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Mapping quantitative trait loci for murine growth: a closer look at genetic architecture

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Summary

Over 20 years ago, D. S. Falconer and others launched an important avenue of research into the quantitative of body size growth in mice. This study continues in that tradition by locating quantitative trait loci (QTLs) responsible for murine growth, such as age-specific weights and growth periods, and examining the genetic architecture for body weight. We identified a large number of potential QTLs in an earlier F2 intercross (Intercross I) of the SM/J and LG/J inbred mouse strains. Many of these QTLs are replicated in a second F2 intercross (Intercross II) between the same two strains. These replicated regions provide candidate regions for future fine-mapping studies. We also examined body size and growth QTLs using the combined data set from these two intercrosses, resulting in 96 microsatellite markers being scored for 1045 individuals. An examination of the genetic architecture for age-specific weight and growth periods resulted in locating 20 separate QTLs, which were mainly additive in nature, although dominance was found to affect early growth and body size. QTLs affecting early and late growth were generally distinct, mapping to separate chromosome locations. This QTL pattern indicates largely separate genetic and physiological systems for early and later murine growth, as Falconer suggested. We also found sex-specific QTLs for body size with implications for the evolution of sexual dimorphism.

1. Introduction

One of the greatest problems in the history of genetics was reconciled with the realization that continuous phenotypic variation could reflect the influence of many genes of small effect (reviewed in Falconer & Mackay, 1996). This realization has since challenged geneticists and evolutionary biologists with the task of defining the genetic architecture of organisms in order to understand the development of evolutionarily important phenotypes, such as body size, and processes such as complex disease states. Genes of small effect are called quantitative trait loci (QTLs) and provide insight into questions about the genetic control of complex traits. For example, a QTL analysis can allow us to address specific questions concerning genetic architecture, such as the number of loci potentially affecting the trait, the distribution of gene effects, and the underlying patterns of gene action, including additivity, dominance, sex-specificity, epistasis and pleiotropy.

Body size has been considered the quintessential quantitative phenotypic trait; that is, a trait that does not exhibit classic Mendelian inheritance attributable to a single genetic locus, but is strongly heritable. Body weight variation in mice is normally distributed and seems to be controlled by many genes, each having a relatively small additive effect on the phenotype (Falconer, 1953), thus making it an ideal trait for a QTL analysis. Falconer and colleagues (1978) found that body size growth in rodents appears to occur through two general physiological mechanisms that act at different life stages (Atchley *et al.*, 1997; Atchley & Zhu, 1997). Other studies of rodent growth report a second principal component contrasting early with later age-specific weight

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(Cheverud *et al*., 1983; Atchley *et al*., 1984; Leamy & Cheverud, 1984; Riska *et al*., 1984), suggesting a trade-off between growth during these two periods. Recently, Atchley and colleagues (1997) reported the results of an index selection experiment for high and low early growth, holding later growth constant, and for high and low later growth, holding early growth constant. These experiments illustrate the ability to separate the evolution of these two growth periods.

A common approach for detecting QTLs for growth and body size in mice incorporates an intercross between inbred strains that vary for the trait of interest followed by an examination of associations between trait values and marker genotypes (Keightley *et al*., 1996; Cheverud *et al*. (1996); Brockman *et al*., 1998; Morris *et al*., 1999) also used this approach to examine the QTLs influencing body weight in two strains of inbred mice selected for large, LG/J (Goodale, 1938), and small, SM/J (MacArthur, 1944), body size that would be expected to have allelic differences at a large number of loci affecting growth (Chai, 1956*b*). From an F2 intercross between the LG/J and SM/J strains, a large number of QTLs were located, each with relatively minor effects on murine growth an body weight. Specifically, 11 QTLs were found to affect early growth and 12 QTLs were found to affect late growth. Further, early growth QTLs mapped to different chromosomal positions from the late QTLs, with only four instances where early and late growth mapped within the same intermarker interval. Because of the distinct nature of the loci affecting early and late growth, the hypothesis of antagonistic pleiotropy was rejected in favour of separate genetic modules for early and late growth.

In the present paper, we compare the QTLs mapped in a second F2 intercross of the LG/J and SM/J mouse strains with the results of the first QTL analysis. This second F2 intercross provides an independent evaluation of the original QTLs and allows the formation of *a priori* hypotheses to test for replication of QTLs affecting body size. We also combine the data from these two intercross experiments, thereby doubling our sample size in order more accurately to define the number and positions of QTLs, as well as their genotypic, sex-specific and pleiotropic effects on murine growth.

