, do we find the same significant chromosomal regions as those influencing growth in the early population? Genomewide LR test statistics for multi-trait CIM for the four growth rates (EG, WG, MG, and LG) are in Figure 3.3. Loci are listed in Table 3.9 and denoted by GainXL.

Our results suggest eight chromosomal regions, three of which are suggestive, influence multiple growth rates in the late population. Two of the eight regions are significant in both combined-sex and sex-specific analyses: Gain1L on chromosome 2 and

Gain8L on chromosome 13. Both QTL also have significant genotype-by-sex interactions, suggesting different genetic effects for the two sexes. The remaining six loci are detected only in the combined-sex analysis and are located on chromosomes 6, 7, 8 and 12 (Gain2L through Gain7L). We found no significant single-trait growth rate QTL. Table 3.10 indicates each locus's influence on growth rates from pleiotropy tests (Jiang and Zeng, 1995). QTL influence only a subset of the four growth rates, similar to loci in the early population. Late gain (LG) is influenced by four loci: Gain1L, Gain2L, Gain4L and Gain7L. This number agrees with the number of effective loci for females (4.4) but is lower than the number of effective loci for males (7.4). Gain5L and Gain6L on chromosome 8 influence only pre-weaning growth (EG and WG). Gain7L influences only 3-to 5-week gain and 4- to 8-week gain (MG and LG). Five of the eight loci are pleiotropic, influencing both pre- and post-weaning growth: Gain1L, Gain2L, Gain3L, Gain4L, and Gain8L. Gain3L and Gain8L are positively pleiotropic loci, where the L^+ allele increases both pre-weaning and post-weaning growth. The remaining three QTL (Gain1L, Gain2L, and Gain4L) are negatively pleiotropic, decreasing pre-weaning growth while increasing post-weaning growth in L^+ mice.

Genetic effects of loci are in Table 3.10. Positive estimates of additive effects indicate the L^+ allele increases the trait, while negative effects indicate the L^- allele increases the trait. The L^+ allele is partially dominant for weaning gain and middle gain; however, the L^+ allele is highly overdominant for early gain at Gain3L, Gain5L, and Gain6L and underdominant for late gain at Gain2L and male genetic effects for late gain at Gain4L. As expected, the L^+ allele increases post-weaning growth (MG and/or LG) at all loci but does

not uniformly increase pre-weaning growth. Instead, the L^- allele increases pre-weaning growth, relative to the L^+ allele, at most loci influencing EG or WG. This increased pre-weaning growth is likely to compensate for decreased growth after 28 days of age. However, L^+ mice also have compensatory effects at QTL. Five loci influence both EG and WG, all of which have significantly different genetic effects for WG, relative to EG, in both L^+ and L^- mice. At Gain4L, Gain5L, and Gain6L, the L^- allele's effect on 7- to 21-day gain is significantly different from genetic effects for EG, likely to accelerate growth after the restricted period of 0- to 10-days. At Gain3L and Gain8L, L^+ mice have increased weaning gain (WG) and middle gain (MG) genetic effects, relative to early gain (EG) effects, indicative of compensatory growth after 10-days of age. This agrees with growth trajectories of L^+ and L^- mice, which suggest both lines have a burst of compensatory growth between 10- and 28-days of age (Rhees and Atchley, 2000).

Gain1L and Gain2L have sex-specific QTL-by-ontogeny effects, where only one sex has genetic effects significantly changing over ontogeny. At Gain1L, males' genetic effects for LG are different from those for EG. However, females' genetic effects for EG are low. As such, the QTL may only influence late gain in females, potentially explaining the significant QTL-by-sex interaction. Despite Gain2L having no significant QTL-by-sex interaction (table 3.9), only females have a significantly genotype-by-environment test for WG and LG effects. The sex-specificity in changing genetic effects may reflect the high dominance value for LG in females and may reflect a female-specific fitness effect in this genomic region (table 3.10).

Chromosomal regions in Table 3.9 have been previously characterized (table 2.2), with age-specific growth QTL described in similar regions of mouse chromosomes 2, 6, 7, and 8 (Cheverud *et al.* 2001; Corva *et al.* 2001; Ishikawa and Namikawa 2004; Rocha *et al.* 2004a). As with early F_2 mice, the nature of loci differed from those previously described. For example, Brockmann and colleagues (2004) found a positively pleiotropic QTL on chromosome 2 in the same region as negatively pleiotropic Gain1L. Interestingly, several chromosomal regions described here were replicated in another age-specific mapping using mice selected for hypertrophic growth (Rocha *et al.* 2004a). Replicated loci are in similar regions of chromosomes 6 (27-28 cM), 7 (15 cM), 8 (24-26 cM), 12 (53-55 cM), and 13 (54-55 cM). Such chromosomal regions may contain selection-response genes in the ICR background, as both this and Rocha's report used crosses derived from ICR randombred mice subject to selection.

In both early and late F_2 mice, we found three sets of growth loci: one set influencing pre-weaning growth rates, one set influencing post-weaning growth rates, and a set influencing both pre- and post-weaning growth. It is likely chromosomal regions in Table 3.7 and 3.9 directly influence or regulate hyperplasia and hypertrophy, among other cellular mechanisms of growth. However, we did not directly assess cell number or cell size in F_2 mice and cannot characterize all loci influencing pre-weaning growth as hyperplastic loci or all loci influencing post-weaning growth as hypertrophic loci, especially negatively pleiotropic QTL. Potential follow-up work will focus on fine mapping regions, determining hyperplastic/hypertrophic candidate genes, and measuring candidate genes' influence on cell number and cell size. QTL in both populations influence compensatory growth in all four

lines. QTL influence compensatory growth in two ways: (1) QTL have increased genetic effects for WG and/or MG, such as Gain3L's influence on WG in L^+ mice; or (2) QTL influence only compensatory growth, such as MGain1E and MGain2E influencing only MG in E^+ mice.

While the pattern of three growth loci sets is common to both populations, specific chromosomal regions do not overlap. Chromosomes 7 and 8 are the only chromosomes with significant QTL in both populations. Locations of molecular markers in early and late populations (tables 3.1 and 3.2) clearly show the chromosome 8 loci (EGain5E, WGain6E, Gain5L, Gain6L) do not overlap between populations. The molecular marker D8Snp305 was genotyped in both populations. Both QTL in early mice (EGain5E and WGain6E) are distal to D8Snp305 while Gain5L and Gain6L in the late population are proximal to D8Snp305.

Gain4E in the early population and Gain4L in the late population are both located on chromosome 7 and are the only overlapping genomic region. Gain4E is approximately 20cM distal to Gain4L; however, there are no chromosome 7 markers genotyped in both populations, so we cannot easily compare linkage maps. We cannot eliminate the possibility the two QTL represent the same casual genetic variation. Early F_2 mice had a longer chromosome 7, in terms of recombination units, relative to both the late population and the Mouse Genome Informatics (83 cM versus 100 cM). This may lead to a more distal placement of the QTL, relative to Gain4L's location. However, if the regions do overlap, the causal genetic variation(s) may be unique to each population.

Loci influencing Growth Curves—Both Populations

Next, we ask if growth curve traits are under the same genetic control as growth rate. Do the same maker intervals influence growth rate and growth curve traits within each population? Genomewide LR test statistics plots for the three growth curve traits (mature body weight, maximum growth rate, and time of maximum growth rate/inflection point of the growth curve) in the early population are in Figure 3.4, with loci listed in Table 3.7. Genomewide LR test statistics plots for the three growth curve traits in late mice are in Figure 3.5, with loci listed in Table 3.9.

Six loci influence growth curve traits in early F_2 mice, three of which have significant QTL-by-sex-specific interactions (table 3.7). Unlike growth rate QTL, the early population has no sex-specific growth curve QTL. Rather, marker intervals with sex-specific growth rate QTL on chromosomes 8, 11, and 17 (EGain5E/WGain6E, LGain7E and LGain9E) have combined-sex growth curve QTL (Curve4E, Curve5E, and Curve6E). Early mice also have several single-trait loci, one influencing maximum growth rate (MGR) and mature body weight (MBW): Rate2E and MBW1E on chromosomes 5 and 6, respectively.

The late F_2 population had six QTL influencing growth curve traits, two of which have significant QTL-by-sex effects (table 3.9). Combined-sex loci were located on chromosomes 5, 7, 8, and 13 (Curve1L through Curve6L), with a single-trait loci influencing IP (IP1L). Growth curve QTL in either population do not overlap with growth curve QTL in other strains (Wu *et al.* 2004; Zhao *et al.* 2004; Wu *et al.* 2005; Zhao *et al.* 2005), again illustrating genetic background in results of QTL mappings.

Both populations had growth rate QTL and growth curve QTL in the same marker interval; in this report, we refer to these marker intervals as "common growth intervals". Preliminary pleiotropy-versus-close linkage analyses suggest these common growth intervals are pleiotropic for the two phenotypic datasets. However, additional recombinants and molecular markers are necessary to separate linkage from pleiotropy.

Genetic Effects in Common Growth Intervals

Patterns of genetic effect covariation for common growth intervals agree with correlated selection responses. Selection for increased growth has increased both mature body weight and maximum growth rate in E^+ and L^+ mice, relative to control, E^- and L^- mice (Rhees and Atchley, 2000). We therefore expect an up-selected allele (E^+ or L^+) would increase mature body weight (MBW) and maximum growth rate (MGR). E^+ mice have not significantly altered the inflection point of their growth curves, relative to control mice. Rather, E^- and L^+ mice have achieved maximum growth rate (at the inflection point) later in ontogeny, relative to E^+, L^-, and control mice (Rhees and Atchley, 2000). We therefore expect the L^+ and E^- alleles at growth curve QTL would increase inflection point (IP).

Additive values for three common interval growth rate/growth curve trait QTL in early mice are diagramed in Figure 3.6 (genetic effects listed in Tables 3.8 and 3.11). The E^+ allele at negatively pleiotropic loci Gain4E (chr 7) increases WG and decreases post-weaning growth rates MG and LG in both sexes. As expected, the E^+ allele at Curve2E (chr 7) increases maximum growth rate while decreasing inflection point. The E^+ allele at Curve2E decreases MBW (table 3.11), which is contrary to expectation. EGain5E/WGain6E and

Curve3E on chromosome 8 agree with previous correlated responses, increasing pre-weaning growth, MBW, and MGR while decreasing inflection point.

Sex-specific additive values for four common interval growth rate/growth curve trait QTL are diagramed in figure 3.7 (genetic effects in Tables 3.10 and 3.12) and also follow expected patterns. The L^+ allele at Gain3L (chr 7, left panel) and Gain8L (chr 13) are positively pleiotropic. Their corresponding growth curve trait QTL increase MBW and MGR in L^+ mice. Gain6L (chr 8) decreases pre-weaning growth (EG and WG) while Curve4L decreases mature body weight and maximum growth rate in L^+ mice. Finally, the L^+ allele at negatively pleiotropic Gain4L (chr7, right panel) decreases EG and WG while increasing LG. In the same marker interval, Curve3L increases inflection point and decreases MGR in L^+ mice. Although not shown, the other sex not presented in Figure 3.7 have similar patterns.

Genetic effects for growth curve QTL are in Tables 3.11 and 3.12. Multi-trait QTL do not consistently influence all three growth curve traits but influence only a subset of the three traits. Rhees and Atchley (2000) demonstrated correlated responses in age of maximum weight gain (t') were accompanied by changes in MGR. However, the two traits are not influenced by the same QTL at all loci. The genetic architecture of maximum body weight (MBW) and maximum growth rate (MGR) also partially overlap, which does agree with previous research (Rhees and Atchley, 2000).

Both up-selected alleles (E^+ and L^+) primarily increase MBW and MGR (table 3.11 and 3.12), as expected from correlated selection responses (Rhees and Atchley, 2000). There are several exceptions, primarily at loci illustrating compensatory growth in the down-

selected allele. The E^+ allele at Curve1E on chromosome 1 decreases maximum growth rate (table 3.11). However, it is the same marker interval as MGain1E, which decreases post-weaning growth in E^+ mice (table 3.8). Hence, E^- mice have increased post-weaning growth at MGain1E and a higher maximum growth rate at Curve1E, which occurs during the 3-to 5-week timeframe spanned by MG. Similarly, Gain6L increases pre-weaning growth while Curve4L increases maximum growth rate and inflection point in L^- mice (tables 3.6 and 3.9). Thus, allelic effects that differ from expectations are due to correlated responses in compensatory growth. As expected, E^+ allele decreases inflection point while the L^+ allele increases inflection point at growth curve QTL. The only exception is Curve6E, which increases IP in E^+ males.

In both populations, the genetic architecture of growth rates and growth curve traits partially overlap. In early mice, marker intervals on chromosome 5 and 15 influence only growth rate while regions on chromosomes 6 and 8 influence only growth curve traits (table 3.7). In the late population, portions of chromosomes 2, 6, 8, and 12 influence only growth rates while portions of chromosomes 5, 13, and 16 influence only growth curve traits (table 3.6). For regions influencing only growth curve traits, we cannot suggest genes in these regions would influence hyperplasia or hypertrophy. However, we do find these regions of chromosomes 5, 6, 13, and 16 influence post-weaning growth (table 2.2; Cheverud *et al.* 1996; Morris *et al.* 1999; Vaughn *et al.* 1999; Brockmann *et al.* 2004).

Other research has found different sets of growth attributes are under overlapping genetic control. Genomic regions influencing body weight and growth rates partially overlap, as do loci influencing weight and tail length (Cheverud *et al* 1996; Rocha *et al.*

2004a). Several growth curve QTL have been mapped in (LG/J x SM/J) F_2 mice, a genetic background also analyzed for growth rate and body weight QTL (Cheverud *et al.* 1996; Vaughn *et al.* 1999). In these previous analyses, QTL influencing growth curves partially overlapped with growth rate or body weight QTL (Cheverud *et al.* 1996; Wu *et al.* 2004; Zhao *et al.* 2004; Wu *et al.* 2005; Zhao *et al.* 2005). Our results confirm this.

Epistatic Interactions

Finally, we ask if early and late F_2 mice share epistatic interactions. Three epistatic interactions were significant in the early mapping population (table 3.13), with no significant QTL-by-QTL interactions. All three significant epistatic interactions involve a region of chromosome 4. D4Mit334 (80.2 cM) interacts with D6Mit17 on chromosome 6 (31.8 cM) to influence 1-week to 3-week growth (WG) and maximum growth rate (MGR). D4Mit334 also interacts with D10Mit103 (chr 10, 89 cM) to impact mature body weight (MBW). Least square means for the nine genotypic classes are listed in Table 3.13; means presented reflect means of the residuals after removal of fixed effects. Thus, negative means reflect initial phenotypic values smaller than the overall population mean. Means suggest the two D4Mit334 and D6Mit17 interactions act in the same manner, likely in an additive-by-additive or additive-by-dominant manner. For WG and MGR, the double homozygote E^+/E^+ mice have the greatest mean, while the two $E^+/E^+/E^-/E^-$ classes have the greatest negative mean. In contrast, D4Mit334/D10Mit103 interaction is likely additive-by-dominant, where the genotype at D4Mit334 follows an additive pattern (mean $E^+/E^+ > E^+/E^- > E^-/E^-$) but only for the two homozygous D10Mit103 genotypes (E^+/E^+ and E^-/E^-). Heterozygotes at

D10Mit103 show the opposite pattern, where E^-/E^- mice have the highest mean and E^+/E^+ mice have the lowest mean.

Significant epistatic interactions in the late population are listed in Table 3.14, along with least squared means for the nine genotypic classes for significant interactions. Again, there are no significant QTL-by-QTL interactions. Gain1L on chromosome 2 (D2Mit113) interacts with D5Mit81 (41.5 cM) on chromosome 5 to influence MG and LG. Gain5L on chromosome 8 (D8Mit180) interacts with D3Mit77 to influence WG. This same marker pair also influences early gain (EG), although not significantly (p=0.0006; data not shown). There is an additional epistatic interaction between D14Snp759 (chr 14; 27.2) and D5Mit382 (chr 5; 69.2 cM), influencing MG. Means in table 3.14 suggests D8Mit180/D3Mit77 pair acts primarily additive-by-additive. Means of the two $L^+/L^+/L^-/L^-$ classes are highest, with the double L^- homozygote mean being the lowest. The remaining three interactions likely act in an additive-by-dominant manner. At D5Mit81, L^+/L^+ homozygotes follow an additive pattern (mean of D2Mit113 genotype: $L^+/L^+ > L^+/L^- > L^-/L^-$). However, D5Mit81 heterozygotes show the reverse pattern (mean of D2Mit113 genotype: $L^+/L^+ < L^+/L^- < L^-/L^-$). We see a similar pattern at D14Snp759 and D5Mit382.

