QUANTITATIVE TRAIT LOCI AFFECTING POSTNATAL GROWTH IN MICE

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Early quantitative genetic studies of mammalian growth had indicated that postnatal growth occurred in at least three phases (see Figure 1 ; Atchley and Rutledge, 1980 ; Atchley *et al.,* 1984 ; Riska *et al.,* 1985), an early phase lasting until 3 or 4 weeks in mice during which growth rate increases, a later, decelerating growth phase, lasting to about 10 weeks in mice or the time of epiphyseal fusion, and, after skeletal growth is complete, a third phase due primarily to the addition of fat to the tissues. Here we review the evidence for individual quantitative trait loci (QTLs) affecting growth during these three phases in the intercross of LG/J and SM/J mouse strains.

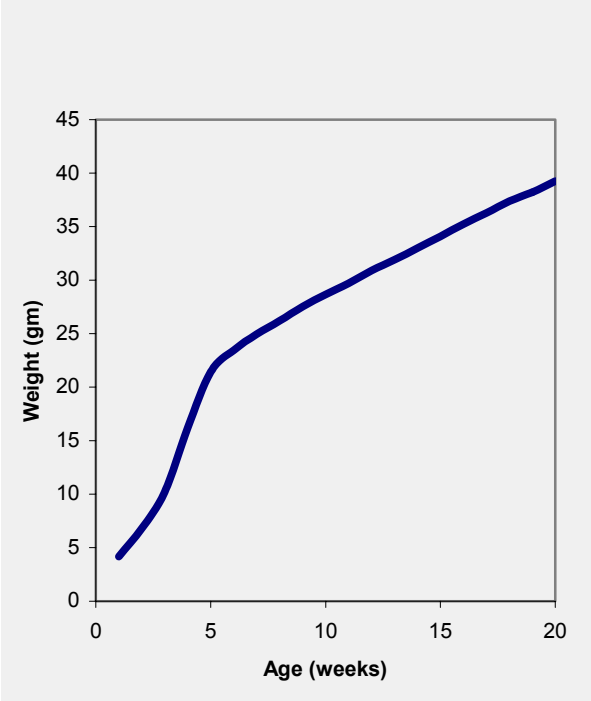

Figure 1. Murine growth

Earlier studies by Atchley and colleagues on a randombred strain of ICR mice (Cheverud *et al.,* 1983 ; Leamy and Cheverud,. 1984 ; Riska *et al.,* 1985) had indicated that early and later postnatal weights and growth had only a small positive or even negative genetic correlation indicating either that early and later weights are affected by separate sets of genes or perhaps by genes exhibiting antagonistic pleiotropy, having opposite effects on different growth periods.

MATERIALS AND METHODS

We searched for QTLs affecting various growth periods in the intercross of LG/J and SM/J inbred mice (Chai, 1956 ; Cheverud *et al.,* 1996). These strains were inbred from separate populations selected for large (LG/J ; Goodale, 1938) and small (SM/J ; MacArthur, 1944) body size at 9 weeks. Earlier work on the strains indicated that they differed by at least 11 effective loci for adult body weight (Chai, 1956). Thus, this cross should exhibit the effects of many genes, each of relatively small effect. In two replicate F_2 intercross experiments, we generated over 500 animals from repeated mating of 40 to 50 F_1 hybrids. Animals were weaned at three weeks and separated into single sex cages. Individual animals were weighed from 1 to 10 weeks on the weekly anniversary of their birth. Early growth is between 1 and 3 weeks, middle growth between 3 and 6 weeks, and late growth between 6 and 10 weeks. Soon after 10 weeks, the animals were either sacrificed (first experiment) or mated (second experiment). After sacrifice, the animals were necropsied, their internal organs weighed and saved for DNA extraction, and their skeletons prepared by dermestid beetles. Ninety-six microsatellite loci spread throughout the autosomes (see Figure 2) were scored on the DNA obtained from the spleen or liver.

Animals from the second F_2 intercross were randomly mated to produce 200 independent full-sib F_3 families averaging 8 pups each. Most of these families were paired with another sibship and half of each sibship cross-fostered in the first few days after birth. This cross-fostering design (Riska *et al.,* 1985) allows for the measurement of maternal effects. Several different populations were generated from these F_3 animals. A randomly mated advanced intercross line was generated and has been maintained at an effective size over 125 animals. This population has reached the F_{22} generation and is being used for fine-mapping QTL studies. At this time, the population has accumulated recombination expanding the local genetic map 10 times relative to the F_2 generation. In addition, 55 recombinant inbred lines were begun. Twenty-four of these lines are still extant and 18 have reached an inbreeding coefficient greater than 0.986. We are scoring these lines for 500 microsatellite markers and characterizing them for growth, body size, obesity, and diabetes-related traits.