2. Materials and methods

(i) *Mouse strains and microsatellites*

The history of the mouse strains used in these experiments and the details of animal husbandry are available in Cheverud *et al*. (1996). Briefly, the mouse strains used in both the mapping experiments originated from the intercross of the Large (LG/J) and Small (SM/J) mouse strains acquired from Jackson Laboratories. SM/J had been selected for small body size at 60 days while LG/J was selected for large body size at 60 days (Chai, 1956*a*,*b*). Both strains have been systematically inbred for over 104 generations (Festing, 1996). In this study, two independent intercross experiments are considered using these inbred mouse strains and are referred to as Intercross I and Intercross II, where Intercross II is the replicate study. Both F2 study populations were initiated by mating 10 SM/J males with 10 LG/J females to produce F1 hybrid animals. The F1 hybrids were randomly mated starting at 10 weeks of age producing 535 F2 animals in Intercross I and 510 F2 animals in Intercross II for a total of 1045 F2 animals in the combined mapping data set.

At 70 days, mice were necropsied to obtain DNA from the liver or spleen. DNA extraction and polymerase chain reaction amplification protocols can be found in Routman & Cheverud (1995). Seventy-five polymorphic microsatellite loci arranged in 55 intervals were scored in Intercross I while 96 polymorphic loci arranged in 72 intervals were scored in Intercross II. The relative positions of these markers are given in Dietrich *et al*. (1992, 1996). All 19 autosomes were represented in each experiment and the relative positions of these microsatellite loci in this intercross are shown in Fig. 1. Microsatellite markers were chosen to cover the genome as completely as possible with additional markers added in Intercross II to fill in larger intervals from Intercross I. These additional markers are denoted by an asterisk following the name of the microsatellite marker.Three markers on chromosomes 3, 18 and 19 were scored in Intercross I that were not used in Intercross II due to difficulty in scoring. These markers are enclosed in parentheses in Fig. 1. The combined data set defines a total map distance of approximately 1780 cM with an average interval length of approximately 23 cM. Centimorgan distances are provided using Haldane's units (Weir, 1996).

(ii) *Growth measurements*

The F2 hybrids were weighed at 10 weekly intervals starting at 7 days of age. Weights were recorded to the nearest 0·1 g with a digital balance integrated with a microcomputer. The 10 age-specific weights were corrected for a series of covarying environmental effects such as F1 dam, litter size at birth, parity and sex (Cheverud *et al*., 1996; Kramer *et al*., 1998). The raw weights were corrected by the effects of each covariate by adding the difference in means between each class and a standard class to each individuals' record.

Various growth rates were also calculated from the age-specific weights. Early growth was defined as growth between weeks 1 and 3. The Middle growth

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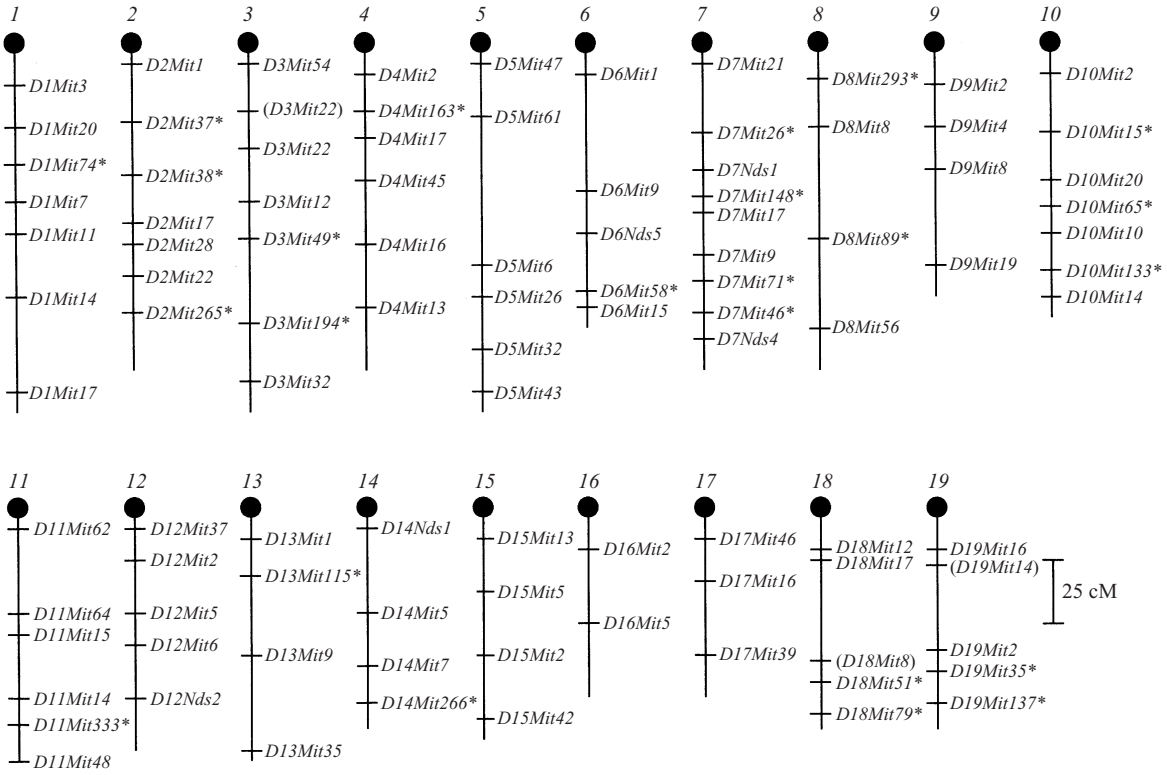

Fig. 1. Relative positions of microsatellite markers scored in the intercross of LG/J with SM/J. Markers denoted with an asterisk are new markers added in the analysis of Intercross II and were not scored in Intercross I. Markers enclosed in parentheses were scored in Intercross I but not scored in Intercross II.

period was defined as growth from 3 to 6 weeks and the Late growth period consisted of growth from 6 to 10 weeks. Six-week weight gain was defined as growth from 1 to 6 weeks. The data were analysed for each age-specific weight as well as for Early, Middle, Six-week and Late growth periods.