Significant epistatic interactions do not occur in the same genomic regions between populations. However, two regions with direct effect QTL in the early population also had significant epistatic interactions in the late population. Regions containing the early population's loci MGain2E (chr 5, 40 cM) and Gain3E (chr 5, 64 cM) had significant epistatic interactions in the late population, influencing post-weaning growth (MG and LG). We did not find that the number of epistatic interactions increased with ontogeny, as previous

analyses found (Brockmann *et al.* 2004; Yi *et al.* 2006). However, we found far fewer epistatic interactions than other studies (Brockmann *et al.* 2000, 2004), potentially due to differences in genetic background of mapping populations (Wade, 2001). Alternatively, linear models such as that used here may not find all significant genetic interactions due to poor resolution (Cheverud, 2000). We did not look for sex-specific interactions, due to small sample numbers for genotypic classes when analyzing sexes separately. Such analyses may find additional epistatic genomic regions, especially given the high proportion of sex-specific genetic effects in the hyperplastic population.

Summary of Effects

The number and genetic effects of QTL are listed in table 3.15. Due to sex-specific genetic effects, sexes are summarized separately. A total of ten genomic regions (defined as unique marker intervals) influence growth in early F_2 mice while eleven genomic regions influence growth in the late population. Both populations have three sets of loci: one set influencing only pre-weaning growth, one set influencing only post-weaning growth and one set influencing both. While this pattern confirms previous findings, chromosomal regions did not consistently agree with any one specific previous experiment, illustrating the importance of genetic background in results of QTL mapping (Cheverud *et al.* 1996; Vaughn *et al.* 1999; Rocha *et al.* 2004a; Brockmann *et al.* 2004; Yi *et al.* 2006). QTL plus epistatic interactions explained between 10 and 50% of phenotypic variation in the seven traits analyzed here. However, there are clear population and trait differences. For example, genetic effects explained more variation in the restricted growth period in both populations, potentially due to increased genetic effects in compensatory growth periods.

DISCUSSION

This experiment describes the genetic consequences of age-specific selection for growth rate in early and late ontogeny. Selection for age-specific growth has produced significant divergence in body weight, growth rates, hyperplasia, and hypertrophy between selection treatments, among others. Herein, we demonstrate age-specific selection has also produced significant divergence in genes influencing growth. To summarize: (1) QTL influencing growth do not overlap between the early and late population, verifying age-specific selection has fixed different genetic variation; (2) QTL influencing early and late growth in both populations are likely to be involved in cellular differences in growth such as hyperplasia, hypertrophy, or apoptosis; (3) QTL are primarily age-specific or negatively pleiotropic; (4) within each population, two sets of growth phenotypes are under partially overlapping genetic control, including direct and epistatic effects; (5) epistatic interactions partially overlap between populations, suggesting age-specific growth shares some genetic interactions; and (6) both populations show clear evidence of compensatory growth, both via QTL that only influence compensatory growth, as well as different genetic effects for growth intervals not under selection.

This report is the first to examine the genetic architecture of growth in mice subject to age-specific selection. Previous mappings have looked for growth QTL in mice selected for post-weaning change, such as 6-week body weight or gain between 3- and 6-weeks of age. Despite differences in selection regimes, results in the hypertrophic population generally agree with these previous studies, in terms of proportions of sex-specific QTL effects,

variation explained by QTL, and locations of QTL. However, both populations differed from earlier work in the detection of negatively pleiotropic QTL.

Genetic effects in the Early Population

The early and late populations show several fundamental differences in terms of genetic architecture. Direct and epistatic effects in the early population explain little phenotypic variation in pre-weaning growth, compared to pre-weaning variation in the late population and previous studies (Cheverud *et al.* 1996; Vaughnn *et al.* 1999; Brockmann *et al.* 2004). The low proportion of variance attributed to genomic components may be due to low power to detect small effect QTL, i.e., low sample size, or increased genetic effects for compensatory growth at early growth loci in late selection lines.

Alternatively, selection for early gain may have acted upon "heritable" environmental effects, such as maternal contributions via uterine and postnatal effects. While maternal effects are perceived as environmental effects by offspring, they are influenced by genetic variation and can respond to selection (Funk-Keenan and Atchley, 2005 and references therein). Despite performing within family selection, maternal effects may change as a correlated response to altered growth. Indeed, Rhees and colleagues (1999) found evidence that early-selected females have altered uterine and postnatal maternal effects as correlated responses to selection. However, uterine effects alone did not significantly alter growth. Rather, uterine environment and fetal genotype interacted to impact growth, suggesting uterine components responded to selection for early growth. In contrast, nursing components changed as a correlated response to selection (Rhees *et al.* 1999). In other mouse strains, selection for altered growth has fixed genetic variation at QTL influencing maternal effects

(Peripato *et al.* 2002; Wolf *et al.* 2002). These maternal effects loci explained a large proportion of pre-weaning growth variance, relative to direct effect genes (Wolf *et al.* 2002).

The early population had a high proportion of sex-specific genetic effects, located on chromosomes 5, 8, 11, 15, and 17. In contrast, QTL in the late population were predominantly present in both sexes, in proportions comparable to previous experiments (Vaughn *et al* 1999; Ishikawa *et al.* 2005). Although sexual dimorphism in mouse growth is well-documented (e.g., Cheverud, 2005), sexual dimorphism at genetic variation in early growth loci has only been documented for one QTL (Vaughn *et al.* 1999). Our results suggest selection for early growth has acted on sexually dimorphic pathways, rather than a few isolated genes. Other quantitative traits, such as longevity, have a high percentage of sex-specific QTL effects in the mouse, suggesting sexually dimorphic pathways exist for other quantitative traits (Miller *et al.* 1998; Jackson *et al.* 1999). Such pathways may allow segregating variation for growth traits to be maintained separately in the two sexes.

Some researchers suggest CIM (and MCIM, by extension) analyses increase the likelihood of false-positive sex-specific loci as the number of marker cofactors increases (Curtsinger, 2002). We limited the number of molecular cofactors in each analysis and forced CIM to fit the same number of marker cofactors for all analyses. In addition, interval mapping, which fits no molecular cofactors, produced the same sex-specific effects as CIM (data not shown).

Cellular-Specific Growth and IGF2/GH

Proteins in the IGF2 and IGF1/GH pathways play key roles in cell proliferation and cell enlargement, respectively, and are key candidate genes for hyperplasia and hypertrophy

(Cheverud *et al.* 1996; Cheverud, 2005). Proteins in both pathways are also involved in targeted growth (Hornick *et al.* 2000), making them key candidate genes for QTL with compensatory genetic effects. IGF2 is highly expressed during gestation and early postnatal growth, with a temporal expression pattern that mirrors the ontogenetic pattern of hyperplasia (Baker *et al.* 1993). IGF2 expression starts to diminish at weaning, at approximately the time GH becomes an active mitogen (Sara and Hall, 1990; Singh *et al.* 1991). Both GH and IGF1, which is regulated by GH, are expressed from birth until later in ontogeny. However, GH does not stimulate growth or IGF activity until after weaning, most likely due to low receptor number early in ontogeny (Hyatt *et al.* 2004). GH receptor numbers are also decreased during feed restriction, leading to a decrease in IGF-1 concentration and activity (Hornick *et al* .2000). Upon re-feeding, GH receptors levels increase, allowing IGF1 to act (Hornick *et al.* 2000).

IGF2 is especially relevant as a candidate growth gene since it is genomically imprinted. At a genomically imprinted gene, one parental allele is active (transcribed) and the other allele is silent (not transcribed). Which parental allele is silent or active is determined on a gene-by-gene basis. This differential expression of parental alleles is achieved via differential DNA methylation at the two alleles. Methylation levels of both the maternal and paternal *IGF2* gene vary temporally and spatially in prenatal ontogeny, where methylation and *IGF2* levels are correlated (Weber *et al.* 2001). Historically, genomic imprinting and other parent-of-origin effects were largely ignored in QTL mapping experiments. However, recent QTL experiments have shown genetic variance and/or epigenetic variation at genomically imprinted genes is associated with phenotypic variation,

denoting a potential role in selection response (Van Laere *et al.* 2003; Tuiskula-Haavisto *et al.* 2004; Mantey *et al.* 2005).

In mice, *IGF2* is on mouse chromosome 7, in a 1 Mb region with other imprinted genes. Negatively pleiotropic QTL Gain4L and Curve3L are in the same chromosomal region, as are previously described positively pleiotropic QTL, including a dietary-dependent post-weaning growth QTL (Cheverud *et al.* 1996; Vaughn *et al.* 1999; Ishikawa and Namikawa, 2004; Cheverud *et al.* 2004). Genomically imprinted genes in close proximity to QTL are key candidate genes, since environmental effects can alter epigenetic states and produce long-lasting phenotypic effects (Waterland and Jirtle, 2003). Methylation patterns at imprinted genes may also vary during the course of ontogeny (Waterland *et al.* 2006), suggesting a role in age-specific growth. However, no research has specifically looked for epigenetic variation at imprinted genes in terms of changes throughout normal development.

The mouse *GH* gene is located on chromosome 11 at 65 cM. A late-gain QTL in the early female mice (LGain7E; 57 cM) is in the same genomic region. Interestingly, LGain7E influences only compensatory growth in the hyperplastic population, where the E^- allele increases late gain (table 3.8). However, additional work is necessary to determine if either QTL represents genetic variation in the *IGF2* or *GH* genes.

Response to Age-Specific Selection for Growth

There is considerable evidence that phenotypic, additive, non-additive, and maternal variance and covariance dynamically change over ontogeny (Atchley and Zhu, 1997 and references therein). Such ontogenetic changes are due, in part, to differing temporal activity of genes and other causal factors (Atchley and Hall, 1991). Thus, if natural selection

operates at different times in development, it will act on different genes, produce different cellular changes, and alter different genetic covariances. These temporal variance and covariance changes are the mechanism of growth regulation via compensatory growth. According to Riska *et al.* (1984), variance in an age-specific weight is a function of variance of previous growth plus covariance(s) among growth rates. Thus, if variance decreases as ontogeny continues, it is likely due to negative covariance between growth rates. Compensatory growth is any growth that reduces variation, as detected by negative phenotypic correlations between growth rates at different intervals (Atchley, 1984; Riska *et al.* 1984). The decrease in phenotypic variance is accompanied by genetic and environmental reductions in variance (Riska *et al.* 1984). As a result, mammalian compensatory growth leads to negative genetic and environmental correlations, suggesting a genetic component to growth regulation.

Principal components analyses show a similar pattern. The majority of additive genetic variation in growth is explained by an overall size factor and several smaller factors. These smaller factors contrast growth in different intervals. For example, a factor may increase growth from birth to 4 weeks while decreasing growth from 5 to 10 weeks (Cheverud *et al.* 1983; Ishikawa and Namikawa, 2004). We therefore expect a proportion of growth QTL would reflect the negative genetic covariation in ontogeny. However, previous molecular analyses find growth is primarily controlled by positively pleiotropic age-specific loci, each increasing growth during a specific time period in ontogeny (Cheverud, 2005 and references therein).

In contrast, our results suggest negatively pleiotropic QTL, in addition to age-specific loci, influence age-specific growth. These QTL may be involved in perceiving hormonal cues and/or altering growth accordingly. Earlier age-specific growth mapping found no negatively pleiotropic growth QTL, likely because there was no selection over multiple phases of growth. As such, there was no selection to achieve targeted growth and previous age-specific QTL mappings did not have the necessary genetic background to find negatively pleiotropic loci (Cheverud *et al.* 1996; Vaughn *et al.* 1999). The early and late populations in this report have negative phenotypic correlations between growth across cellular phases ($p<0.0001$). In addition, selection lines and inbred strains derived from the original selection experiment have clear phenotypic and cellular evidence of compensatory growth (Atchley *et al.* 1997; Atchley *et al.* 2000; Rhees and Atchley, 2000).

Our results suggest compensatory growth is influenced by previously described growth QTL but acts by altering genetic effects for times not under selection. Interestingly, compensatory growth seems to act within each cellular growth phase. Mice from the original restricted index selection experiment demonstrate compensatory growth in two intervals, between 10-12 days of age and between 30-40 days of age (see Atchley *et al.* 1997; Rhees and Atchley, 1999; Atchley *et al.* 2000). We found significantly changes in genetic effects for these two periods. However, we found no significant QTL for growth between 10- and 28-days of age (data not shown), potentially because this interval spans both hyperplastic and hypertrophic growth.

Results here shed new light on a common result of previous QTL mappings. Age-specific experiments typically find early growth loci have high dominance variance

components and directional dominance (Cheverud *et al.* 1996; Vaughn *et al.* 1999; Brockmann and Bevovja, 2002; Rocha *et al.* 2004a). As ontogeny continues, directional dominance is reversed at a subset of loci to lead to shift towards additivity (Rocha *et al.* 2004a). We find early growth loci in only the late population (i.e., Gain3L, Gain5L, and Gain6L) have high dominance components and directional dominance. High dominance components may thus reflect compensatory growth early in ontogeny due to selection later in ontogeny rather than true directional dominance for early growth.

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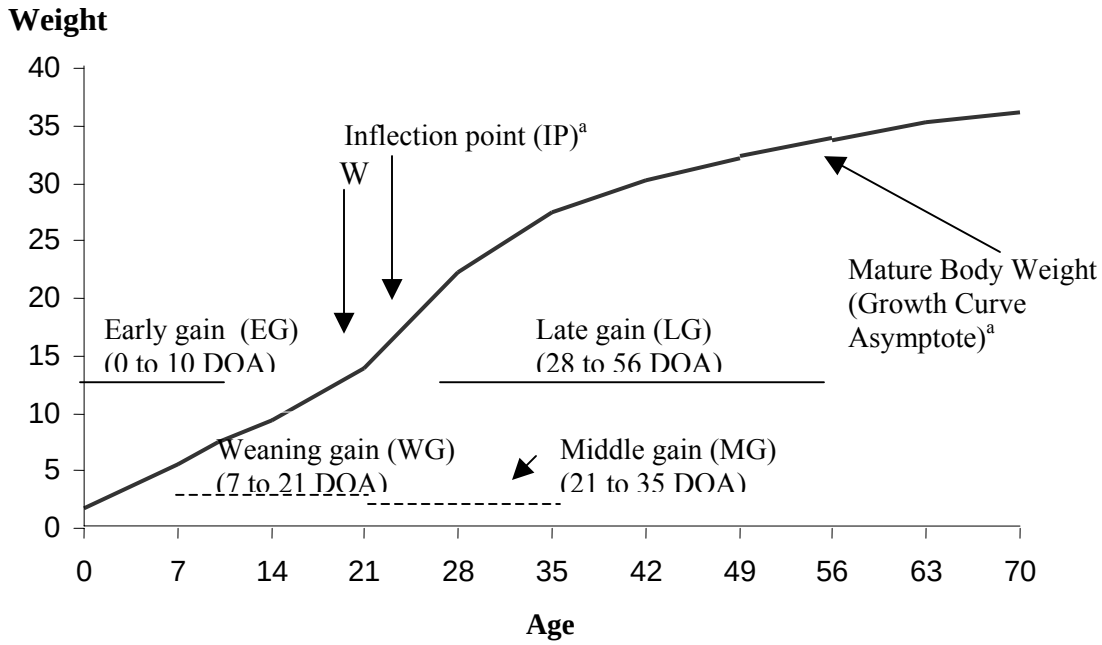

Figure 3.1 Representative growth curve of F_2 mice. Two solid horizontal lines reflect time in ontogeny under selection: 0 to 10 days of age (DOA) or 28 to 56 DOA. Two dashed horizontal lines reflect additional periods in ontogeny analyzed: 7 to 21 DOA and 21 to 35 DOA. "W" reflects weaning at 21 DOA. Inflection point and Mature Body Weight reflect relative occurrence of growth curve traits to fixed time point (weaning) and growth rates analyzed. Inflection points (days) and mature body weight (grams) are individual-specific, as denoted by [a].

Table 3.1 Microsatellite markers genotyped in early F_2 mice and their chromosomal locations in Haldane units (cM). The location of the first marker on each chromosome was obtained from Mouse Genome Informatics (MGI). "N" denotes number of F_2 mice for which a molecular marker was genotyped. Markers with significant segregation ratio distortion ($p<0.01$) are denoted with an asterisk.

