

# Pleiotropy of quantitative trait loci for organ weights and limb bone lengths in mice

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**Leamy, Larry J., Daniel Pomp, E. J. Eisen, and James M. Cheverud.** Pleiotropy of quantitative trait loci for organ weights and limb bone lengths in mice. *Physiol Genomics* 10: 21–29, 2002. First published May 21, 2002; 10.1152/physiolgenomics.00018.2002.—We investigated the genetic basis of several limb bone lengths and weights of organs in mice produced from a cross of the F<sub>1</sub> between CAST/Ei (wild strain) and M16i (selected for rapid growth rate) back to M16i. From previous correlation studies, we hypothesized that quantitative trait loci (QTLs) would exhibit greater pleiotropy within than between the limb length and organ weight character sets. Using interval mapping procedures and significance testing at the chromosome-wise level, we discovered 14 putative QTLs affecting weight of the liver, spleen, heart, and/or kidney, 9 of which affected more than one organ; and 12 QTLs for limb lengths, all of which affected the length of two or more of the limb bones in these mice. As was hypothesized, most QTLs affected either organ weights or limb lengths independently of each other, although five QTLs were found that affected both sets of characters. The direction of the effect of these QTLs was almost always consistent within and between characters, with little evidence for antagonistic pleiotropy.

interval mapping procedures; antagonistic pleiotropy; modularity

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WHAT GENES CONTROL COMPONENTS of body weight such as organ weights and bone dimensions? Recent quantitative trait locus (QTL) studies using rodent models have shown that there is an abundance of genes scattered throughout the genome that influence body weight itself, particularly at later stages of growth (1, 2, 4, 6, 8, 18–20, 31). Many of these QTLs may be regulatory genes that alter the expression of other genes coding for hormones, growth factors, and their receptors, and in some cases they may represent genes with pleiotropic effects on obesity and related characters (1, 2, 31, 33).

Although our understanding of the number and effects of QTLs influencing body weight continues to

accumulate, we know much less about the genes influencing its components. Phenotypic and genetic correlations among weights of organs such as the liver, kidney, or testis in mice typically are positive and significant (2, 10, 11, 22), however, so we would expect that at least some QTLs for a given organ weight would also affect other organ weights. But only a few QTL studies have been conducted using organ weight characters (for example, see Refs. 1, 2, 31), and in these studies, the characters have been analyzed individually rather than jointly. Furthermore, organ weights sometimes are expressed as a percentage of body weight (31) to adjust for variation in overall size, and QTL studies using absolute values of organ weights are not strictly comparable to those using relative weights. As a consequence, we do not yet have a good understanding of the extent of pleiotropy of the QTLs that influence weights of various organs.

Besides structures such as internal organs, bones also are important contributors to body weight, and in fact various bone dimensions have long been used as overall measures of size in allometry and related studies (25). Phenotypic and genetic correlations between skeletal measures such as limb bone lengths are usually positive and moderate to high in magnitude, so QTLs affecting the length of one limb should often have pleiotropic effects on lengths of other limbs. However, limb lengths form a rather tightly integrated group of characters that in multivariate factor or clustering analyses tend to sort out separately from other dimensions (23, 24). We would therefore expect that QTLs affecting the length of the limbs would only occasionally have pleiotropic effects on organ weights as well (and vice versa).

The purpose of this study was to test these expectations for pleiotropy by conducting a whole genome scan for QTLs affecting several organ weights and limb bone lengths in a population of mice. This population was produced from a cross of highly divergent strains: M16i, consisting of large and moderately obese mice; and CAST/Ei, a wild strain of small mice with lean bodies. Beyond the detection of these QTLs and an assessment of the magnitude of their effects, a multivariate approach to interval mapping is combined with formal pleiotropy tests to clarify pleiotropic patterns of

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effects of the QTLs within and between the organ weight and limb length sets of characters.