(iii) *QTL analysis: replication*

The presence and relative positions of potential QTLs were determined in Intercross I using the interval mapping methods described in Lander & Botstein (1989) as realized in the Mapmaker/QTL 1.1b software (Paterson *et al*., 1988; Lincoln *et al*., 1992*a*, *b*). This procedure, as performed on the data from Intercross I, is described in detail in Cheverud *et al*. (1996). The level of statistical significance was determined via simulation and QTL effects were accepted liberally so as to reduce the chance of rejecting true positive QTL effects. Replication of these potential QTLs was analysed using Intercross II by performing a multiple regression analysis at each previously identified QTL location. The analysis included either an age-specific weight or a growth period as the dependent variable and the additive and dominance scores (discussed below) as the independent variables (Haley & Knott, 1992). This statistical test is based on an *a priori* hypothesis that a QTL found in Intercross I will also be found in the same location in Intercross II and is therefore protected from multiple comparison problems. A QTL was considered replicated if its point-wise probability was less than 5%.

(iv) *QTL analysis: combined data*

Interval mapping was performed on the combined data from Intercross I and Intercross II using the map distances generated from the combined data using Mapmaker 3.0b (Lander *et al*., 1987; Lincoln *et al*., 1992*a*, *b*). This data set included 1045 individuals and the full complement of 96 microsatellite markers. While the relative positions of the markers used are given by Dietrich *et al*. (1992, 1996), map distances may vary between crosses. Therefore, the microsatellite positions for Intercross I and II were mapped independently as well as in combination using the Mapmaker 3.0b program. Map distances were measured for Intercross I, Intercross II and the combined data set. Significant differences in map distance between the intercrosses were tested for using the procedure of Ott (1991) as modified by Williams *et al*. (1995). Differences were found only for chromosomes 2 and 3 and thus a blocking variable was incorporated into the interval mapping model to account for this source of variation. Each of these chromosomes was relatively poorly marked in Intercross I (Cheverud *et al*., 1996).

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(v) *QTL analysis: interval mapping*

We employed the regression interval mapping method of Haley & Knott (1992) with the combined data set by imputing genotype scores every 2 cM throughout the intervals between mapped molecular markers. The marker genotypes and their levels of recombination were used to obtain the probability that an arbitrary position lying between flanking markers is homozygous SM/J, heterozygous, or homozygous LG/J. These probabilities were then multiplied by -1, 0 and 1, respectively, and summed to obtain an additive genotypic score (X_a). Likewise, the probability of heterozygosity at the arbitrary intermediate location was calculated and used as a dominance genotypic score (X_d). The growth periods or age-specific weights were then jointly regressed onto the additive and dominance genotypic scores to obtain estimates of additive (a) and dominance (d) genotypic values and the probability of a gene affecting the character at a specific location. The probability was obtained using the SECTOR option in SYSTAT 7.0 (Cohen & Cohen, 1983; Cohen & Wilkinson, 1997) which provides F and χ^2 statistics and associated probabilities for the full multivariate trait set and F values and associated probabilities for each individual trait.

(vi) *QTL analysis: significance levels*

As discussed by Lander & Kruglyak (1995), we adjusted point-wise probabilities for the presence of multiple comparisons. We calculated two threshold levels to distinguish between chromosome-wide and genome-wide significance. Lander & Kruglyak (1995) recommended LOD thresholds for a F2 intercross at 2·8 for suggestive linkage, roughly corresponding to our 5% chromosome-wide value, and 4·3 for significant linkage at a 5% genome-wide significance level. However, Lander & Kruglyak's (1995) thresholds are overly conservative, assuming a dense marker map not obtained here. Further, QTLs described here are being followed up in an additional advanced intercross population (Darvasi & Soller, 1995), not by further saturating the F2 population with markers. We derived chromosome-wide and genome-wide significance levels for our combined experiment by first calculating the number of independent markers on each chromosome or the effective marker number (M_{eff}; Cheverud, 1999*a*). The effective number is less than the total number of markers scored because of intercorrelations among linked markers. The effective marker number is

$$M_{\text{eff}} = M[1-(V(\lambda)/M))], \quad (1)$$

where M is the number of markers scored and ($V(\lambda)$) is the variance of the eigenvalues of the intermarker correlation matrix for each chromosome (Cheverud, 1999*a*). Each 5% chromosome-wide significance threshold was obtained by dividing 0·05 by the effective number of markers on that chromosome. Five per cent LOD score thresholds are given in the last column in Table 2. The genome-wide 5% threshold was obtained by dividing 0·05 by the effective number of markers summed across all the chromosomes. Summing these values is justified in a F2 population because chromosomes are in linkage equilibrium. The 5% genome-wide threshold is 3·49 on the LOD scale. These experiment-specific values match well with those predicted by Lander & Kruglyak (1995) based on map densities similar to those realized in our experiment.