Interval mapping of quantitative traits (Lander and Botstein, 1989) followed the procedures described by Haley and Knott (1992). Statistical significance thresholds for genome-wide and chromosome-wide levels were determined by simulation and by using a Bonferroni correction based on the number of independent comparisons performed (Cheverud, 2001a). At the less stringent chromosome-wide level, we expect one false positive result per trait. We consider this possibility acceptable given the large number of independent significant results detected at this level.

RESULTS

In our replicated QTL study of growth in the LG/J and SM/J intercross (Cheverud *et al.,* 1996 ; Vaughn *et al.,* 1999), we found that early and later growth periods were typically affected by different sets of genes mapped across the mouse chromosomes (see Figure 2). We did not find evidence of antagonistic pleiotropy. These early growth genes are independent of genetic maternal effects because all animals were born from genetically identical F_1 hybrid mothers. Several early growth genes, 5 of 12, exhibited overdominance with the heterozygote growing most quickly while later growth was typically either co-dominant or the LG/J allele was dominant to the SM/J allele. The observed decrease of relative dominance values with age may be a result of the directional selection for adult size imposed on the progenitor strains. We also found many instances of epistatic interaction (unpublished results) among loci affecting these growth rates.

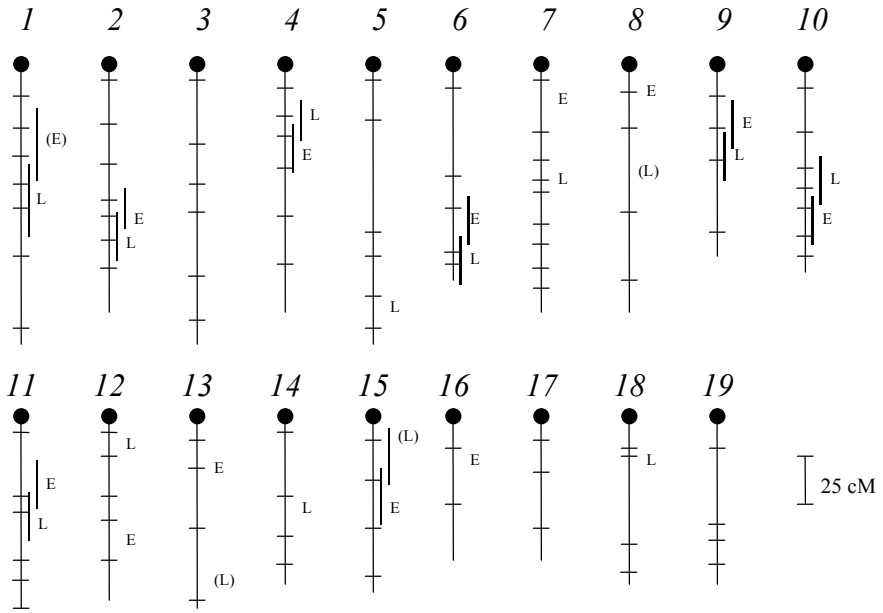

Figure 2. Relative positions of QTLs affecting Early (E) and Late (L) growth. Parenthetical entries represent locations with exclusive effects on early or late weights but not on growth itself (from Vaughn *et al.*, 1999)

By mating the F_2 intercross animals and cross-fostering half of their litters we were able to detect maternal effects on postnatal growth (Kramer *et al.,* 1998). As expected (Cheverud *et al.,* 1983) these effects were strong during the pre-weaning growth period (responsible for about 30% of the variance before 3 weeks) and then declined as animals grew older

(responsible for 5% of the variance at 10 weeks). Our breeding and cross-fostering design allowed us to map genes responsible for the genetic maternal effects (Wolf *et al.,* 2002). We detected 4 maternal QTLs affecting early postnatal growth from 1 to 2 weeks. These QTLs accounted for 24% of the variance in early growth. These maternal effect loci also showed strong epistatic interactions amongst one another and with several other genomic locations. Several of these maternal effect loci are in the same general location of QTLs affecting adult weight and adiposity (see Figure 3 ; Cheverud *et al.,* 1996, 2001 ; Vaughn *et al.*, 1999).
Animals continue to gain weight during the third growth phase, after skeletal development is complete. Most of this weight gain seems to be due to increased adiposity. The SM/J strain adds relatively much more weight and fat after 10 weeks than the LG/J strain (Cheverud *et al*., 1999). A QTL mapping study in the second F_2 intercross allowed us to map body size components. The reproductive fatpad was weighed at necropsy as a measure of adiposity and tail length was measured as an indicator of skeletal size. We found 8 QTLs for adiposity and 9 for tail length (see Figure 3; Cheverud *et al.* 2001). Most of these QTLs also affected adult body size. Again, as when earlier growth periods were compared, there was no antagonistic pleiotropy between adiposity and skeletal size. In fact, only two QTLs seemed to affect both adiposity and skeletal size. In each instance, further fine-scale mapping (unpublished results) indicates that these joint effects resolve into separate QTLs. Typically, when dominance was detected, the allele with the larger effect was dominant. We also discovered extensive and widespread epistatic interactions among these loci, especially among the adiposity loci (Cheverud *et al.,* 2001).