Chr	Marker	cM	N	Chr	Marker	cM	N
1	D1Snp1022	20.0	448	10	D10Mit188*	4.0	523
	D1Mit132	37.5	493		D10Mit86*	18.6	499
	D1Mit49	47.3	482		D10Mit64	47.9	550
	D19Mit72	77.9	490		D10Mit103	88.9	551
	D1Snp4538	110.1	551	11	D11Mit133	2.0	453
2	D2Mit1	1.0	441		D11Snp11	11.2	481
	D2Mit267	12.2	549		D11Mit112	32.7	549
	D2Snp37	27.8	483		D11Mit285	57.4	549
	D2Mit13	65.7	525		D11Mit333	75.9	548
	D2Mit482	68.5	482		D11Mit69	92.8	538
	D2Mit343	103.6	453	12	D12Mit182	2.0	551
	D2Mit145	108.4	453		D12Mit201	36.7	540
	D2Mit266	128.6	551		D12Snp60	66.7	551
3	D3Mit46	13.8	382	13	D13Mit275	9.0	551
	D3Umi11*	47.1	343		D13Mit88	13.3	483
	D3Mit299*	67.5	382		D13Mit191	47.1	551
	D3Mit45*	104.9	302		D13Snp61	74.3	551
4	D4Mit101	3.2	546		D13Mit230	79.2	551
	D4Mit172	20.9	482	14	D14Mit132	1.0	551
	D4Mit213	29.1	540		D14Mit54	17.3	383
	D4Mit178	49.8	551		D14Mit32*	43.5	367
	D4Mit334	80.2	487		D14Snp74	65.9	381
5	D5Mit81	28.0	551	15	D15Mit13	7.0	551
	D5Mit309	48.4	545		D15Mit94	21.7	518
	D5Mit239	62.1	546		D15Mit122	42.4	551
	D5Mit158	67.5	551		D15Mit35	75.3	551
	D5Mit221	90.2	550	16	D16Mit32	1.0	438
6	D6Snp4.7	5.0	537		D16Snp572	16.0	551
	D6Mit17	31.8	551		D16Mit135	29.6	483
	D6Mit368	65.0	550		D16Mit139	43.5	483
	D6Mit198*	80.3	475		D16Mit217	56.3	478
7	D7Mit266	10.0	551	17	D17Mit213	9.0	479
	D7Mit228	23.8	551		D17Mit16	20.9	551
	D7Nds1	52.1	490		D17Mit177	34.2	551
	D7Mit220	68.4	551		D17Snp7*	67.4	483
	D7Mit109	98.7	514	18	D18Mit19	2.0	381
8	D8Snp305	30.0	483		D18Mit69	11.9	366
	D8Mit120	58.6	487		D18Mit62	15.1	382
	D8Mit49	69.6	483		D18Mit235	24.0	548
9	D9Snp298	13.0	458		D18Mit152	42.3	551
	D9Mit226	17.6	551		D18Mit4	67.8	464
	D9Mit207	38.0	551	19	D19Mit68	6.0	442
	D9Mit347	65.0	495		D19Mit39	34.5	551
	D9Mit19*	80.8	383		D19Mit71	70.4	538

Table 3.2 Microsatellite markers genotyped in late F_2 mice and their chromosomal locations in Haldane units (cM). The location of the first marker on each chromosome was obtained from Mouse Genome Informatics (MGI). "N" denotes number of F_2 mice for which a molecular marker was genotyped. Markers with segregation ratio distortion ($p<0.01$) are denoted with an asterisk.

Chr	Marker	cM	N	Chr	Marker	cM	N
1	D1Mit232	20.0	501	9	D9Mit228	21.0	305
	D1Mit181	39.3	416		D9Mit336	39.0	369
	D1Mit253	41.3	416		D9Mit347	63.8	366
	D7Mit252	58.9	415		D9Mit201	84.4	519
	D1Mit139	92.1	519	10	D10Mit282	12.0	425
	D1Mit268	115.5	518		D10Mit112	37.8	511
	D1Snp453	131.7	519		D10Mit61	42.2	511
2	D2Mit1	1.0	512		D10Mit94	54.3	510
	D2Mit4	8.0	519		D10Mit231	58.9	502
	D2Mit99	58.0	274	11	D11Mit112	32.0	502
	D2Mit103	68.5	291		D11Mit285	56.4	519
	D2Mit105	74.9	301		D11Mit102	81.7	422
	D2Snp81	93.3	416	12	D12Mit285	15.0	519
	D2Mit113	125.0	518		D12Mit143	36.9	519
3	D3Mit328	5.0	515		D12Mit141	66.7	516
	D3Mit3	22.1	411		D12Snp60	74.4	518
	D3Mit98	51.3	519	13	D13Mit16	10.0	515
	D3Mit77	60.8	518		D13Mit202	41.0	519
	D3Mit345	69.0	519		D13Mit77	73.1	475
4	D4Mit105	6.0	411	14	D14Mit230	6.5	506
	D4Mit172	22.7	519		D14Snp759	27.2	518
	D4Mit197	34.6	519		D14Mit116	56.0	313
	D4Mit9	53.4	519		D14Mit95	80.7	519
	D4Mit311	84.3	519	15	D15Mit13	5.0	417
5	D5Mit146	1.0	427		D15Mit55*	23.6	519
	D5Mit388	26.8	518		D15Snp70*	46.4	427
	D5Mit81	41.5	457		D15Mit35	82.2	476
	D5Mit382	69.2	514	16	D16Snp395	2.0	450
	D5Mit425	83.8	518		D16Mit157*	15.3	518
6	D6Snp4.7	5.0	500		D16Mit139	32.8	519
	D6Mit116	14.6	517	17	D17Mit59*	10.0	389
	D6Mit261	45.2	519		D17Mit16	21.9	519
	D6Mit230	54.3	519		D17Mit52	27.3	503
	D6Mit15	89.1	490		D17Mit39	59.0	519
7	D7Mit294	8.0	519		D17Mit123	80.6	470
	D7Snp210	23.2	519	18	D18Mit19*	1.0	403
	D7Mit194	52.1	433		D18Mit119	19.7	410
	D7Mit101	77.4	519		D18Mit194	35.6	519
8	D8Mit222	8.0	518		D18Mit152*	49.4	490
	D8Mit180	34.0	519	19	D19Mit28	12.0	519
	D8Snp305	50.1	440		D19Mit46	32.6	518
	D8Mit120	80.4	404		D19Mit89	42.4	519
					D19Mit91	50.7	518

Table 3.3 Mean, variance, skewness, corrected kurtosis, and coefficient of variation for selection criterion traits of early-selected mice. Mice are separated into parental generations and F_1 populations derived from those generations. Units for EG and LG are grams.

	Early Gain (EG)					Late Gain (LG)				
	Mean	Var	Skewness	Kurtosis	CV	Mean	Var	Skewness	Kurtosis	CV
Trait										
Generation 8										
E^+ females	7.82	0.93	-1.08	3.07	13.4	9.97	7.5	-0.21	0.78	27.4
E^+ males	7.48	0.68	-0.14	1.13	11.0	13.2	8.1	-0.18	-0.52	21.6
E^- females	5.09	0.52	-0.005	-0.40	14.4	10.08	8.2	0.41	-0.62	28.5
E^- males	5.26	0.53	0.67	0.07	13.4	12.2	12.3	0.14	-0.10	29.3
F_1 females	4.98	1.16	0.09	-1.20	26.2	12.8	6.4	0.49	-0.28	19.9
F_1 males	6.31	1.6	-0.27	-1.28	23.8	15.2	9.6	1.03	-0.03	20.8
Generation 12										
E^+ females	7.99	0.77	0.94	2.8	12.1	10.1	3.5	0.11	-0.39	18.3
E^+ males	8.10	0.75	0.25	1.5	15.8	14.4	4.7	0.45	0.19	17.9
E^- females	5.14	0.57	-0.34	0.05	10.1	12.5	7.9	0.67	-1.34	14.4
E^- males	5.45	0.49	0.12	0.54	13.7	13.8	6.4	0.04	-0.58	19.9
F_1 females	5.7	0.89	-0.03	-1.5	15.7	12.9	6.7	0.75	-0.35	16.8
F_1 males	6.78	0.95	0.25	-0.99	14.2	13.6	8.1	0.56	0.14	16.5

Table 3.4 Mean, variance, skewness, corrected kurtosis, and coefficient of variation for selection criterion traits of late-selected mice. Mice are separated into parental generations and F_1 populations derived from those generations. Units for EG and LG are grams.

	Early Gain (EG)					Late Gain (LG)				
	Mean	Var	Skewness	Kurtosis	CV	Mean	Var	Skewness	Kurtosis	CV
Trait										
Generation 8										
L^+ females	4.66	0.11	0.07	-2.15	6.9	16.34	2.27	-0.50	-1.91	9.2
L^+ males	4.37	0.90	-0.86	-1.32	7.7	18.16	8.99	-0.66	-1.41	16.5
L^- females	5.55	0.57	0.174	0.12	13.6	5.25	7.35	0.37	-0.04	11.6
L^- males	5.67	0.35	0.15	0.28	10.5	5.98	8.31	-0.16	-0.17	8.2
F_1 females	4.31	0.88	0.25	-0.36	12.9	9.89	6.86	0.06	-0.32	6.7
F_1 males	4.36	0.94	0.12	-0.06	16.6	12.93	4.14	0.12	0.65	7.9
Generation 12										
L^+ females	5.66	0.50	-0.21	0.83	10.6	16.28	3.56	0.30	-0.06	9.8
L^+ males	5.80	0.43	-0.75	0.41	11.5	19.45	7.38	-1.59	6.36	13.7
L^- females	5.12	0.43	-0.42	0.75	14.3	5.15	6.34	0.07	0.15	12.8
L^- males	5.89	0.67	0.004	-0.23	12.2	5.91	8.7	0.32	-0.17	9.2
F_1 females	4.98	0.75	0.09	0.24	13.9	9.89	3.2	0.43	0.24	7.5
F_1 males	5.34	1.03	0.52	-0.14	15.7	12.93	3.1	0.22	-0.43	9.6

Table 3.5 Mean, variance, skewness, excess kurtosis, and coefficient of variation for growth traits in early F_2 mice. Units for traits are grams for the four growth rates and Mature Body Weight (EG, WG, MG, LG, MBW), grams/day for Maximum Growth Rate (MGR), and days for Inflection Point (IP).

	Early F_2 females n=254					Early F_2 males, n=297				
	Mean	Var	Skewness	Kurtosis	CV	Mean	Var	Skewness	Kurtosis	CV
Trait										
Early Gain (EG) (0 to 10 DOA gain)	5.77	1.28	-0.75	0.27	19.62	5.84	1.7	-0.69	0.04	22.18
Weaning Gain (WG) (7 to 21 DOA gain)	8.2	2.3	-0.4	0.48	18.52	8.48	4.2	-0.61	0.28	24.31
Middle Gain (MG) (21 to 35 DOA gain)	11.45	2.7	0.11	-0.51	14.23	15.21	4.1	-0.38	0.23	13.3
Late Gain (LG) (28 to 56 DOA gain)	9.67	4.2	-0.22	0.58	21.2	13.20	6.1	0.27	-0.1	18.7
Mature Body Weight (MBW)	31.77	7.2	0.46	0.73	8.5	38.93	10.6	-0.27	0.17	8.4
Max Growth Rate (MGR)	0.824	0.007	0.31	0.27	10.2	1.02	0.011	-0.36	0.80	10.3
Inflection Point (IP)	22.71	4.12	0.39	0.81	9.0	25.06	5.14	1.06	2.68	9.1

Table 3.6 Mean, variance, skewness, kurtosis, and coefficient of variation for growth traits in late F_2 mice. Units for traits are grams for the four growth rates and Mature Body Weight (EG, WG, MG, LG, MBW), grams/day for Maximum Growth Rate (MGR), and days for Inflection Point (IP).

	Late F_2 females n=256					Late F_2 males, n=263				
	Mean	Var	Skewness	Kurtosis	CV	Mean	Var	Skewness	Kurtosis	CV
Trait										
Early Gain (EG) (0 to 10 DOA gain)	5.31	1.7	-0.63	-0.52	24.66	5.58	1.28	-0.85	0.48	20.3
Weaning Gain (WG) (7 to 21 DOA gain)	8.11	3.25	-0.53	0.05	22.28	8.61	3.30	-0.55	0.20	21.1
Middle Gain (MG) (21 to 35 DOA gain)	11.43	5.23	-0.28	-0.01	20.1	15.13	4.52	-0.37	0.51	14.1
Late Gain (LG) (28 to 56 DOA gain)	9.58	6.8	0.08	-0.26	21.64	13.26	6.2	0.09	0.16	18.6
Mature Body Weight (MBW)	30.94	9.9	0.22	-0.02	10.13	38.97	11.86	0.09	0.59	8.83
Max Growth Rate (MGR)	0.814	0.009	0.12	0.48	12.26	1.02	0.01	-0.23	-0.42	11.3
Inflection Point (IP)	23.00	5.9	0.2	0.5	10.5	25.42	4.98	1.89	11.82	8.8

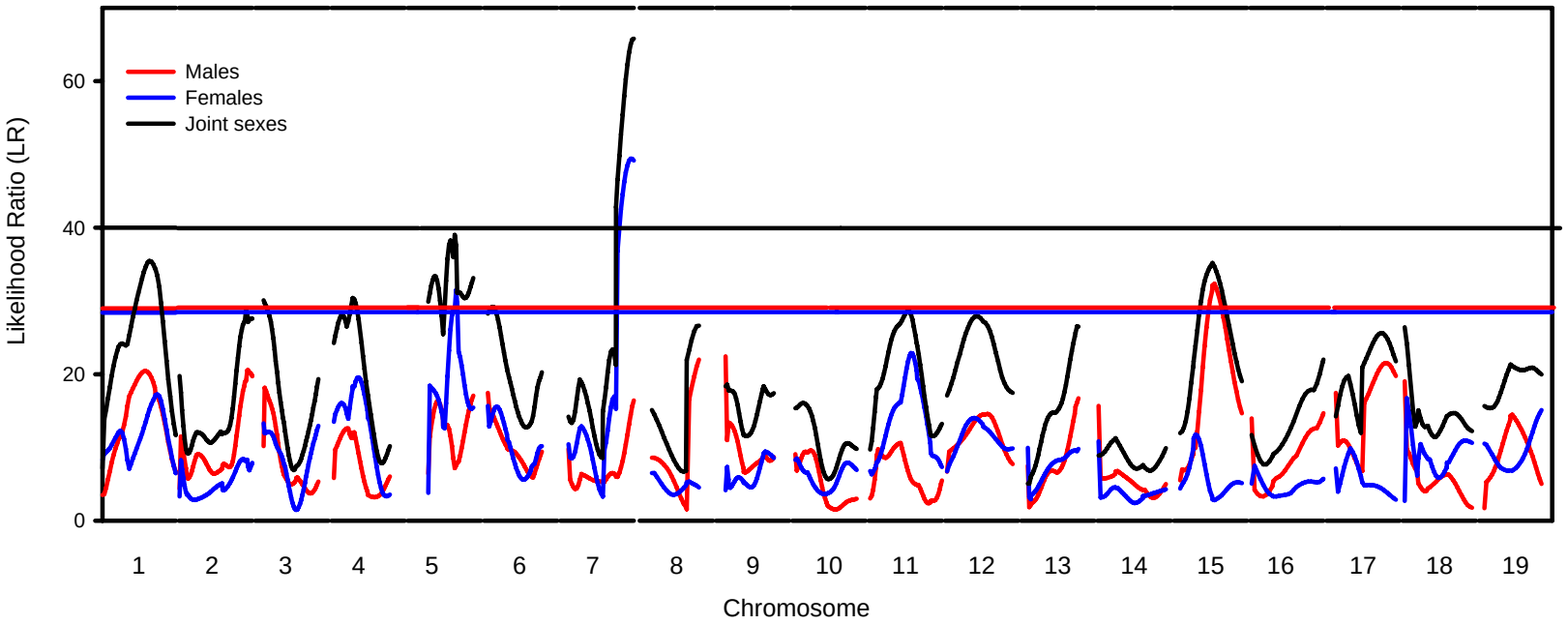

Figure 3.2 Genomewide Likelihood Ratio (LR) Test Statistic for growth rate QTL in the early population. Data represents LR test statistics for QTL influencing 4 weight gains (EG, WG, MG, and LG) across 19 autosomes for combined-sex (black), male-specific (red) and female-specific (blue) analyses. Horizontal lines reflect 5 % significance LR thresholds of 27.2, 29.2, and 37.7 for female-specific, male-specific, and combined-sex analyses, respectively. All significance thresholds were obtained via 1000 permutations.

Table 3.7 Multi-trait and single-trait quantitative trait loci influencing growth rate and growth curve traits in the early population

[a] denotes if locus influences multiple trait dataset (Gains or Growth Curve Traits) or one of the seven single traits (listed in Table 2.3). Possible single-trait QTLs are named: MBW (Mature Body Wgt.), Rate (Maximum Growth Rate), IP (time of maximum growth rate, or inflection point), EGain, WGain, MGain, or LGain (the four individual growth rates)

[b] denotes QTL's approximate map distance from centromere.

[c] denotes one-LOD confidence interval.

[d] denotes cM location of peak LR test statistic. If joint-trait QTL, reflects joint-trait LR test statistic from MCIM.

[e] denotes suggestive QTL ($0.05 < p < 0.1$)

[f] denotes if QTL was detected in combined-sex or sex-specific analysis. If combined-sex QTL has an asterisk, denotes a significant QTL-by-sex interaction effect.