(vii) *Sex-specific QTLs*

We also examined sex-specific effects by testing the interaction between sex and the additive and dominance genotypic scores. If the mapping results indicated a significant interaction, males and females were mapped separately with a concordant adjustment of the Bonferroni-corrected LOD threshold values.

(viii) *QTL analysis*

The tests described above are for a single QTL on each chromosome. If it appeared that an additional QTL for the same trait may also reside on the chromosome, specific two-QTL models were fitted to the data using the additive and dominance genotypic values for two chromosomal positions at a time (Haley & Knott, 1992). If the two-QTL model fitted significantly better than the one-QTL model at the 5% level, the two-QTL model was accepted. Significance was determined using a χ^2 test with 2 df on twice the difference in natural logarithms of the probabilities of each model.

(ix) *Pleiotropy*

When QTLs for two or more traits map to the same chromosome, it is necessary to consider whether they map to the same position, indicating pleiotropy, or to different positions. A formal description and simulation model for testing pleiotropy between QTL effects can be found in Cheverud (1999*b*). Pleiotropy was tested by identifying the most likely chromosomal position for each trait considered separately (traits A and B) and then for the combination of both traits (AB). A χ^2 value was obtained for trait A at its mostly likely position and at the most likely combined trait position (AB), controlling for variation in trait B (Cohen & Cohen, 1983; Cohen & Wilkinson, 1997). Likewise, a χ^2 value was obtained for trait set B at its most likely position and at the most likely combined trait position (AB), controlling for variation in trait

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A. The differences in χ^2 values between the separate-position model and the combined-position model for traits A and B were summed to produce a χ^2 value for pleiotropy with 1 df. A significant result indicates that the two traits are likely to be affected by two different QTLs while a non-significant result indicates that a single QTL affecting both traits cannot be rejected. This test was performed on the Early, Middle and Late growth periods and for groups of age-specific weights whenever they mapped to the same chromosome.

3. Results

(i) *Variation in age-specific weights and growth*

Table 1 provides the means and phenotypic standard deviations for age-specific weights and growth rates after correction for covariates described in Cheverud *et al*. (1996) and Kramer *et al*. (1998). Correlations between age-specific weights decline as the time between weights increases. Correlations among Early and Late age-specific weights are low, sharing less than 10% of their variance in common. Early growth is independent of Middle and Late growth. The Middle growth period shares less than 10% of its variance with Late growth. Six-week weight gain is the sum of Early and Middle growth periods accounting for the correlation with its components.

(ii) *Replicated QTLs*

The results from Intercross I indicated that QTLs affecting age-specific weights and growth rates were detected on 17 of the 19 chromosomes with only chromosomes 5 and 16 not containing any QTLs at the 10% level. In Intercross II we found QTLs on 15 of the 19 chromosomes. The replicated QTLs from Intercross II for each trait are presented in bold type in Table 2. A QTL was considered replicated if it was significant at the 5% level at the same location as the QTL found in Intercross I. We found no instances of a QTL having an opposite effect between intercrosses. In general, we had greater replication success for the most robust QTLs from Intercross I and for the later age-specific weights than for earlier age-specific weight QTLs. We detected nearly all of the previously identified QTLs on six chromosomes from Intercross I (chromosomes 4, 6, 7, 8, 9 and 10). These QTLs were generally associated with the highest LOD scores. The mean LOD score for the replicated QTLs was 5·25 ($\pm$0·36) and the mean for the QTLs that did not replicate was 3·34 ($\pm$0·19). Replication was generally poor for QTLs that were of marginal significance in Intercross I or on chromosomes that were not as well covered by microsatellite markers. However, a few QTLs in Intercross I that did exhibit a high LOD score also failed to replicate in Intercross II. Only chromosome 3 failed to replicate any QTLs found in Intercross I. In Intercross I, the one-QTL model was not significantly better than the null model for chromosome 3, but a two-QTL model did fit significantly better than the null model (Cheverud *et al*., 1996). This was not replicated in Intercross II.