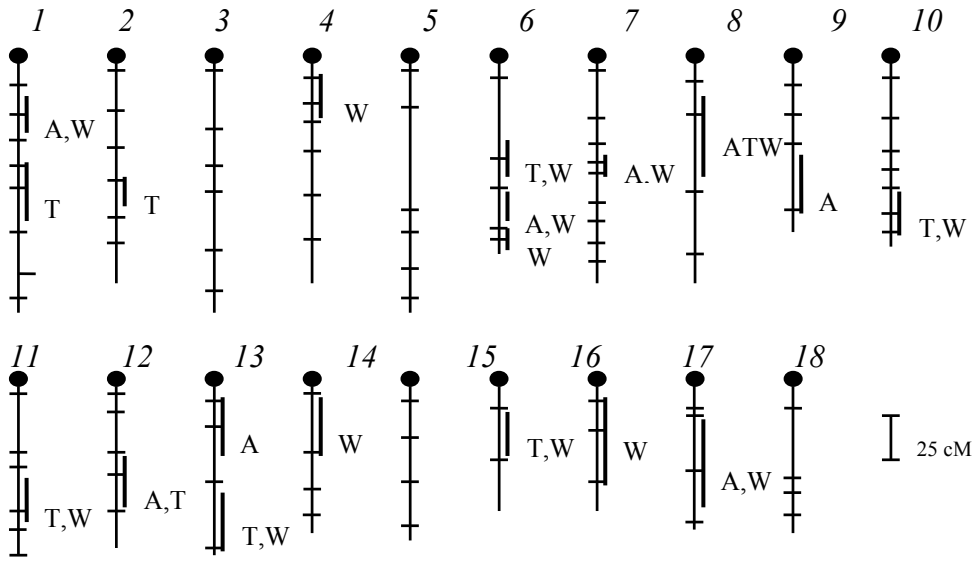

Figure 3. QTLs for adiposity (A), tail length (T) and body weight (W) (from Cheverud *et al*., 2001)

DISCUSSION

Our QTL mapping studies in the LG/J by SM/J intercross have uncovered many loci of relatively small effect on growth. This is not surprising given the earlier results of Chai (1956) and the generation of these strains through selection. Most quantitative variation for complex traits, such as diseases in humans or production traits in agricultural animals, is likely to be of this kind. We found variable dominance depending on the growth period, with early growth often exhibiting overdominance and later growth exhibiting dominance for the allele resulting in larger size. There were large numbers of epistatic interactions among loci affecting these traits. Perhaps most importantly, we found that variability in different growth periods and physiological systems are controlled by independent sets of genes. Thus, the organization of pleiotropy for growth and body composition appears modular rather than exhibiting antagonistic pleiotropy.

This same finding of modular developmental organization has also been found for morphological traits in the LG/J by SM/J cross. While a proportion of QTLs affecting cranial morphology affect all regions of the skull, the majority of loci affect only the neurocranium or the face (Leamy *et al.,* 1999). The bony neurocranium grows largely in response to the growth of the brain it envelops and the brain completes growth early in eutherian mammals, completeing growth by about 3 weeks in mice (Riska *et al.,* 1984 ; Atchley *et al.,* 1984). Most facial growth occurs during the middle and later growth periods, after 3 weeks, under the influence of the growth hormone axis. Just as we found for the growth periods, the parts of the cranium growing at different times are affecting largely by distinct sets of genes.

On an even smaller morphological scale, the mandible is composed of several parts interacting either with the muscles of mastication or the developing teeth (Atchley and Hall, 1991). Again, we found that a minority of genes affect the entire mandible but that most genes have effects restricted to developmentally distinct regions, such as the muscle attachment areas of the ascending ramus or the alveolus supporting the teeth (Cheverud *et al.,* 1997 ; Cheverud, 2001b).

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