Chr	QTL Name	Sex [f]	Trait [a]	Dis [b]	CI [c]	LR [d]
1	Curve1E	Combined	GCT	65	37-86	30.3
	MGain1E[e]	Combined	Middle Gain	77	49-80	18.5
5	Rate2E	Combined	Max Growth Rate	36	0-66	15.2
	MGain2E	Combined	Middle Gain	40	28-46	25.8
	Gain3E	Combined*	Gains	64	48-66	37.8
6	MBW1E	Combined	Mature Body Wgt.	5	0-11	19.5
7	Curve2E	Combined	GCT	82	72-94	34.0
	Gain4E	Combined	Gains	86	72-94	56.1
8	Curve3E	Combined*	GCT	30	30-52	26.0
	EGain5E	Males	Early Gain	68	56-68	14.5
	WGain6E	Males	Weaning Gain	68	56-68	15.3
	Curve4E [e]	Combined	GCT	68	60-68	24.6
11	Curve5E [e]	Combined*	GCT	42	29-61	29.5
	LGain7E	Females	Late Gain	57	45-70	18.0
15	Gain8E	Males	Gains	42	35-52	33.8
17	Curve6E	Combined*	GCT	52	36-64	30.2
	LGain9E	Males	Late Gain	54	36-66	15.7

Table 3.8 Effects of quantitative trait loci influencing growth rate in the early population.

[a] denotes chromosome and peak LR map distance from centromere
[b] QTL effect for trait are listed only if the trait's LR at a joint-mapped QTL exceeded significance threshold for pleiotropy (see Jiang and Zeng, 1995)
[c] Additive estimates reflect the effect of the E^+ allele, divided by the sex-specific phenotypic standard deviation of trait (Table 3.5)
[d] Dominance estimates reflect difference of heterozygote from average of two parents, divided by the sex-specific phenotypic standard deviation of trait (Table 3.5)
[e] denotes chromosomal region was detected only during single trait mapping (95th percentile LR between 13 and 15)
[f] denotes suggestive locus ($0.05 < p < 0.1$)
[g] QTL was significant in one of the two sex-specific analysis and in combined-sex analysis. Genetic effects for both sexes are presented.
[h] Trait with subscript has genetic effects significantly different from traits pleiotropically influenced by same QTL. If two of three traits have subscripts, genetic effects for all three traits were significantly different. If followed by "m" or "f" in parentheses, denotes only one sex had significantly different genetic effects.

			Males			Females		
QTL Name	Chr, Peak LR[a]	Trait[b]	a/ σ_p[c]	d/ σ_p[d]	d/a	a/ σ_p[c]	d/ σ_p[d]	d/a
MGain1E [e, f]	1, 77 cM	MG	-0.384	-0.186	0.48	-0.280	-0.128	0.45
MGain2E [e]	5, 40 cM	MG	0.331	0.220	0.67	0.257	0.140	0.55
Gain3E [g]	5, 64 cM	EG	0.042	0.013	0.31	0.155	0.040	0.26
		WG [h]	-0.052	-0.018	0.35	-0.229	0.080	-0.35
		MG [h]	0.211	0.060	0.28	0.306	0.238	0.78
Gain4E [g]	7, 86 cM	WG [h]	0.245	0.113	0.46	0.399	0.228	0.57
		MG	-0.095	-0.218	2.3	-0.255	-0.078	0.31
		LG	-0.169	-0.150	0.89	-0.417	-0.117	0.28
EGain5E [e]	8, 68 cM	EG	0.239	0.020	0.08			
WGain6E [e]	8, 68 cM	WG	0.285	0.020	0.07			
LGain7E [e]	11, 57 cM	LG				-0.409	-0.170	0.41
Gain8E	15, 42 cM	MG [h]	-0.139	0.065	-0.47			
		LG	-0.149	-0.122	0.82			
LGain9E [e]	17, 54 cM	LG	0.352	-0.131	-0.37			

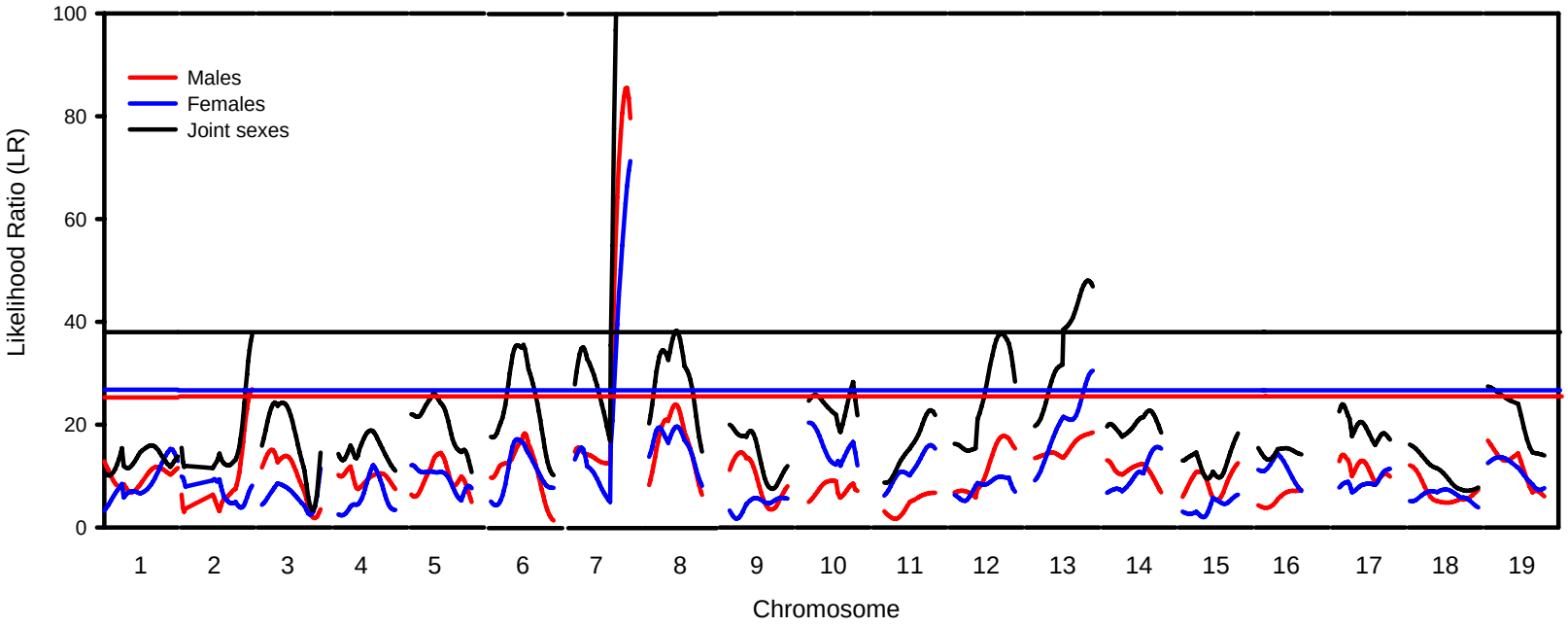

Figure 3.3 Genomewide Likelihood Ratio Test Statistic for growth rate QTL in the late population. Data represents LR test statistics for QTL influencing 4 growth rates (EG, WG, MG, and LG) across 19 autosomes for combined-sex (black), male-specific (red) and female-specific (blue) analyses. Horizontal lines reflect 5 % significance LR thresholds of 25.3, 26.8, and 38.5 for male-specific, female-specific, and combined-sex analyses, respectively. All significance thresholds were obtained via 1000 permutations.

Table 3.9 Multi-trait and single trait quantitative trait loci influencing growth rate and growth curve traits in the late population

[a] denotes if locus influences multiple trait dataset (Gains or Growth Curve Traits) or one of the seven single traits (listed in Table 2.3). Possible single-trait QTLs are named: MBW (Mature Body Wgt.), Rate (Maximum Growth Rate), IP (time of maximum growth rate, or inflection point), EGain, WGain, MGain, or LGain (the four individual growth rates)

[b] denotes QTL's approximate map distance from centromere.

[c] denotes one-LOD confidence interval.

[d] denotes cM location of peak LR test statistic. If joint-trait QTL, reflects joint-trait LR test statistic from MCIM.

[e] denotes suggestive QTL ($0.05 < p < 0.1$)

[f] denotes if QTL was detected in combined-sex or sex-specific analysis. If combined-sex QTL has an asterisk, denotes a significant QTL-by-sex interaction effect.

Chromosome	QTL Name	Sex [f]	Trait [a]	Dis [b]	CI [c]	LR [d]
2	Gain1L	Combined *	Gains	113	107-115	38.5
5	Curve1L[e]	Combined	GCT	30	23-40	28.6
6	Gain2L[e]	Combined	Gains	40	30-54	35.3
7	Gain3L[e]	Combined	Gains	18	10-29	35.2
	Curve2L[e]	Combined	GCT	23	10-35	29.1
	Gain4L	Combined	Gains	74	68-78	161.5
	Curve3L	Combined	GCT	76	60-70	87.8
8	Gain5L[e]	Combined	Gains	24	16-32	34.2
	Curve4L	Combined	GCT	42	36-54	35.7
	Gain6L	Combined	Gains	44	36-54	38.3
12	Gain7L	Combined	Gains	60	48-70	37.7
13	Curve5L	Combined *	GCT	30	22-71	41.3
	Curve6L	Combined *	GCT	53	38-63	46.2
	Gain8L	Combined *	Gains	67	55-73	48.0
16	IP1L	Combined	Inflection Point	2	0-10	19.6

Table 3.10 Effects of quantitative trait loci influencing growth rate in the late population

[a] denotes chromosome and peak LR map distance from centromere
[b] QTL effect for trait are listed only if the trait's LR at a joint-mapped QTL exceeded significance threshold of 5.99 (see Jiang and Zeng, 1995)
[c] Additive estimates reflect the effect of the L^+ allele, divided by the sex-specific phenotypic standard deviation of trait (Table 3.6)
[d] Dominance estimates reflect difference of heterozygote from average of two parents, divided by the sex-specific phenotypic standard deviation of trait (Table 3.6)
[e] denotes chromosomal region was detected only during single trait mapping (95^{th} percentile LR between 13 and 15)
[f] denotes suggestive locus ($0.05 < p < 0.1$)
[g] QTL was significant in one of the two sex-specific analysis and in combined-sex analysis. Genetic effects for both sexes are presented.
[h] Trait with subscript has genetic effects significantly different from traits pleiotropically influenced by same QTL. If two of three traits have subscripts, genetic effects for all three traits were significantly different. If followed by "m" or "f" in parentheses, denotes only one sex had significantly different genetic effects.

			Males			Females		
QTL Name	Chromosome, Peak LR[a]	Trait[b]	a/σ_p[c]	d/σ_p[d]	d/a	a/σ_p[c]	d/σ_p[d]	d/a
Gain1L[f, g]	2, 113 cM	EG	-0.160	-0.004	0.02	-0.009	0.105	-11.2
		LG[h (m)]	0.259	0.017	0.06	-0.098	-0.104	1.06
Gain2L[f]	6, 40 cM	WG[h (f)]	-0.199	0.149	-0.74	-0.110	-0.008	0.07
		LG	0.020	-0.073	-3.65	0.092	-0.385	-4.18
Gain3L[f]	7, 18 cM	EG[h]	0.064	0.077	1.18	0.062	0.235	3.81
		WG	0.182	0.089	0.49	0.172	0.161	0.93
		MG	0.167	-0.171	-1.02	0.074	-0.147	-1.97
Gain4L	7, 74 cM	EG[h]	-0.132	0.074	-0.55	-0.123	0.055	-0.44
		WG[h]	-0.412	0.702	-1.70	-0.403	0.409	-1.01
		LG	0.266	-0.479	-1.80	0.144	-0.096	-0.66
Gain5L	8, 24 cM	EG[h]	-0.118	-0.192	1.62	-0.025	-0.149	5.84
		WG	-0.221	-0.194	0.87	-0.230	-0.098	0.42
Gain6L	8, 44 cM	EG[h]	-0.095	-0.232	2.44	-0.032	-0.170	5.35
		WG	-0.177	-0.297	1.67	-0.199	-0.048	0.24
Gain7L[f]	12, 60 cM	MG	0.375	0.045	0.12	0.200	-0.175	-0.87
		LG	0.297	0.224	0.75	0.157	0.121	0.77
Gain8L[g]	13, 67 cM	EG[h]	0.064	-0.176	-2.76	0.163	-0.023	-0.14
		WG	0.092	-0.018	-0.19	0.164	0.200	1.22
		MG	0.197	0.224	1.13	0.355	-0.038	-0.10

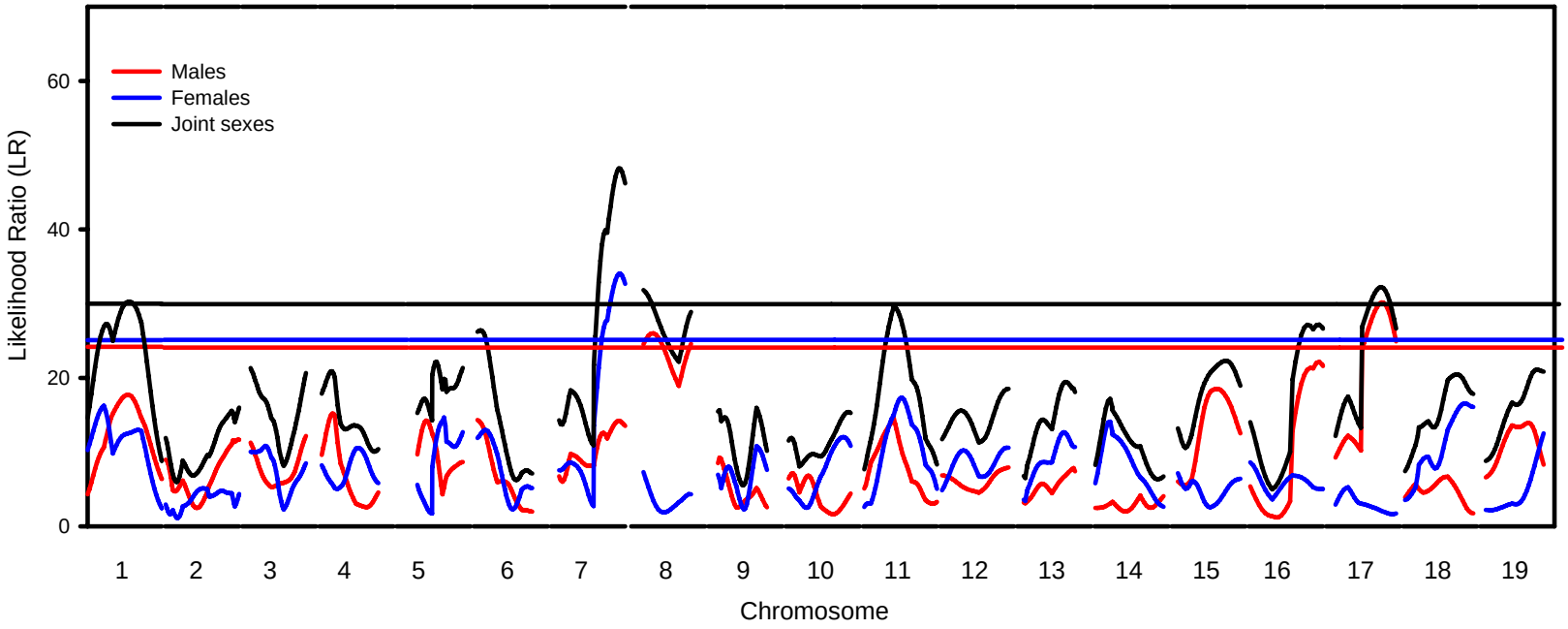

Figure 3.4 Genomewide Likelihood Ratio Test Statistic for growth curve trait QTL in the early population. Data represents LR test statistics for QTL influencing 3 growth curve traits (mature body weight, maximum growth rate, and time of maximum growth rate, or inflection point) across 19 autosomes for combined-sex (black), male-specific (red) and female-specific (blue) analyses. Horizontal lines reflect 5 % significance LR thresholds of 24.2, 25.1, and 30.3 for male-specific, female-specific, and combined-sex analyses, respectively. All significance thresholds were obtained via 1000 permutations.