In a number of instances, QTLs located in intercross II were not significant at the 5% level but were significant at the 10% level. This occurred when the QTL was at a slightly different location on the chromosome compared with Intercross I. While these QTLs are not considered to have replicated in this

Table 1. *Means $\pm$ standard deviations and correlations for age-specific weights and growth periods*

		Age (weeks)												
Age	Weight (g)		1	2	3	4	5	6	7	8	9	10	E	M	SW
1	4·83 $\pm$ 0·65														
2	8·26 $\pm$ 1·00	0·75													
3	12·55 $\pm$ 1·49	0·69	0·83												
4	19·20 $\pm$ 2·13	0·63	0·70	0·80											
5	23·36 $\pm$ 2·33	0·52	0·62	0·70	0·87										
6	27·37 $\pm$ 2·55	0·45	0·53	0·60	0·76	0·90									
7	29·73 $\pm$ 3·90	0·40	0·48	0·54	0·69	0·85	0·94								
8	31·45 $\pm$ 3·30	0·34	0·42	0·48	0·63	0·79	0·88	0·94							
9	33·67 $\pm$ 3·58	0·32	0·39	0·46	0·59	0·75	0·85	0·91	0·94						
10	35·52 $\pm$ 3·92	0·30	0·38	0·43	0·56	0·72	0·82	0·88	0·92	0·94					
E	7·73 $\pm$ 1·14	0·34	0·66	0·91	0·68	0·62	0·53	0·48	0·44	0·42	0·35				
M	14·78 $\pm$ 2·04	0·05	0·05	0·02	0·37	0·62	0·81	0·77	0·75	0·73	0·71	0·01			
SW	22·54 $\pm$ 2·33	0·21	0·36	0·46	0·65	0·85	0·97	0·91	0·87	0·84	0·78	0·48	0·87		
L	8·15 $\pm$ 2·33	0·01	0·06	0·07	0·11	0·21	0·29	0·46	0·58	0·66	0·81	0·07	0·30	0·31	

Growth periods are designated as Early (E), Middle (M), Six-week (SW) and Late (L). Correlations $>$ 0·08 are significant at the 5% level.

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Table 2. *Quantitative trait loci for age-specific weights and growth periods*

Chromosome	Length (cM)	Traits	Common position	Distance from centromere (cM)	Chromosome-specific LOD
1	152	1, 2, 4, 5, ***6, 7, 8, 9, 10, M, L***	*D1Mit11* + 10	84	2·12
2	146	***2, 3***, 7, 8, 9, 10, ***E, M, SW, L***	*D2Mit38* + 8	80	2·04
3	124	No QTLs			1·98
4	128	2, 3, ***4, 5, 6, 7, 8, 9, 10, E, M, SW, L***	*D4Mit17* + 8	50	1·97
5	132	L	*D5Mit32* + 10	130	2·02
6	100	1, ***2, 3, 4, 5, 6, 7, 8, 9, 10, E, M, SW, L***	*D6Nds5* + 14	88	1·90
7	100	***1, 2, 3, 4, 5, 6, 7, 8, 9, 10, E, M, SW, L***	*D7Nds1* + 4	50	2·07
8	104	2, ***3, 4, E***	*D8Mit293* + 2	24	1·81
		5, 6, 7, 8, 9, 10, M, SW	*D8Mit8* + 8	56	
9[M]	94	5, ***6, 7, 8, 9, 10, E, M, SW, L***	*D9Mit4* + 8	42	1·82
10	94	***4, 5, 6, 7, 8, 9, 10, E, M, SW, L***	*D10Mit10* + 4	84	1·97
11	118	***1, 2, 3, 4, 5, 6, E, M***, SW	*D11Mit62* + 32	36	2·02
12[M]	74	10, ***L***	*D12Mit2* + 0	24	1·95
		3, E	*D12Mit6* + 20	74	
13	98	***3, 4, 5, 7, 8, E***	*D13Mit1* + 4	14	1·83
		6, 9, 10, ***M***, SW	*D13Mit9* + 14	86	
14	88	***5, 6, 7, 8, 9, 10, M, SW***, L	*D14Mit5* + 10	58	1·84
15[M]	76	1, 2, ***3***, 4, ***5***, 6, 7, 8, ***9***, 10, E, M, SW, L	*D15Mit5* + 16	46	1·82
16[M, F]	30	2, 3, 4, 5, 6, 7, 8, 9, 10, E, M, SW	*D16Mit2* (+0[M]; +30[F])	14; 44	1·53
17	54	7, 8, 9, **M, SW**	*D17Mit16* + 4	18	1·67
18	46	***8, 9, 10, M, L***	*D18Mit17* + 14	38	1·72
19	66	No QTLs			1·84

Significant QTL effects for age-specific weights (in weeks) and growth periods (Early (E), Middle (M), Six-week (SW) and Late (L)) for each chromosome are shown. Sex-specific QTLs are denoted by ([M]) or ([F]). The common position is the most likely location for the traits where pleiotropic effects were unresolved. Replicated QTLs are shown in ***bold italics***. Also included are the map distances from the centromere on each chromosome. Significance testing was performed on each chromosome separately using chromosome- and genome-wide criteria as describe in the text. The chromosome-wide threshold is provided in the last column and the genome-wide threshold is 3·49.

part of the study, the combination of effects from Intercross I and Intercross II may produce a significant effect in the overall mapping of the combined data set due to the increase in sample size

(iii) *Combined interval mapping*

(a) *Number of genes and effects* The combined interval mapping analysis is presented in Table 2. We found 20 QTLs affecting age-specific weight and/or growth on 17 of the 19 autosomes in the mouse genome with no QTLs being found on chromosomes 3 or 19. The raw and standardized additive (a, $2a/s_p$) and dominance (d, d/s_p) genotypic values for each QTL are available from the authors upon request.