## MATERIALS AND METHODS

**Population and characters.** The M16i and CAST/EI (CAST) strains of mice used in this study are genetically quite different. The M16i strain was generated from long-term selection for rapid 3- to 6-wk weight gain in ICR mice (9, 15), followed by relaxed selection and then 15 generations of full-sib mating. The minimum inbreeding coefficient for the M16i strain was 0.95, and it probably approached 1.0 because of the inbreeding that took place during selection. Mice in the CAST strain were derived from a wild population of the subspecies *Mus musculus castaneus* and had undergone at least 35 generations of inbreeding via full-sib mating prior to use in this study.

Mice of both strains were reared in an environment of 21°C, 55% relative humidity, and a light/dark cycle of 12:12 h, according to NIH guidelines for animal care in the Mouse Genetics Laboratory at North Carolina State University. Food (Purina Mouse Chow 5015 from mating until weaning, and Purina Laboratory Chow 5001 from weaning until death) and water were provided ad libitum. CAST males were crossed with M16i females to produce F<sub>1</sub> males that in turn were backcrossed to M16i females, resulting in over 400 mice that reached 12 wk of age.

Each mouse produced from this backcross was killed at 12 wk of age and weighed (WT12), and a tail clip was collected and frozen for later DNA extraction. In addition, the heart, liver, spleen, and kidney (right side only) were weighed (wet weights) in all mice. The skeletons of all mice were prepared by exposure to dermestid beetles. This permitted the measurement (to the nearest 0.01 mm) of the total length of the right sides of the femur, tibia, humerus, and ulna limb bones. Organ weights (but not limb lengths) also were taken on the original M16i parents, but the CAST mice did not breed well, and no measurements were taken on them. Thus the organ weight and limb length data presented below are only from the backcross mice used in the QTL analysis.

All mice were genotyped for a total of 92 microsatellite markers located on 19 autosomes (see APPENDIX) using standard protocols involving PCR and gel electrophoresis. Although this marker coverage of the autosomal genome is somewhat sparse, Darvasi and Soller (7) have pointed out that a fairly wide marker spacing is optimal for minimizing costs and detecting QTLs in an initial QTL-marker linkage study. Additional markers usually are not useful if there is a QTL effect on a chromosome; more recombinant progeny generally are more helpful in detecting additional QTLs and locating them more precisely (30). An additional consideration regarding these microsatellites is that they should occur in a 1:1 ratio in this backcross population, and results of  $\chi^2$  analyses suggested that in fact all markers did show this expected ratio when considering the number of comparisons made and thus that there was no detectable segregation distortion.

**Preliminary statistical analysis.** Prior to the QTL analysis, the distributions of all nine characters (lengths of 4 limb bones, weights of 4 internal organs, and WT12) were examined and found to be normal for body weight and the four organ weights but nonnormal for the four limb lengths. No transformation of the limb length characters was attempted, however, since normality is not an assumption of the randomization procedure used to test for statistical significance

of putative QTLs (see below). The distributions of the characters also were tested for the effects of litter size, sex, sire, and family (within sire) differences. Since significant effects were found for these factors for one or more characters, all nine characters were adjusted by obtaining residuals from a general linear model. In this model, litter size, sex, sire, and family were treated as categorical factors. Thus the QTL analysis and results presented below used each of the characters adjusted for the effect of all of these covariates, including sex (i.e., sexes are pooled). Altogether, 419 mice were available for the analysis, including 212 males and 207 females.

Basic statistics first were calculated for the adjusted values of all nine characters to provide some description of their central tendency and variation. All pairwise correlations among these characters also were calculated and tested for significance using the sequential Bonferroni procedure (34). A principal components analysis (16) was conducted to describe patterns of covariation among the characters and to see whether the limb length characters sorted out separately from the organ weights in this population of mice.

**QTL analyses.** QTL analyses were conducted using the interval mapping approach described by Haley and Knott (14), except that canonical correlation rather than regression techniques were used to initially identify the most likely sites of QTLs. Unlike the single-character regression approach, use of the multivariate technique of canonical correlation allowed us to simultaneously analyze all four characters in each of our two character sets (body weights and limb lengths) to identify QTLs jointly (pleiotropically) affecting more than one of these characters (see below for details). Two separate analyses were run: one for the four limb length characters, and another for the four organ weight characters. (A separate QTL analysis was done for body weights at various ages including at 12 wk, the results from which are not published here.)