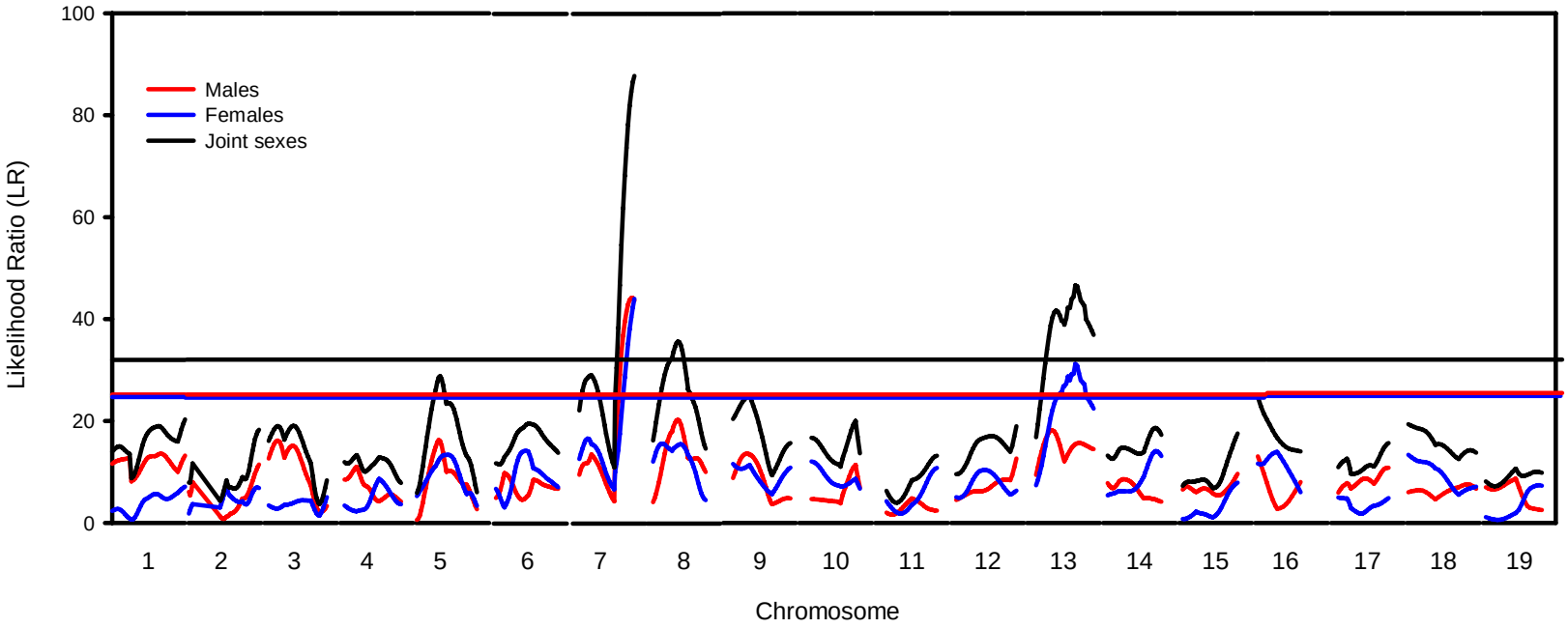

Figure 3.5 Genomewide Likelihood Ratio Test Statistic for growth curve trait QTL in the late population. Data represents LR test statistics for QTL influencing 3 growth curve traits (mature body weight, maximum growth rate, and time of maximum growth rate, or inflection point) across 19 autosomes for combined-sex (black), male-specific (red) and female-specific (blue) analyses. Horizontal lines reflect 5 % significance LR thresholds of 24.7 and 25.2, and 31.8 for female-specific, male-specific, and combined-sex analyses, respectively. All significance thresholds were obtained via 1000 permutations.

Table 3.11 Effects of quantitative trait loci influencing growth curves traits in the early population.

[a] denotes chromosome and peak LR map distance from centromere

[b] QTL effect for trait are listed only if the trait's LR at a joint-mapped QTL exceeded significance threshold of 5.99 (see Jiang and Zeng, 1995)

[c] Additive estimates reflect the effect of the E^+ allele, divided by the sex-specific phenotypic standard deviation of trait (Table 3.5)

[d] Dominance estimates reflect difference of heterozygote from average of two parents, divided by the sex-specific phenotypic standard deviation of trait (Table 3.5)

[e] denotes chromosomal region was detected only during single trait mapping (95^{th} percentile LR between 13 and 15)

[f] denotes suggestive locus ($0.05 < p < 0.1$)

[g] QTL was significant in one of the two sex-specific analysis and in combined-sex analysis. Genetic effects for both sexes are presented.

[h] Trait with subscript has genetic effects significantly different from traits pleiotropically influenced by same QTL. If two of three traits have subscripts, genetic effects for all three traits were significantly different. If followed by "m" or "f" in parentheses, denotes only one sex had significantly different genetic effects.

			Males			Females		
QTL Name	Chr, Peak LR[a]	Trait[b]	a/ σ_p[c]	d/ σ_p[d]	d/a	a/ σ_p[c]	d/ σ_p[d]	d/a
Curve1E	1, 65 cM	MBW [h]	-0.236	0.110	-0.47	-0.307	0.059	-0.19
		MGR	-0.246	0.158	-0.64	-0.284	0.255	-0.90
Rate2E [e]	5, 36 cM	MGR	0.190	0.314	1.65	0.229	0.241	1.05
MBW1E [e]	6, 5 cM	MBW	0.185	0.131	0.71	0.253	-0.068	-0.27
Curve2E [g]	7, 82 cM	MBW	-0.122	-0.191	1.56	-0.234	-0.059	0.25
		MGR [h]	0.183	-0.070	-0.38	0.270	0.207	0.76
		IP	-0.256	-0.108	0.42	-0.463	-0.178	0.38
Curve3E [g]	8, 38 cM	MBW	0.403	0.032	0.08	-0.009	-0.066	7.21
		MGR [h (m)]	0.319	-0.115	-0.36	0.103	-0.067	-0.65
Curve4E [g]	8, 68 cM	MBW	0.255	-0.046	-0.18	0.024	0.026	1.06
		MGR [h (m)]	0.286	-0.002	-0.01	0.065	0.125	1.92
		IP [h (m)]	-0.275	-0.169	0.62	-0.096	-0.211	2.20
Curve5E	11, 41 cM	MBW	-0.090	0.193	-2.14	-0.247	-0.029	0.12
		IP	0.014	0.272	6.40	-0.180	-0.111	0.61
Curve6E [g]	17, 52 cM	MBW	0.235	0.033	0.14	0.080	0.016	0.20
		IP	0.417	-0.236	-0.57	-0.015	0.093	-6.26

Table 3.12 Effects of quantitative trait loci influencing growth curves traits in the late population

[a] denotes chromosome and peak LR map distance from centromere
[b] QTL effect for trait are listed only if the trait's LR at a joint-mapped QTL exceeded significance threshold of 5.99 (see Jiang and Zeng, 1995)
[c] Additive estimates reflect the effect of the L^+ allele, divided by the sex-specific phenotypic standard deviation of trait (Table 3.6)
[d] Dominance estimates reflect difference of heterozygote from average of two parents, divided by the sex-specific phenotypic standard deviation of trait (Table 3.6)
[e] denotes chromosomal region was detected only during single trait mapping (95^{th} percentile LR between 13 and 15)
[f] denotes suggestive locus ($0.05 < p < 0.1$)
[g] QTL was significant in one of the two sex-specific analysis and in combined-sex analysis. Genetic effects for both sexes are presented.
[h] Trait with subscript has genetic effects significantly different from traits pleiotropically influenced by same QTL. If two of three traits have subscripts, genetic effects for all three traits were significantly different. If followed by "m" or "f" in parentheses, denotes only one sex had significantly different genetic effects.

			Males			Females		
QTL Name	Chr, Peak LR[a]	Trait[b]	a/ σ_p[c]	d/ σ_p[d]	d/a	a/ σ_p[c]	d/ σ_p[d]	d/a
Curve1L	5, 30 cM	IP	-0.199	-0.369	1.86	0.205	0.086	0.42
Curve2L	7, 23 cM	MBW [h (m)]	0.233	-0.098	-0.42	0.147	0.078	0.53
		MGR	0.238	-0.055	-0.23	0.177	-0.087	-0.49
Curve3L	7, 76 cM	MGR [h]	-0.208	0.492	-2.36	-0.426	0.270	-0.63
		IP	0.351	-0.460	-1.31	0.248	-0.229	-0.92
Curve4L	8, 42 cM	MBW [h (m)]	-0.319	-0.314	0.99	0.016	-0.137	-8.47
		MGR	-0.254	-0.044	0.17	-0.192	0.200	-1.04
Curve5L	13, 30 cM	MBW [h]	0.358	0.440	1.23	0.391	-0.125	-0.32
		MGR [h]	0.226	0.360	1.59	0.290	-0.376	-1.30
		IP	0.142	0.145	1.03	0.133	0.310	2.32
Curve6L [g]	13, 53 cM	MBW [h]	0.332	0.253	0.76	0.481	0.029	0.06
		MGR	0.249	0.353	1.42	0.421	-0.159	-0.38
IP1L	16, 2 cM	IP	-0.142	-0.242	1.71	-0.208	0.182	-0.88

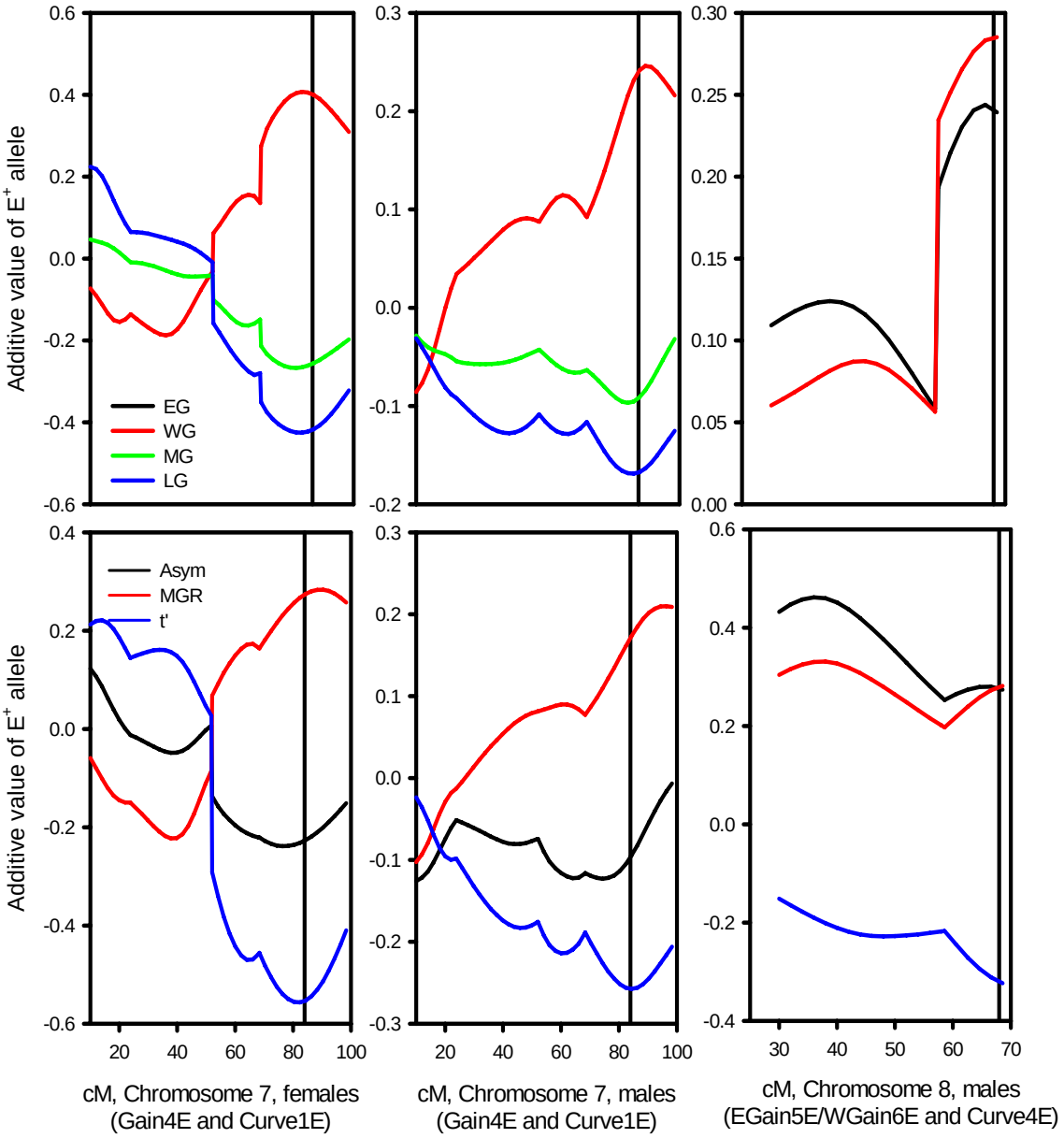

Figure 3.6 Covariation of additive effect of the E^+ allele at common growth intervals with multi-trait QTL in the early population. Top row reflects additive estimates for growth rate loci while bottom row reflects additive estimates for growth curve trait loci. Variables from each trait set are included only if trait is listed in Tables 3.8 or 3.11. Black vertical line denotes peak LR test statistic for QTL. Parameter estimates are standardized by variable's phenotypic standard deviation.

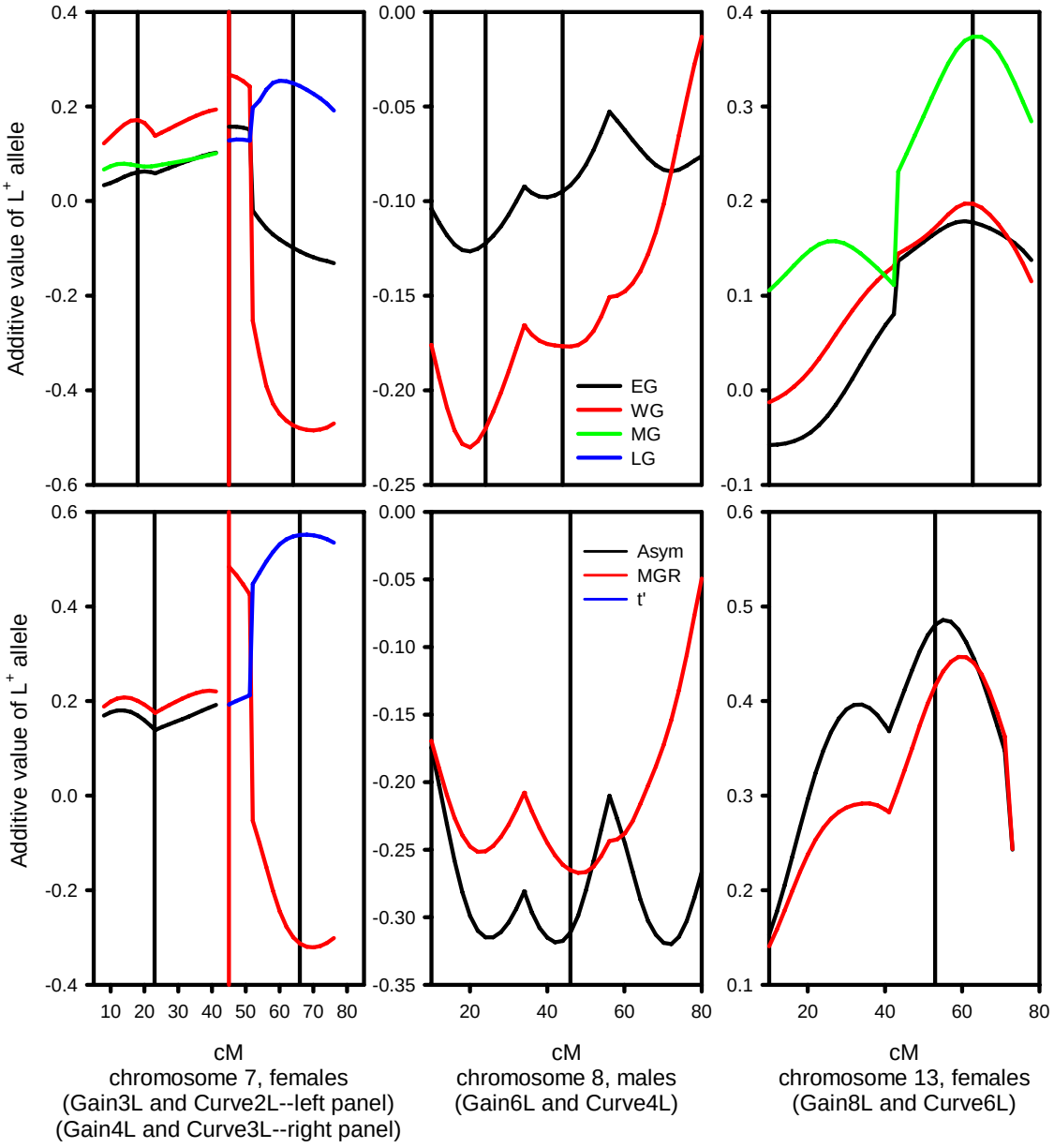

Figure 3.7 Covariation of additive effect of the L^+ allele at common growth intervals with multi-trait QTL in the late population. Top row reflects additive estimates for growth rate loci while bottom row reflects additive estimates for growth curve trait loci. Variables from each trait set are included only if trait is listed in Tables 3.10 or 3.12. Black vertical reference line denotes peak LR test statistic for QTL. Parameter estimates are standardized by variable's phenotypic standard deviation. Chromosome 7's panels have two black references lines to reflect two QTL for growth rate (Gain3L and Gain4L) and growth curve traits (Curve2L and Curve3L), with red reference line reflecting split panel for the two QTL sets.

Table 3.13 Least-square means for nine genotypic classes at significant marker-by-marker interactions ($p < 0.0002$) in the early population. The effect of each interaction is estimated as the reduction in sums of squares and is listed below the trait. Values reflect means for the nine genotypic classes, scaled by the highest means (i.e., deviations from genotypic class with the greatest positive mean).