A wide range of genetic architectures were found in the combined mapping analysis, a few of which will be highlighted below. For example, simple additivity was discovered at *D1Mit11* + 10 for Late growth, LG/J dominant to SM/J at *D11Mit62* + 32 for Early growth, SM/J dominant to LG/J at *D11Mit64* + 10 for Late growth, and overdominance at *D4Mit17* + 6 for Early growth. Considering growth rates, the most extreme dominance value is −0·22 g (0·09 SD) at *D14Mit5* + 10 cM for Late growth rate. Generally, the LG/J allele is dominant to the SM/J allele, indicating that higher weight is dominant to lower weight at most loci. Significant LOD scores for Early growth rates ranged from 1·82 on chromosome 9 to 8·15 on chromosome 15 while those for Late growth ranged from 2·52 on chromosome 18 to 7·92 on chromosome 2. The highest LOD score (22·64) was observed for week 7 weight on chromosome 6 at *D6Nds5* + 14. In general, chromosomes 6 and 7 produced the most highly significant results. Further, nearly half of all QTLs exceeding the chromosome-wide threshold also exceeded the genome-wide threshold.

We also examined the age-specific sums for additive and dominance genotypic values. The sum of the additive genotypic values increases with age, from 1·24 g multiple homozygote difference at 1 week (25% of the mean) to a 23·38 g multiple homozygote at week 10 (66% of the mean). This is also reflected in the Early and Late growth periods (0·65 g difference for Early growth and 5·27 g difference for Late growth). Sums of standardized additive genotypic values also increased with age from approximately 2 SD in the first 2 weeks to over 6 SD by week 10.

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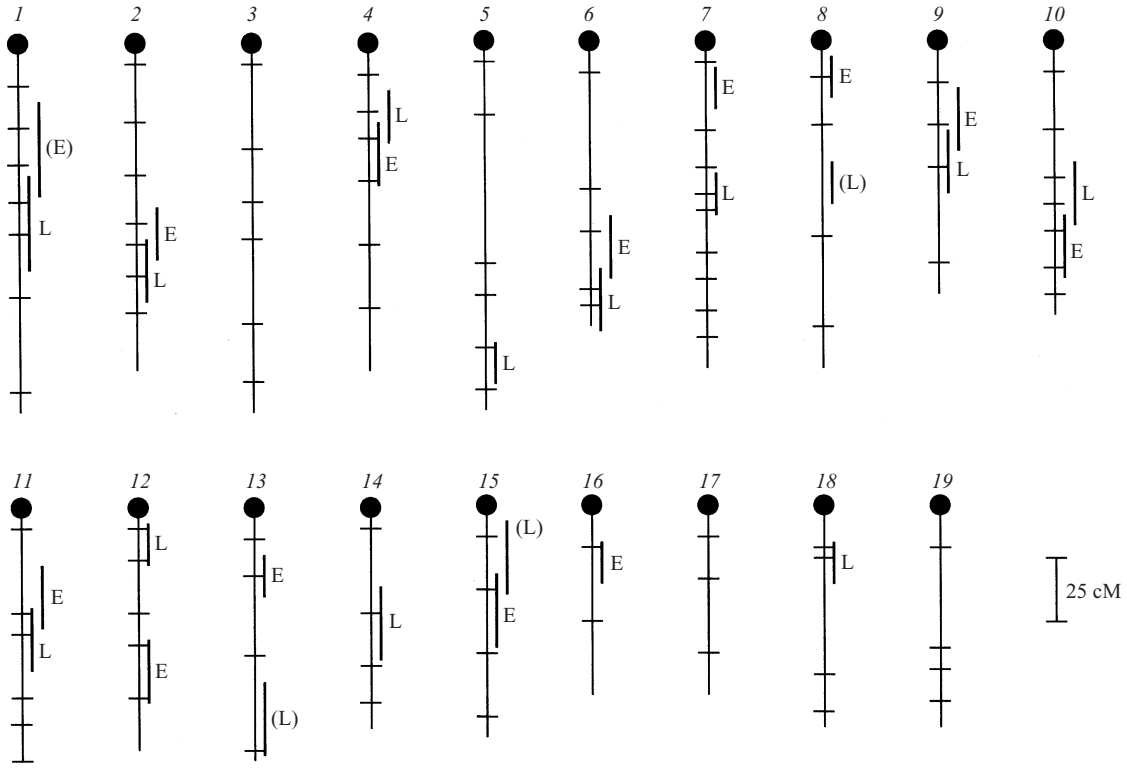

Fig. 2. Relative positions of QTLs affecting Early (E) and Late (L) growth. Parenthetical entries represent locations with exclusive effects on early or late weights but not on growth itself.

While some of this increase in homozygote difference between early and late ages can be accounted for by the increase in the number of QTL detected at later ages (from 6 at week 1 to 13 at week 10), the average additive genotypic values also increased with age from approximately 0·10 SD during the first few weeks to approximately 0·85 SD during the last few weeks.

In terms of the dominance genotypic values, there is a trend for dominance to increase during weeks 1–3 and then level off at later ages. When the Early and Late growth periods are considered, the dominance value is much larger in the Early growth period (2·52 SD), due to several overdominant loci, than in the Late growth period (0·97 SD). A similar pattern is also observed in the raw dominance values. This indicates that the multiple heterozygote is born larger than either homozygote and grows at approximately the same rate as the LG/J homozygote and much faster than the SM/J homozygote during the Early growth period. Heterozygote growth then slows relative to the multiple homozygotes during the Middle and Late growth periods. This is reflected in the much larger dominance ratios (d/a) at week 1 and week 2 (1·4 and 1·9, respectively) than at week 10 (0·01).

In a few cases, it appeared that more than one QTL could be present for a particular trait. We analysed these instances using two QTL models. In no case did a two-QTL model fit better than the one-QTL model.

(b) *Pleiotropy* In this analysis, we found a total of 12 QTLs affecting Early growth and 11 QTLs affecting Late growth explaining 19% and 18% of the variance, respectively, among the F2 hybrids. The distribution of these Early and Late growth QTLs among the 19 autosomes is shown in Fig. 2. In addition, we include four positions with exclusive effects on early and late weights but not on growth itself in Fig. 2. These are indicated in parentheses. A total of 12 chromosomes (1, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13 and 15) contained QTLs affecting both Early and Late growth and were analysed for pleiotropy. Of these 12 chromosomes harbouring QTLs affecting both Early and Late growth, those found on chromosomes 7, 8, 12 and 13 indicated separate genes for the two traits. In the other eight instances the QTLs could not be resolved into independent positions, retaining the possibility that a single gene affects both Early and Late growth. However, if the distributions of effects on Early and Late growth are unrelated, as indicated in Table 1, five co-occurrences of separate Early and Late growth genes in 72 intervals would be expected by chance alone. Of these eight Early/Late QTL pairs, none exhibit a significant opposite effect, that is, the genetic architecture of these QTLs does not exhibit antagonistic pleiotropy. Genotypic effects on Middle and Six-week growth periods generally mapped either with Early or Late growth periods, rarely mapping to distinct positions. In two cases, on chromosomes 8

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and 17, Six-week and Middle growth periods mapped without either Early or Late but did map with Middle age-specific weights.

We also examined pleiotropic relationships between groups of age-specific QTLs where the confidence intervals did not overlap or where there was only minor overlap. A break within the rows of a given chromosome between ages in Table 2 denotes separate QTLs for age-specific weights only. Otherwise, the QTLs for the age-specific weights are assumed to be affected by the same QTL. Generally, pleiotropy could not be rejected between these groups except on chromosomes 12 and 13.

(c) *Sex-specific QTLs*. Sex-specific results, based on the significance of sex by genotype interaction, were observed on four of the chromosomes: 9, 12, 15 and 16 (Table 2). Typically, when the sexes were analysed separately, one sex was observed to have a significant QTL for a given age-specific weight or growth rate while the other sex had no effect on the QTL. Chromosome 16 produced no significant QTLs in Intercross I. However, when the data from the two experiments were combined and analysed for sex-specific effects, significant QTLs were indicate at opposite ends of the chromosome for males and females for the Middle and Late growth periods and for Middle and Late age-specific weights (Table 2).

4. Discussion

Falconer *et al*. (1978) investigated whether genetic changes in body weight, viewed as an adjustment of growth regulation, operated by changing cell number, cell size, or via both mechanisms. Their results indicated that both mechanisms were involved such that the cells of mice selected for increased body size at 6 weeks of age were more numerous and larger than control mice. Additionally, mice that were selected for small body size showed that opposite effect, their cells being smaller and less numerous than those of unselected lines. Thus, it was concluded from this and other genetic studies by Atchley and colleagues (Atchley *et al*., 1997; Atchley & Zhu, 1997) that body size growth involved separate physiological mechanisms: an early mechanism facilitating increase in cell number and a later-developing mechanism leading to an increase in cell size. Our studies of body size growth in the SM/J by LG/J intercross are consistent with expectations based on this earlier work.