Marker		D4Mit334		
		E^+/E^+	E^+/E^-	E^-/E^-
D6Mit17	E^+/E^+	1	-0.13968	-0.87385
Trait: WG	E^+/E^-	-0.10543	0.107605	0.889988
F=6.76	E^-/E^-	-0.82686	0.428326	-0.39276
D6Mit17	E^+/E^+	1	-0.19851	-1.56976
Trait: MGR	E^+/E^-	0.052489	-0.00305	0.862339
F=7.14	E^-/E^-	-0.66048	0.729351	-0.53604
D10Mit103	E^+/E^+	0.412366	-0.1362	-0.24705
Trait: MBW	E^+/E^-	-0.39925	0.34449	1
F=6.87	E^-/E^-	0.683907	0.094931	-1.33524

Table 3.14 Least-square means for nine genotypic classes at significant marker-by-marker interactions ($p < 0.0002$) in the late population. The effect of each interaction is estimated as the reduction in sums of squares and is listed below the trait. Values reflect means for the nine genotypic classes, scaled by the highest means (i.e., deviations from genotypic class with the greatest positive mean).

Marker		D3Mit77		
		L^+/L^+	L^+/L^-	L^-/L^-
D8Mit180	L^+/L^+	0.187988	0.62166	0.932581
Trait: WG	L^+/L^-	-0.42952	-0.0346	-0.30843
F=5.77	L^-/L^-	1	-0.68966	-0.92823
		D5Mit382		
D14Snp759	L^+/L^+	-0.10488	0.478162	0.286232
Trait: MG	L^+/L^-	-1.18591	0.389821	-0.45889
F=5.69	L^-/L^-	0.997903	-1.03725	1
		D2Mit113		
D5Mit81	L^+/L^+	0.510253	-1.36374	0.779748
Trait: MG	L^+/L^-	-0.27124	0.053221	-0.59153
F=7.16	L^-/L^-	-0.75292	0.465148	1
D5Mit81	L^+/L^+	0.306278	-1.93195	1
Trait: LG	L^+/L^-	-0.36057	0.488567	-1.2392
F=6.56	L^-/L^-	-0.64248	0.835979	0.359551

Table 3.15 Summary of direct and epistatic analyses. [a] denotes summed standardized genotypic effect, [b] denotes summed standardized dominance effect [c] Estimated as reduction in residual sums of squares from analysis using most likely QTL position [d] Estimated as reduction in residual sums of squares from analysis using most likely QTL position plus loci involved in epistatic interactions

Trait	# QTLs	a_s [a]	d_s [b]	Direct Effects (%)[c]	Direct plus Epistatic Effects (%) [d]	# QTLs	a_s [a]	d_s [b]	Direct Effect (%)[c]	Direct plus Epistatic Effects (%)[d]
	Early F_2 Females					Early F_2 Males				
Early Gain (EG)	1	0.16	0.04	4.3	4.3	2	0.28	0.03	5.6	5.6
Weaning Gain (WG)	2	0.17	0.31	6.7	9.9	3	0.48	0.12	15.0	18.0
Middle Gain (MG)	4	0.03	0.17	43.0	43.0	5	-0.08	-0.06	15.6	15.6
Late Gain (LG)	2	-0.83	-0.29	15.0	15.0	3	0.03	-0.40	13.0	13.0
Mature Body Weight (MBW)	7	-0.44	-0.12	43.0	46.7	7	0.63	0.26	40.7	41.4
Max Growth Rate (MGR)	5	0.38	0.76	31.4	35.4	5	0.73	0.29	28.2	31.4
Inflection Point (IP)	4	-0.75	-0.41	18.1	18.1	4	-0.10	-0.24	22.6	22.6
	Late F_2 Females					Late F_2Males				
Early Gain (EG)	6	0.03	-0.13	30.1	30.1	6	-0.36	-0.30	31.8	31.8
Weaning Gain (WG)	6	-0.61	0.15	40.8	41.0	6	-0.74	0.55	39.6	40.9
Middle Gain (MG)	3	0.63	-0.36	6.9	13.0	3	0.74	0.10	13.7	17.7
Late Gain (LG)	4	0.30	-0.46	21.0	26.4	4	0.84	-0.31	24.8	39.8
Mature Body Weight (MBW)	4	1.04	-0.16	13.3	13.3	4	0.60	0.28	25.9	25.9
Max Growth Rate (MGR)	5	0.27	-0.15	30.7	30.7	5	0.25	1.11	27.4	27.4
Inflection Point (IP)	4	0.38	0.35	21.4	21.4	4	0.15	-0.93	23.7	23.7

Hepatic Endopolyploidy as a Cellular Consequence of Age-Specific Selection for Rate of Development in Mice

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Running Title: Selection and Hepatic Endopolyploidy

ABSTRACT

Endopolyploidy is the generation of polyploid cells by DNA replication without subsequent cell division and is correlated with hypertrophic growth, or growth via cell size. Thus, selection that alters growth may also change onset and frequency of endopolyploidy as a correlated response to selection. Changes in endopolyploidy in the liver are described as a result of age-specific restricted index selection for rate of development in body weight. Polyploidy changes over ontogeny are described in five mouse lines: two selected for divergence in early growth (0 to 10 days of age), two selected for divergence in late growth (28 to 56 days of age), and one randombred control. Polyploid cell frequency within each line increased as ontogeny continued, as expected. Results suggest a selection treatment-by-ontogeny effect, as lines selected for divergence in early growth have temporary polyploidy changes that dissipate before sexual maturity. However, lines selected for divergence in late growth have increases in two types of polyploid cells, starting near sexual maturity and continuing into adulthood.

INTRODUCTION

Endopolyploidy involves DNA replication without subsequent cell division, resulting in an increase in nuclear DNA content while maintaining cell number. It is a normal physiological process seen in a variety of organisms and organs and is associated with terminal differentiation of cells, increased age, and metabolic load. The frequency of polyploid cells typically increases during periods of high metabolic activity for an organ, e.g., polyploid cardiomyocytes form early in ontogeny during peak heart function (Vinogradov et al. 2001; Abatskaya and Vinogradov, 2004). However, it is not clear if polyploid cells form as a byproduct of normal growth processes or develop to enhance and aid growth (Storchova and Pellman, 2000; Abatskaya and Vinogradov, 2004).

Several theories have been proposed to explain why polyploid cells form. Medvedev (‘86) suggests cells in certain organs may accumulate more somatic mutations, leading to decreased cell cycle regulation. He also proposed polyploidy may arise to create redundant sets of genes or act as a tissue-specific detoxication system. Vinogradov suggests polyploidy may develop to accommodate the normal physiological demands of proliferation and differentiation during ontogeny (Vinogradov, ‘98; Vinogradov, 2001). Alternatively, polyploidy may arise to facilitate increased gene expression via increased transcription factor levels (reviewed in Storchova and Pellman, 2004).

Polyploid cells form by two seemingly independent mechanisms (Vinogradov et al. 2001). Failure of cytokinesis after DNA replication and telophase in a mononuclear diploid cell (2n) will produce a binuclear diploid cell (2x2n). Alternatively, if telophase is omitted after DNA replication, a mononuclear diploid cell (2n) gives rise to a mononuclear tetraploid

cell (4n). Polyploid cells can also form from polyploid cells, i.e., a mononuclear tetraploid cell could give rise to a mononuclear octoploid (8n). Within mammals, there is considerable temporal and spatial variation in the extent and type of polyploid cell detected. In general, smaller mammals such as rodents show higher proportions of polyploid cells, relative to larger mammals (Vinogradov et al. 2001). Within rodents, there are species and even strain differences for frequency and primary type of polyploid cell in the same organ (Vinogradov et al. 2001; Gandillet et al. 2003; Guidotti et al. 2003).

In the mouse liver, polyploid hepatocytes start to accumulate at weaning, possibly due to increased metabolic load in the liver resulting from the change to solid food. At approximately the same time, growth begins to shift from hyperplasia (changes in cell number) to hypertrophy (changes in cell size) (Winick and Noble, '65; Wheatley, '72). At this point, creation of polyploid cells may increase the capacity for gene expression by increasing the number of complete genomes per cell. Not surprisingly, hepatocyte polyploidy level is correlated with increases in cell size, the hallmark of hypertrophic growth (Gupta, 2000; Vinogradov et al. 2001).

In some invertebrate species, variation in degree of endopolyploidy is associated with phenotypic variation in quantitative traits, e.g., body size in *C. elegans* (Flemming et al. 2000). Given that variation in endopolyploidy affects phenotypic variation, selection may act on genetic variation for onset and extent of mammalian endopolyploidy. Herein, we describe results to determine if age-specific selection for growth has altered onset and frequency of polyploid hepatocytes. We look for polyploidy changes in four mouse strains that differ in rate of development in two phases of ontogeny (Atchley et al., '97), as well as liver cell

number and cell size (Atchley et al. 2000). We use flow cytometry to analyze polyploid hepatocytes in the G_1 phase of the cell cycle and ask the following questions: (i) Has long-term genetic selection for changes in growth rates lead to changes in number of polyploid mouse hepatocytes? (ii) Has selection for differential growth involving hyperplasia or hypertrophy affected the frequency and time of onset of endopolyploidy during ontogeny?

METHODS AND MATERIALS

Generation of the Selection strains

Fifteen restricted index selection lines were created from an ICR Harlan Sprague-Dawley randombred mouse population (Atchley et al., '97). The selection criteria were body weight gain between birth and ten days of age (early gain, or EG) and body weight gain between 28 and 56 days of age (late gain, or LG). EG is growth primarily via changes in cell number while LG is growth via changes in cell size (Atchley et al. 2000). Four replicated selection lines and a randombred control line were produced, each subjected to a different selection treatment. Lines were each replicated three times to assess for genetic drift, producing a total of 15 selection lines. Each replicate was maintained as a separate entity and no crosses were made between replicates. Within family selection was performed to reduce maternal effects.

The four selection treatments were as follows: $E^{+}L^{0}$ mice were selected for increased early gain, EG, while holding late gain, LG, constant. Its reciprocal line, $E^{-}L^{0}$, was selected for decreased EG while holding LG constant. $E^{0}L^{+}$ mice were selected for increased LG while holding EG constant; $E^{0}L^{-}$ mice were selected for decreased LG while holding EG constant. Statistically significant differences have accumulated in body weight and weight

gain, tail length, organ cell number and cell size, uterine and maternal effects, reproductive onset and longevity between the selection lines as a result of genetic selection (Atchley et al., '97; Ernst et al., '99; Rhees et al., '99; Atchley et al. 2000; Ernst et al. 2000; Miller et al. 2000; Rhees and Atchley, 2000). After 35 generations of genetic selection, the best performing replicates from each of the four selection lines were brother-sister mated to create inbred strains. The resultant inbred strains are designated in this report as E^+, E^-, L^+, and L^-.

Cell Cycle Samples

Tissue samples were taken to compare mice from a control line to mice from inbred strains (E^+, E^-, L^+, and L^-) in the 23rd generation of brother-sister inbreeding. In this report, we use the terms "line" and "strain" interchangeably, but "selection strain" always refers to the inbred strains derived from the four selection treatment lines. The original randombred control lines created in the selection experiment (Atchley et al., '97) are no longer available. Hence, control samples (C) analyzed here are randombred ICR mice recently obtained from Harlan Spraque-Dawley. For the current analysis, fifteen pairs of males and females from the same selection strain were randomly mated. For control samples, 15 randomly mated pairs of mice were obtained from the same population of randombred ICR mice as the founding stock of the selection experiment (Atchley et al., '97). Mothers' ages ranged from 10 to 15 weeks of age. Litters were standardized at birth to 7-8 pups per litter. Pups were forcibly weaned at 21 days of age and same sex mice were housed 3-4 mice per cage.

Liver samples were obtained at birth (0 days of age, or 0 DOA), 10 DOA, 28 DOA and 56 DOA to reflect the original selection criterion (Atchley et al., '97). All liver samples were collected in a temporally equivalent manner to minimize documented circadian rhythm

effects on the cell cycle (Farsund, '75). Mice were weighed prior to sacrifice and the resultant body weight used later as a covariate in subsequent analyses. Body weight at birth was calculated as the total litter weight divided by the total number of pups in the litter. With the exception of 0 DOA samples, only females were analyzed because these strains have well-documented sexual dimorphism in liver growth and weight (Atchley et al. 2000). Because of the low number of cells per newborn liver, multiple liver samples from the same litter were pooled for 0 DOA samples (between 2 and 6 livers).

Litter mothers were sacrificed approximately one week after weaning of pups. Liver samples were taken from mothers to assess effect of genetic selection on polyploidy in older mice (advanced age, or AA, samples). At the time of sacrifice, mothers' ages ranged from 16 to 23 weeks.

Single cell suspensions were produced from each liver sample by gentle homogenization to maximize cell yield while minimizing cellular damage. Cell count and viability were measured using trypan blue staining on a hemocytometer. Four sets of $2x10^6$ cells from each sample were fixed overnight in methanol at -20°C.

Polyploidy cannot be determined simply by measuring cellular DNA in a population of cells because cells in different phases of the cell cycle can have the same DNA content (C). A second stain must be done to verify cells are in the same cell cycle phase. Therefore, cells were dual-stained for DNA and cyclin E_1, a G_1 phase-specific protein (Pozarowski and Darzynkiewicz, 2004). The cyclin E_1 stain, along with DNA stain, will separate non-polyploid G_2/M diploid cells from the higher ploidy polyploid cells. Fixed cells were sequentially blocked in normal donkey serum (5% in PBS, Jackson Immunoresearch),

incubated with polyclonal anti-cyclin E_1 primary antibody (1:250, Upstate Biotechnology) and incubated with donkey anti-rabbit FITC-conjugated secondary antibody (1:100, Jackson Immunoresearch, $F(ab')_2$ fragment). All incubations were performed for one hour at room temperature with gentle shaking. After antibody incubation, cellular DNA was stained with 1 mg/mL propidium iodide (PI) solution with RNase A to prevent RNA staining.

To assess staining variation, two replicates of $2x10^6$ cells were dual-stained for cyclin E_1 and DNA for all samples taken after 0 DOA. A control set of $2x\ 10^6$ cells was stained with PI alone for each sample to assess FITC background stain, as PI was the main cause of high FITC background. Samples were sorted on a FACSCalibur System (Becton Dickinson), with 20,000 cells being counted per dual-stained sample.

Data Analysis

Three cell counts were analyzed for each sample: cyclin E_1 positive/ 2C cells (2C), cyclin E_1 positive/4C cells (4C), and cyclin E_1 positive/8C cells (8C). Cell counts were standardized by the total number of cyclin E_1 positive cells and multiplied by 100 to yield relative proportions of 2C, 4C, and 8C cells per sample. While there is variation in cyclin E_1 expression (Darzynkiewicz et al. 2001), data analysis should not be affected since we are using the presence of cyclin E_1 expression over background level as the criterion for inclusion. The three proportions analyzed herein (2C, 4C, and 8C) will not add up to 100% since there are additional cyclin E_1 positive cells not analyzed (3C, 6C and 10C cells). In this report, we refer to the cellular proportions as 2C, 4C, and 8C rather than 2N, 4N, and 8N. This is because polyploid proportions contain both binuclear and mononuclear cells. For example, the 4C cellular proportion contains both binuclear diploid cells (2 x 2n) and

mononuclear tetraploid cells, (4n) which differ in ploidy number but have the same DNA content (C).

Analysis of variance was performed on each ploidy proportion using PROC MIXED in SAS (SAS Institute, 2003). Stain replication and interactions involving stain were not significant at any age. Consequently, they were dropped from statistical models. The statistical model was

$$Y_{ijkl}=\mu + \alpha_i + \gamma_j + \chi_{ij} + \delta_{k(i)} + \varepsilon_{ijkl}$$

where Y_{ijk} is the observation for individual *l* from line *i* and litter *k* (nested within line *i*) at age *j* with line-by-age interaction χ_{ij}. Litter nested within line was fit as a random effect. A second MIXED model was run fitting body weight as a covariate. Inclusion of body weight in the model did not change significance of means or contrasts, so results are presented for the non-body weight model.

Least squares means for each ploidy proportion for line and age combinations were calculated. Significance tests for differences between lines used a Bonferroni correction due to the high number of comparisons. P-values for model sources of variation were calculated from Type III sums of squares because of possible high correlation between sources of variation. Contrasts were performed for each age and ploidy combination to assess for age-specific changes between line pairs (Bonferroni-corrected). Contrasts may show different significance patterns than least squares means; performing fewer contrasts may lead to higher power for detection compared to differences in least squares means.

After initial means and contrasts, orthogonal contrasts were performed in PROC MIXED to assess for line-by-age interaction for ontogenetic samples. Polynomial contrasts

were performed due to the pattern of cellular proportion changes over ontogeny. Control samples were not used to avoid compounding selection and drift effects. Ages of sampling (0, 10, 28, 56) are not equally spaced; thus, coefficients for polynomial contrasts were computed in PROC IML in SAS (SAS Institute, 2003). For each cellular proportion, three orthogonal sets were performed: line (2 contrasts: E^+ versus E^-, L^+ versus L^-), age (3 contrasts: linear, quadratic, cubic), and line*age (6 interactions). Since contrasts are orthogonal, no Bonferroni correction was performed.