Interval mapping of the combined data set revealed many genes with relatively small effects on age-specific body weights and growth. More QTLs were found for later ages than for early ages (6 vs 13 for week 1 and week 10, respectively). A larger proportion of the phenotypic variance was detected at later ages because more QTLs were detected and their standardized additive genotypic effects were larger. The maximum effect for a single QTL was on the order of 11% with an average effect per QTL of approximately 2·5% of the phenotypic variance after correction for covariates. We were also able to detect QTLs accounting for only 1% of the phenotypic variance due to the large sample size of this study. These results are consistent with earlier biometric studies of this intercross (Chai, 1965*a*, *b*; Kramer *et al*., 1998). Chai (1965*a*, *b*) estimated at least 11 effective genes for body size at 60 days. At 63 days we found 14 genes controlling body size variation. A complete biometrical, quantitative genetic analysis was performed for Intercross II by Kramer *et al*. (1998) and, again, similar trends in variance components were reported. For example, the total phenotypic variation was shown to increase with age as did the heritability. However, the additive and dominance variance components are generally lower in the mapping study than were found in the biometrical analysis. This indicates that there may still be more QTLs of smaller effect segregating in this intercross than we could detect despite our ability to identify QTLs contributing only about 1% to the total phenotypic variance. Alternatively, significant amounts of epistatic interaction were found for week 10 weight in Intercross I (Routman & Cheverud, 1997; Cheverud, 1999*a*). The observed difference in percentage variance accounted for between the biometrical study and mapping study could be due in part to epistatic interactions. Thus, body weight in this F2 intercross of the LG/J and SM/J mouse strains behaves as a classic quantitative trait with variation due to many loci, each of relatively small effect, and shows distinct and repeatable patterns of additive and dominance effects.

Another aspect of genetic architecture is the occurrence of genetic correlation between traits. Our results indicate that, in general, there are distinct genetic systems responsible for early and later growth. This is further evidenced by the relatively low phenotypic correlations between early and later weights and growth in this F2 population. Low phenotypic correlations between early and later growth have been found in other studies of rodent growth (Rutledge *et al*., 1972; Cheverud *et al*., 1983; Atchley *et al*., 1984; Riska *et al*., 1984) and can have two genetic sources. Correlations will be low when the loci do not display pleiotropic effects and/or when loci displaying positive pleiotropy are balanced by loci displaying negative pleiotropy. In the combined F2 population we found a general trend for loci to affect either Early or Late growth. Four chromosomes contained only an Early or a Late growth QTL. An additional four chromosomes contained both Early and Late QTLs but were separated into distinct loci with our test for pleiotropy. In another eight cases

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where Early and Late QTLs were found on the same chromosome, we could not eliminate the potential for pleiotropic control. In the previous intercross experiment, Cheverud *et al.* (1996) found four co-occurrences of Early and Late growth. However, Early and Late growth QTLs were found in combination on 10 chromosomes and a formal test of pleiotropy was not performed. In other studies where pleiotropy for body size QTLs were found, significant negative correlations for Early and Later growth have been reported (Rutledge *et al.*, 1972; Cheverud *et al.*, 1983; Atchley *et al.*, 1984; Riska *et al.*, 1984). However, we did not find negative correlations between Early and Late growth in this study. A more highly resolved map in these regions would be needed to confirm or reject pleiotropy for these QTLs.

Intraspecific variation in size may reflect genetic variation, differential investment of mothers, environmental factors as well as gender of offspring. Sexual dimorphism is present in the strains used in our intercross experiments; males are about 8·0 g larger than females in the LG/J strain and about 4·0 g larger than females in the SM/J strain. Few mapping studies have examined sex-specific effects in mammals even though variation between males and females for phenotypic traits is the raw genetic material that produces sexual dimorphism. We found four cases where sex interacted with genotype. On chromosomes 9, 12 and 15, the QTL is primarily due to a male-specific locus. On chromosome 16, separate male- and female-specific QTLs were found. Interestingly, the QTLs on chromosome 16 were not resolved until the sexes were analysed separately. The male-specific QTL appears near the centromeric end of the chromosome and displays additivity. The female-specific QTL is near the telomeric end of the chromosome with the SM/J allele dominant to the LG/J allele.

As we move closer to being able to identify precisely the genes contributing to complex genetic traits, it becomes increasingly important to correctly and precisely identify the QTL region responsible for affecting the trait. In the initial F2 intercross experiment with LG/J and SM/J mice, Cheverud *et al.* (1996) located and quantified numerous QTLs affecting body size. QTLs were accepted liberally in this initial scan in order not to overlook QTLs responsible for significant variation in body size. Our second F2 intercross provided an *a priori* hypothesis to test the consistency of these initial loci. Although we were able to detect replicated QTLs with an original LOD score of 1·50, we found that the average LOD score was nearly 2 orders of magnitude higher than the average LOD value for QTLs that did not replicate. While these results indicate that replication was most successful on the larger LOD results from Intercross I, failure to replicate does not necessarily prove the hypothesis of no genetic effect at a particular location (Lander & Kruglyak, 1995). Linkages between markers and phenotypes will often have weak effects, which, by chance may be weaker or stronger in a second study. By combining the data from the two intercrosses and interval mapping with twice as many individuals, we found 20 distinct QTLs affecting body size and various growth periods during murine development. We are now in a position to examine epistatic interactions among these QTLs and to map the replicated QTLs in an advanced intercross line in preparation for future positional cloning.

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