Maternal samples were analyzed separately using a simpler model, fitting only line and replicate stain as a source of variation. As with ontogenic samples, stain was not a significant source of variation. Maternal samples were initially analyzed without a random litter effect since no population structure information was available for the control ICR females purchased from Sprague Dawley. A second model was run to fit a random litter effect nested within line for the four selection strains, with missing data for the ICR females. The two models did not differ in terms of significance for means and contrasts so results for no litter effect are presented.

RESULTS

Effect of Selection on Body Weight

The first question to be explored is the effect of long-term selection on body weight. Body weight means for the four ages are shown in Table 3.1, together with Bonferroni-corrected tests for line differences. Body weight difference at 0 DOA was not significant among the five lines. For 10 DOA body weight, the randombred control line was significantly heavier than the four selection strains. This decrease in selection strain body

weight, relative to control mice, may be due to inbreeding and presumably decreased fitness. None of the four selection strains differed significantly for 10 DOA body weight. For 28-day body weight, both the control and early down-selected strain (E^-) were significantly heavier than the two late selection strains L^+ and L^- ($p<0.05$). The early up-selected strain was only marginally heavier than the two late-selected strains ($p<0.1$).

At 56 days, L^- body weight was significantly less than body weight for the other four lines ($p<0.0001$). The two early selected strains (E^+ and E^-) were not statistically different for 56 DOA body weight. Both up-selected strains (L^+ and E^+) had similar 56-day body weights, which were not statistically different from the control line. Selection appears to have compensated for the decreased weight in the selection strains at younger ages due to inbreeding. The similarity of 56-day body weights in the two up-selected lines reconfirms the suggestion of "developmental homoplasy" previously detected between the selection experiment lines (Atchley et al. 2000). Developmental homoplasy refers to a condition where the same complex phenotype was produced by two quite different developmental mechanisms, e.g., hyperplasia and hypertrophy in this instance.

Ontogenetic Changes in Endopolyploidy

Next, we address whether the relative frequency of endopolyploid cells changes during ontogeny. That is, does the number of genomes increase as a function of increasing age? Cellular proportion comparisons across lines are compounded by effects of selection and genetic drift. Thus, ontogenic changes in endopolyploidy are tested within a line. Means and Bonferroni-corrected significance tests for the three ploidy proportions at the four ontogenic ages are given in Table 3.2. None of the ploidy changes from 0 DOA to 10 DOA

were significant within any of the five lines. From 10 DOA to 28 DOA, the proportion of 2C cells increased in all five lines, with statistically significant increases in the L^- and control lines ($p < 0.001$). While the proportion of 4C cells decreased in all five lines, the only statistically significant decrease was in the L^- strain, where the 4C mean decreased from 25.4% to 18.1% ($p < 0.001$). 8C proportion was relatively unchanged between 10 DOA and 28 DOA.

Between 28 and 56 DOA, the proportion of 2C cells significantly decreased for all five lines. There was a corresponding significant increase in 4C cell proportion for all five lines ($p < 0.001$). The proportion of 8C cells at 56 DOA significantly increased in the two early-selected strains and the control line ($p<0.005$). Neither of the late selected strains (L^+ and L^-) showed any significant changes in 8C cell proportion from 0 to 56 DOA.

As expected, proportions of polyploid cells (4C and/or 8C) within a line increased as ontogeny continued but only after sexual maturity. None of the five lines had significant cell proportion changes between birth and 10 DOA. After 10 DOA, polyploid cell numbers decreased in all lines until after 28 DOA, after which they increase. The increase in 2C and decrease in 4C cells between 10 and 28 DOA reflect the fact that growth during this interval is primarily by hyperplastic growth, i.e., changes in cell number. After 28 DOA, polyploidy frequency increases, as does cell size (Atchley et al. 2000). The increase in polyploid cells after 28 DOA also reflects the liver's increased metabolic activity after weaning.

Changes in Endopolyploidy over Ontogeny: Early-Selected Lines

We next investigate the effect of age-specific genetic selection for growth on polyploidy frequency. That is, has selection for divergence in hyperplastic or hypertrophic

growth lead to changes polyploidy frequency? Age-specific contrasts between line pairs for ontogenic samples are in Table 3.3. 0 DOA contrasts were not significant for any line pair and therefore are not included in Table 3.3. 2C and 8C proportion contrasts between the early pair of strains (E^- and E^+) and between the E^+ and control lines were significant, where E^+ had increased 2C and decreased 8C proportions relative to the other two lines ($p<0.05$). 4C proportion contrast in E^+ mice was also significantly different from control 4C proportion ($p<0.01$), potentially illustrating a decrease due to decreased cell size. As with ontogenic comparisons, the increase in 2C and decrease in 4C and 8C proportions at 10 DOA likely reflect continued hyperplasic growth in E^+ mice.

At 28 DOA, 4C proportion in E^- mice was significantly different from 4C proportion in E^+ ($p<0.05$) and the control mice ($p<0.01$). The contrasts, in addition to the means in Table 3.2, suggest a reduction in 4C cellular proportion in E^- mice, relative to control and E^+ mice. The E^- strain shows compensatory growth starting near 30 days of age (Ernst et al. 1999; Rhees and Atchley, 2000), seemingly an after-weaning burst of hyperplasia (Atchley et al. 2000). This occurrence may explain the decrease in polyploidy proportions.

At 56 DOA, contrasts suggest E^+ mice had significantly reduced 2C proportion, relative to control and E^- mice ($p<0.0001$). Neither 4C nor 8C contrast were significant for the early selected pair. However, the E^- 4C mean was significantly less than the E^+ 4C mean (Table 3.2). The strains also significantly differed for the total proportion of polyploid cells, reflected by the proportion of 4C cells + 8C cells ($p=0.03$, data not shown), suggesting an overall increase in polyploid cells in E^+ at 56 DOA, but no changes in a specific polyploid

type. This polyploidy increase in E^+ may reflect a post-weaning burst of growth via cell size, as suggested by the similarity between E^+ and L^+ 56-day body weight.

Changes in Endopolyploidy over Ontogeny: Late-Selected Lines

Contrasts between late mice are in Table 3.3. The late-selected mice had no significant differences in means or contrasts at 10 DOA. At 28 DOA, L^- mice had fewer 4C cells, relative to the control line (Table 3.3; $p<0.01$). Atchley and colleagues (2000) previously demonstrated the L^- line has decreased cell size at 28 DOA, relative to L^+ mice, before the selection period begins (Atchley et al. 2000). This contrast, as well as the decreased mean of 4C cells in L^- mice (Table 3.2), reflects decreased polyploidy frequency, presumably due to the documented decreased hepatocyte size in L^- mice.

Contrasts between the two late-selected strains were significant for 2C and 8C proportions at 56 DOA ($p<0.0001$ and $p<0.05$, respectively). L^- mice had more 2C and fewer 8C cells, relative to the L^+ line. The two strains differed significantly for 2C proportion mean but did not differ for 8C proportion mean after Bonferroni-correction (Table 3.2). The 4C means for the L^+ line was also greater than L^- mean at 56 DOA (Table 3.2); however, there was no significant contrast to this effect (Table 3.3).

Selection Treatment-by-Age Interactions

Contrasts to examine interactions between line and age are in Table 3.4. None of the early selection contrasts was significant for any of the three cellular proportions. The late-selected lines' contrasts were significant for 2C ($p<0.01$) and 8C ($p<0.05$) proportions, with L^+ having fewer 2C and more 8C cells than L^- mice. These significant contrasts average over all four ontogenetic ages; thus, they should be interpreted with caution. The linear and

quadratic age contrasts are highly significant ($p < 0.001$) for all three cellular proportions, indicating both linear changes and quadratic changes in cellular proportions as ontogeny continues.

Line-by-age contrasts show changes in the 2C cellular proportion between the early-selected mice occur in both linear and polynomial manner (both quadratic and cubic). By comparison, the late-selected pair only show 2C proportion changes linearly. The polynomial increases in 2C proportion likely reflect continued exponential growth in the E^+ mice. Quadratic equations typically model growth by exponential cell growth, i.e., growth by cell number, and indicate hyperplasia's role in the 2C proportion increase (Villalba et al. 2000). Both early and late selection pairs show only linear changes in 4C proportion, likely a reflection of cell enlargement and indicative of the relationship between cell size and polyploidy.

Changes in Endopolyploidy: Advanced Age Samples

Means for the three ploidy proportions for maternal samples, together with Bonferroni-corrected tests comparing line differences, are in Table 3.5. The two early selected strains did not differ significantly from each other or the control line for any ploidy class. The only significant mean differences were those involving strain L^+, whose 4C proportion was significantly increased relative to the control and L^- strain means ($p<0.001$). In addition, 8C proportion mean of L^+ was significantly decreased relative to that of the control, E^+, and L^- means ($p<0.05$).

Contrasts between maternal samples (AA, Table 3.3) show the same pattern. Contrasts between L^+ and the control line were significant for all three ploidy proportions,

where L^+ mice had a greater proportion of 2C and 4C cells but lower proportion of 8C cells, relative to control mice ($p<0.05$, $p<0.001$). The increase in 2C proportion (Table 3.3) in AA L^+ mice, relative to control mice, may reflect continued growth by cell number; L^+ mice have a marked increase in cell number between 28 and 70 DOA (Atchley et al. 2000). L^+ also had significantly increased 4C proportion and decreased 8C proportion, relative to L^- ($p<0.001$). Contrasts between L^- and the control were not significant for any of the three ploidy proportions.

By advanced adulthood, polyploid proportions in early-selected mice are not statistically different from control mice. Contrasts (Table 3.3) and ontogenetic means (Table 3.2) both suggest temporary polyploid changes, starting around 10 DOA and ceasing at sexual maturity. Alternatively, late-selected mice have more lasting changes in polyploid cell frequency. L^+ mice have increases in 4C and decreases in 8C proportions, relative to control and late-down selected mice, starting at 56 DOA and continuing into advanced adulthood. The L^- and control lines from the restricted index selection experiment had similar cell sizes at 70 DOA (Atchley et al. 2000), so we may expect them to have similar polyploid profiles. However, the similar polyploid profiles between control and L^- AA samples may also be an effect of litter size. AA control samples were born and raised at a commercial facility where litter size was not standardized. Since mouse litter size in random bred mice is normally greater than 8 pups, control mice were most likely raised and nursed in larger litters relative to the four selection strains. Females reared in larger litters typically produce smaller adult mice (Konig et al., ‘88). Hence, AA control mothers may look

phenotypically more similar to the L^- mothers due to environmental effects, leading to similar polyploidy profiles.

DISCUSSION

Typical mitotic cell cycles have four phases: G_1 (Gap 1), S (DNA synthesis), G_2 (Gap 2), and M (mitosis), where the transition between phases is regulated by cyclins and cyclin-dependent kinases (CDKs). The G_1 to S phase transition involves cyclins D and E, both of which interact with CDK2 and, to a lesser degree, with CDK4 and CDK6. Cyclin E is a late G_1-phase cyclin and until recently, was believed to regulate the cell's entrance into S phase and subsequent expression of S-phase genes that start DNA synthesis.

Typically, mitotic cells must complete M-phase before S-phase and DNA synthesis can start again (Nurse, 1994). Endoreduplicating cells uncouple this requirement for S-phase initiation and skip M-phase altogether, suggesting M- and S-phase protein misexpression as hallmarks of polyploidy. Indeed, endoreduplicating cells from various species show increased expression of S-phase proteins and down regulation of M-phase proteins, relative to mitotic cells (Larkins et al. 2001). The endoreduplicating giant trophoblast cells of the rodent placenta have altered expression of cyclin E and the G_2/M proteins, cyclin A and B, as do polyploid megakaryotes and tumor cells (MacAuley et al., '98; Datta et al., '98; Erenpreisa et al. 2005).

Given the increased expression in cyclin E seen in endoreduplicating cells, it is possible that genetic selection for differential growth could lead to differences in cyclin E expression between lines examined here. However, there were no significant differences in the number of cyclin E-positive cells between any of the five lines at any age. In addition,

recent research had suggested cyclin E expression is not essential for mitotic cell S-phase entry (Sherr, '93; Möröy and Geisen, 2004), suggesting change in cyclin E expression may be an effect of rather than cause for endopolyploidy. There are two subfamilies of mammalian cyclin E (E_1 and E_2). The use of a cyclin E_1 antibody may not allow us to detect all cells expressing cyclin E if there is cyclin E temporal or spatial specificity. However, research shows the two cyclin Es have similar expression patterns in the liver (Boylan and Gruppuso, 2005) and there is no evidence of differential expression of the two cyclins Es in noncancerous cells.

An accurate estimation of 4C and 8C polyploid cells assumes cyclin E_1 is only expressed in the G_1 phase of the cell cycle. Expression of cyclin E_1 in G_2/M phase (so called "ectopic" expression) would lead to mitotic diploid cells that have not yet undergone cellular division being included in the 4C cellular population. We cannot eliminate the possibility of ectopic expression in the selected strains, especially given the unknown cellular targets of genetic selection for altered growth in these mouse strains. However, it seems unlikely outbred control mice would also show such ectopic expression.

Polyploidy and Selection for Cell Size

It is well documented that increases in polyploidy are associated with increasing cell size. Several plant species show a correlation between cell size and increases in endopolyploidy (Melaragno et al., '93; Cheniclet et al. 2005). Mammalian hepatocyte polyploidy level is correlated with increased hepatocyte size, among other mammalian cell types (Gupta, 2000; Vinogradov et al. 2001) and cell volume increases as polyploidy increases (Epstein and Gatens, 1967). Not surprisingly, polyploid frequency starts to

increase at approximately the same time as growth mechanisms shift from hyperplasia to hypertrophy. Correlational analyses found growth rate between weaning and sexual maturity, during the hypertrophic growth phase, was significantly correlated with liver polyploidy. However, pre-weaning growth rate, or growth during the hyperplasic growth phase, is not (Anatskaya and Vinogradov, 2004). Hence, we would expect selection for divergence in cell size is more likely to produce hepatocyte polyploid changes than selection for divergence in cell number.

Given these facts, our hypothesis for hepatocyte endopolyploidy development suggest: (i) There should be no differences in endopolyploidy between the early selected lines, E^+ and E^-, having documented the lines have no hepatocyte size differences (Atchley et al. 2000), and (ii) the late up-selected line, L^+, should show increases in polyploidy frequency, relative to L^-, as a correlated response to increased cell size after 28 DOA (Atchley et al. 2000). Indeed, there were no long-term polyploidy changes in the early selected mice. The only significant differences between the two early-selected lines were times during ontogeny marked by cell number growth. These significance differences suggested an overall decrease in polyploid frequency in the E^+ line, relative to E^-. As selection for increased early growth has increased cell number in the E^+ line, it has lead to a corresponding decrease in polyploidy frequency.

In contrast, selection for increased late growth has increased proportion of 4C cells and decreased proportion of 8C cells, relative to control and late down-selected mice. We are unable to determine which specific polyploid type changed as a result of selection, as the 4C cellular population potentially contains both binuclear diploid (2x2N) and mononuclear

tetraploid (4N) cells. It is surprising to see a decrease in 8C proportion among L^+ mice, relative to control and L^- mice, given the correlation with hypertrophy and increased polyploidy. This decrease may reflect a decrease in a specific 8C ploidy class, such as binuclear tetraploid cells, rather than an overall decrease in 8C cells.

Other organs show hyperplasia-induced polyploidy changes, suggesting selection for hyperplasia may alter polyploidy in other tissues. Growth rate before weaning is negatively correlated with cardiomyocyte polyploidy (Anatskaya and Vinogradov, 2004) and selection for increased early growth may result in fewer polyploid cardiomyocytes. As seen here, altered hyperplastic growth may also lead to specific ploidy-class changes. Brodsky and colleagues (‘85) found mice raised in larger litters had lower heart weight and fewer polyploid cardiomyocytes than mice raised in smaller litters. However, there was clear variation within this decrease, as some polyploidy types were actually more frequent in slower-growing mice.

We cannot eliminate the possibility that genetic drift or genetic background contributed to the increased 4C cellular population in L^+ mice. Different mouse strains show different primary polyploidy cell types at certain ages, illustrating the role of genetic background in polyploidy development (Severin et al., ‘84). Additional work using different replicates subject to the same selection treatment would be necessary to separate the effects of drift and selection.

Body weight was not significant as a source of variation in polyploidy and, when fit as a covariate in the analysis, did not change significant means or contrasts. This seems surprising, given the correlated response in body weight divergence from genetic selection

(Rhees and Atchley, 2000) and the similar genetic architecture between growth rates and body weights (Vaughn et al., '99). Previous correlational analyses suggest body weight does not play a role in hepatocyte polyploidy but does affect polyploidy in other organs (Anatskaya and Vindogradov, 2004). Our results confirm these findings.

Endopolyploidy during Ontogeny

We found polyploid frequency increased as ontogeny continued, with the most ontogenetic changes occurring after 28 DOA when growth had shifted to hypertrophy (Table 3.2). Increasing frequencies of polyploid cells during ontogeny have been detected in a variety of tissues, the degree and type of polyploid cell being species- and strain-specific (Severin et al., '84; Chipchase et al. 2003; Guidotti et al. 2003). Cellular proportions detected here at 0 DOA are very similar to proportions for wildtype mice at birth (Chipchase et al. 2003). Previously published cellular proportions in wildtype mice reflected cells from the G_1 and G_2 cell cycle phases, suggesting the 4C and 8C levels in G_1 detected here in selection strains are higher than those in other strains at birth (Nunez et al. 2000; Chipchase et al. 2003; Lamas et al. 2003) and again emphasize the contribution of genetic background to development of polyploidy.

Endopolyploidy and Gene Expression

Opinion is divided on whether endopolyploidy (and polyploidy in general) conveys a selectable benefit to individuals. Endopolyploidy may arise for any of a dozen reasons (reviewed in Anisimov, 2005) to help or hinder organ function. While most agree endopolyploidy may occur to accelerate development and differentiation at the cellular and

tissue level, few suggest endopolyploidy may accelerate growth by altering expression of genes during metabolic periods.

However, preliminary evidence suggests polyploidy does affect gene expression. Transcriptional and translational activity increases when polyploidy occurs, making the machinery by which to increase gene expression available (D'Amato, '84). Raslova and colleagues (2003) found a subset of genes in polyploid cells had all alleles functional regardless of ploidy number. Galitski and colleagues ('99) found both ploidy-induced (increased expression as ploidy increased) and ploidy-repressed (decreased expression as ploidy increased) genes in polyploid yeast, including decreased expression in the G_1 cyclins genes *CLN1* and *PCL1*.

The role of polyploidy on mRNA variation in the mammalian liver has not been experimentally assessed. In addition, it may not be possible to assay association between mRNA variation at specific genes and mammalian endopolyploidy, given technical limitations to separate live cells into diploid and polyploid cells. However, caution should be used when drawing inferences about mRNA variation in polyploid organs, given the effect of selection and ontogeny on polyploid variation shown here and the potential effect of polyploidy on gene expression.

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Table 4.1: Line means and Bonferroni-corrected tests for body weight at 0, 10, 28, and 56 days. Body weight within each age are ranked and those connected with a line are not statistically different at the p <0.05 level. Sample size (N) for each line and age combination is indicated.

0 DOA			10 DOA			28 DOA			56 DOA		
Weight (g)	LSD	N	Weight (g)	LSD	N	Weight (g)	LSD	N	Weight (g)	LSD	N
1.36	L^+	4	6.58	E^-	25	16.3	L^+	10	22.92	L^-	20
1.40	E^-	10	7.15	L^-	32	17.24	L^-	22	28.31	E^-	36
1.67	E^+	5	7.21	E^+	13	19.95	E^-	30	30.11	C	33
1.76	L^-	10	7.3	L^+	14	19.96	E^+	9	30.78	E^+	16
1.8	C	7	8.58	C	26	22.06	C	26	33.08	L^+	43

Table 4.2 Line means and least significant difference (LSD) tests showing divergence in ploidy cellular proportions in ontogenetic samples. Traits within each proportion are ranked. Proportions were calculated as cells stained for DNA content and cyclin E_1 positive divided by the total number cyclin E_1 stained cells. Means connected by a vertical line are not significantly different at $p< 0.05$. Means in bold are statistically different from the mean at the prior age within the same line at the $p<0.05$ level.

	2C		4C		8C	
Age	Mean	LSD	Mean	LSD	Mean	LSD
0 DOA	46.8	E^+	24.6	E^+	1.8	E^-
	49.7	C	24.8	C	3.5	L^-
	53.8	L^+	26.0	L^+	3.6	C
	59.2	L^-	31.8	E^-	4.9	E^+
	61.2	E^-	32.0	L^-	7.1	L^+
10 DOA	51.3	C	23.8	E^-	2.6	E^+
	56.9	E^-	24.5	E^+	3.9	E^-
	57.2	L^+	25.4	L^-	4.1	L^-
	57.6	L^-	27.2	C	5.2	L^+
	64.9	E^+	28.5	L^+	6.0	C
28 DOA	63.5	E^+	**18.1**	L^-	3.0	L^-
	63.5	E^-	19.7	E^-	3.8	C
	63.6	L^+	23.8	E^+	3.8	E^+
	63.8	C	24.1	C	3.9	E^-
	72.6	L^-	27.3	L^+	4.2	L^+
56 DOA	**39.2**	E^+	**29.2**	E^-	6.0	L^-
	43.7	L^+	**30.4**	L^-	**7.2**	E^-
	47.1	C	**36.0**	C	**7.7**	E^+
	51.5	E^-	**39.0**	E^+	7.9	L^+
	55.4	L^-	**39.9**	L^+	**10.1**	C

Table 4.3 Contrasts for Ploidy Proportions between Line Pairs. Numbers reflect p-value of contrast between line pairs for proportions of 2C, 4C, and 8C cells at 4 ages analyzed. Reciprocal contrasts were equal, so results for $(\tau_i) - (\tau_j)$ are shown. * denotes significance of the contrast at p=0.05 level, ** denotes significance at p=0.01 level and *** denotes significance at p=0.001 level. AA denotes advanced age maternal samples.

	10 DOA			28 DOA			56 DOA			AA		
Line Pair (i,j)	2C	4C	8C	2C	4C	8C	2C	4C	8C	2C	4C	8C
C, E^+	**	**	**				***					
C, E^-					**							
C, L^+										*	***	***
C, L^-					**		***					
E^+, E^-	**		*		*		***					
L^+, L^-							***		*		***	***

Table 4.4 Contrasts for Ploidy Proportions between Line-by-Age Interaction. Numbers reflect p-value of F-test for contrast between line pairs, ages, and line pair-by-age interaction for proportions of 2C, 4C, and 8C cells for 4 ontogenetic ages Reciprocal contrasts were equal, so results for $(\tau_i) - (\tau_j)$ are shown. * denotes significance of the contrast at p=0.05 level, ** denotes significance at p=0.01 level and *** denotes significance at p=0.001 level.

	2C	4C	8C
Line			
$(E^+) - (E^-)$			
$(L^+) - (L^-)$	**		*
Age			
Linear	**	***	***
Quadratic	***	***	***
Cubic			
Line* Age			
Linear $(E^+) - (E^-)$	*	***	
Linear $(L^+) - (L^-)$	***	**	
Quadratic $(E^+) - (E^-)$	**		
Quadratic $(L^+) - (L^-)$			
Cubic $(E^+) - (E^-)$	**		
Cubic $(L^+) - (L^-)$			

Table 4.5 Line means and least significant difference (LSD) tests showing divergence in ploidy cellular proportions in maternal samples. Traits within each proportion are ranked. Proportions were calculated as cells stained for DNA content and cyclin E_1 positive divided by the total number cyclin E_1 stained cells. Means connected by a vertical line are not significantly different at $p < 0.05$. Each line had 15 advanced age samples (the number of females mated for each line, see Methods and Materials)

2C		4C		8C	
Mean	LSD	Mean	LSD	Mean	LSD
30.05	E^+	19.94	C	17.02	L^+
30.14	C	21.79	L^-	28.14	E^-
31.88	L^-	25.20	E^-	29.62	E^+
32.35	E^-	25.81	E^+	31.49	L^-
35.26	L^+	30.79	L^+	35.62	C

CONCLUSIONS

This dissertation describes analyses to better characterize three temporally dynamic factors and their effect on variation in mammalian growth. Included work is on the extra-nuclear maternal contribution to progeny's phenotypes, the interplay between genomic imprinting and maternal effects, the differential genetic control of growth from age-specific selection, and changes in liver endopolyploidy as a response to age-specific growth.

The uterine and postnatal maternal effects discussed in chapter 1 clearly contribute to their offspring's phenotypes in adolescence and into adulthood. Unfortunately, it seems impossible to completely remove maternal influences' on quantitative traits in laboratory studies. At best, researchers can minimize variation from maternal effects by performing embryo transfers (prenatal litter size) and standardizing litter size after birth (postnatal litter size). However, these are not feasible for every experiment. Even selection experiments designed to minimize maternal effects are only partially successful. Rhees and colleagues (1999) showed uterine effects responded to selection for altered early growth. The specific uterine change was conditioned by their progeny's developmental requirements; selection may have acted on genetic variation at maternal effect genes. Genetic variation at maternal effects genes may explain a large proportion of pre-weaning variation between early-selected lines, as seen in other strains (Wolf *et al.* 2002).

Chapters 2 and 3 confirm that the timing of natural selection will impact different developmental components and genetic influences. In chapter 2, selection for age-specific growth has clearly produced significant divergence in genes influencing early and late growth. In addition, age-specific selection has produced compensatory growth genetic

effects, also controlled by different QTL in the two populations. In chapter 3, liver endopolyploidy has increased as a cellular consequence of selection but only in one selection treatment. Hence, the timing of selection was important in producing cellular polyploidy changes.

Results from the QTL mapping in chapter 2 were interesting but require additional work. Despite progress over the past decades, specific genes responsible for age-specific growth and regulation are still unknown. Part of the difficulty lies in where to start fine mapping after initial genomewide screens. Initial results can find QTL regions up to 20cM in length, with up to thousands of putative candidate genes. Recent technology has improved the ability to fine-map via single nucleotide polymorphism arrays (SNP chips). Alternatively, we can explore gene expression differences in candidate genes with a combined QTL mapping/mRNA microarray analysis (e.g., Wayne and McIntyre, 2002).

These technologies may be useful to start fine-mapping key genomic regions from chapter 2, specifically chromosomes 5, 7, and 17. These regions are of interest because they (1) contain direct effects QTL; (2) influence compensatory growth (CG); (3) influence age-specific growth in other mouse strains; and/or (4) are located near hyperplastic or hypertrophic candidate genes. The 22-27 cM region of chromosome 17 influences CG in early mice by direct effects QTL. In late mice, the same region influences the two measures of compensatory growth (WG and MG) by epistasis. Candidate genes in this region include Notch3 (DNA binding, transcription factor, brain development) and Gabbr1 (G-protein receptor, signal transduction). Likewise, chromosome 5 has two regions with direct effects QTL that influence CG in early-selection lines and epistatically influence growth in late-

selected lines. Candidate genes on chromosome 5 include several proteins in the GABA-A pathway (40 cM: Gabra2, Gabra1, Gabrg1, neurotransmitters and cell activity), Gsh1 (40 cM, homeobox and transcription regulation), Tcf1 (64 cM, transcription regulation), and Tbx3 and Tbx5 (64 cM, DNA and protein binding).

Chromosome 7 has QTL of significant effect in both populations and was noted to contain age-specific growth QTL in earlier work (Cheverud *et al.* 1996; Vaughn *et al.* 1999). This genomic region contains the *IGF2* gene, as well as other genomically imprinted genes. IGF2's role in compensatory growth (Hornick *et al.* 2000) makes it a key candidate gene for the chromosome 7 QTL. In addition, fine mapping may find genomically imprinted candidate genes, allowing further exploration of methylation variation at genomically imprinted genes in normal populations. While less than 1% of expressed genes are genomically imprinted, these genes influence fitness traits such as growth, maternal behavior, and diseases and may respond to natural selection. There is considerable degree in methylation variation across populations, or epialleles, in plants and animals that play roles in aging, cancer, and other fitness traits (e.g., Issa, 2000; Ober *et al.* 2003; Kalisz and Purugganan, 2004). Epialleles may play a role in age-specific growth, where methylation level varies over ontogeny and alters expression at genomically imprinted genes.

Chapter 2 also answers several questions about compensatory growth, but raises more. The genetic architecture of CG have not been specifically investigated, so we have no *a priori* theory about genes influencing compensatory growth, such as location, numbers, etc. Our results suggest compensatory growth is primarily influenced by previously described growth QTL. However, these QTL alter genetic effects for times not under

selection to achieve targeted growth. QTL mapping in chapter 2 found several early growth loci with high dominance components and directional dominance, comparable with earlier work (Rocha et al. 2004 and references therein). However, this effect was only in the late population, suggesting it plays a role in compensatory growth. In addition, all but one over- or underdominant QTL were in the late population while growth loci in the early population were primarily additive. As such, high nonadditive components to early growth may only be in an "altered post-weaning growth" background (i.e., Cheverud *et al.* 1996). Compensatory genetic effects may change their mode of action over ontogeny, with early compensatory growth occurring primarily by nonadditive effects. If this is correct, it is not clear why we see this in only early growth genetic effects (between 0 and 10 days) but not in genetic effects for 7-to 21-day gain.

We were only able to search for single-trait epistatic interactions in chapter 2. Searching for multi-trait epistatic interactions may find additional genomic regions influencing a subset of traits, e.g., both pre-weaning growth rates. Searching for multi-trait epistatic interactions may also increase power of detection. However, using linear models to search for multiple-trait epistasis may be difficult; significance thresholds would likely have to be determined via permutation. Non-linear models to detect epistasis have only been used recently, via Bayesian analysis (Yi *et al.* 2006). This method is promising but there is no multiple-trait version and more work may be needed in terms of significance of interactions. Sex-specific epistatic interaction should also be examined, given the hyperplastic population's high proportion of sex-specific genetic effects. I initially searched for sex-specific effects in chapter 2 but became concerned about bias from small sample size in the

nine genotypic classes. As such, high numbers of each sex would be necessary to detect significant interactions, which may be too cost prohibitive.

There are two unanalyzed phenotypic datasets from the F_2 populations, consisting of weekly body weights and tail lengths. These traits may be interesting to look in an epigenetic analysis such as the one outlined by Wu and colleagues (2002). This analysis conditions variance and effects for traits at time "t" on the effects and variance at time "t-1" (Zhu, 1995; Atchley and Zhu, 1997) and is useful to identify QTL generating new genetic variance as ontogeny continues. This may be especially relevant to mapping body weight and tail length, since correlations between the two traits decrease over ontogeny, possibly due to divergence in genetic control as ontogeny continues. Additional analyses may also focus on mapping imprinted QTL via interval mapping (Cui *et al.* 2006) and using functional mapping to follow-up growth curve QTL (Ma *et al.* 2003; Wu *et al.* 2003). Functional mapping uses growth curve traits to estimate the effect of a QTL throughout ontogeny. This would express growth curve QTL effects with respect to time during ontogeny and may allow us to frame results in a cell number/size timeframe.

In Chapter 3, flow cytometry demonstrates only selection for late growth has altered extent of hepatocyte endopolyploidy. Typically, polyploid hepatocytes start to accumulate in the mouse liver near weaning. At the same time, the cellular shift from hyperplasia to hypertrophy occurs and polyploid hepatocytes may accumulate as a result from the cellular shift in growth. Liver polyploidy is highly correlated with cell size (Gupta, 2000; Vinograov *et al.* 2001) and we may expect age-specific selection that alters cell size may also alter hepatic endopolyploidy. We found mice selected for increased cell size (L^+ mice)

have increased 4C cellular proportion at 56 DOA and continuing into adulthood, relative to mice with smaller cell size. We cannot determine which polyploidy class (tetraploid versus binuclear diploid cells) this increased proportion reflects. We did see a decrease in one polyploidy class (8C) in L^+ mice, which seems counterintuitive. This decrease may reflect drift, or a temporary change that dissipates later in adulthood.

There was no evidence of long-term polyploidy changes in the early-selected lines. This is not surprising, as hepatocyte cell size were not significantly different between early-selected mice (Atchley *et al.* 2000). However, early-selected mice did differ in brain cell size at 28 DOA (Atchley *et al.* 2000). Characterizing brain polyploidy changes in the early-selected mice may help us better understand polyploidy formation. For example, is organ polyploidy correlated with only cell size? If polyploidy is correlated with cell size, regardless of organ, we would see endopolyploidy changes in the brains of early-selected mice after weaning, based on documented cell size differences (Atchley *et al.* 2000). Alternatively, organ polyploidy may be correlated with the primary cellular mechanism of growth within an organ, e.g. polyploidy in brain cells is associated with hyperplasia. We would therefore expect to see brain polyploidy before weaning, during hyperplasia cell number changes (Brodsky et al, 1985; Atchley *et al.* 2000).

Despite the clear biological relevance, little is known about the genetic mechanisms through which mammalian polyploidy forms. A few genes involved in plant and *Drosophila* endopolyploidy formation are known, including cell cycle and cyclin regulators such as the fizzy protein (Sigrist and Lehner, 1997; Vinardell *et al.* 2003). Future work could focus on determining causal genetic variation of endopolyploidy in the inbred strains,

especially in fine mapping work from chapter 2. It would also be interesting to assess endopolyploidy's effect on liver gene expression. Due to technical limitations, it is impossible to assess global mRNA expression changes in a homogenous polyploidy population. We could potentially investigate the relationship between changes in endopolyploidy, gene expression, and genetic variation for growth by combined subsequent fine mapping experiments for chapter 2 results with polyploid profiles and mRNA microarrays on each individual. Analyses could focus on changes in gene expression, based on genotypes at candidate endopolyploidy genes.

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