To implement the interval mapping approach, we set index values at 0 for the CAST/M16i heterozygote and at 1 for the M16i homozygote at the sites of all molecular markers. We also calculated index values between flanking markers at each site 2 cM apart using the recombination percentages (see APPENDIX) derived from the Mapmaker 3.0 program (28) and the equations for backcross populations given in Haley and Knott (14). At each site on each chromosome, the canonical correlation analysis generated a (canonical) correlation (with its associated probability) of all characters in a set with the index value at that site. Molecular markers located on chromosomes other than the one being analyzed also were used as conditioning variables in each analysis to account for potential effects of background QTLs (17, 37). The markers chosen (maximum of one per chromosome) were those making the largest, statistically significant, contribution to the variation in the characters analyzed. This multivariate canonical correlation approach for interval mapping of QTLs for a set of characters has been used in previous QTL studies (26, 27), where more details of this approach may be found.

For each of the 19 autosomes, the probabilities of the canonical correlations generated at each site 2 cM apart were converted to likelihood of odds (LOD) scores (30). If the highest LOD score calculated for a given chromosome exceeded a specific threshold value, then a QTL was considered to be present between the flanking markers and most likely at the position associated with that LOD score. Basically, this is a pleiotropy model approach to identify which QTL

Table 1. *Limb lengths, 12-wk body weight, and organ weight for the separate and combined sexes in the backcross mice*

Character	Means			Standard Deviation
	Males	Females	Combined Sexes	
Limb length, mm				
Femur	16.97	16.68	16.83	0.748
Tibia	19.49	19.23	19.36	0.744
Humerus	13.80	13.38	13.59	0.515
Ulna	15.40	14.97	15.19	0.550
Weight, g				
WT12	45.39	35.69	40.60	4.396
Heart	0.259	0.215	0.237	0.032
Liver	3.472	2.669	3.076	0.435
Spleen	0.214	0.211	0.213	0.053
Kidney	0.390	0.273	0.332	0.053

Values are means and standard deviations, as indicated. Sample size was 419 for all characters in the combined sexes. WT12, 12-wk body weight.

location explains the most joint variance in the several characters (21).

One thousand permutation runs of the canonical correlation procedure were made to address the multiple comparisons problem that arises with so many potential QTL tests. These runs yielded 5% (and 1%) suggestive linkage threshold values specific for each chromosome. The 5% suggestive threshold values imply that ~1 (5%) of these 19 chromosomes might be expected to have a significant LOD score by chance alone. For each character set, we also calculated the more stringent 5% experiment-wise threshold value that ensured no greater than 5% type I error in QTL testing across the entire genome (5). Confidence intervals for each QTL were determined as the distance in cM on either side of the QTL locations where there was a drop in the LOD score of 1.0 (30). Once a QTL was found on any chromosome, canonical correlation again was used to test for the presence of two QTLs (26).

After QTL positions were established for each chromosome, multiple regressions of each character on the genotypic index values for the QTL(s) at that site (or sites for two QTLs) on each chromosome were run, again including the same conditioning markers as were used in the canonical correlation analyses. For each QTL and character, these analyses yielded multiple regression coefficients that estimated the

difference between the heterozygote and the homozygote, or equivalently, the effect of replacing one CAST allele (in the heterozygote) with an M16i allele (in the homozygote). These differences henceforth are denoted as *a*, although it should be noted that they are not the same as the additive genotypic value defined as one-half the difference between the homozygotes (12). If the *a* values for two or more characters were significant for a given QTL, then it was assumed that this QTL was pleiotropically affecting these characters. In this manner, we tested for pleiotropy of QTLs within the organ weight and limb length sets of characters (tests of pleiotropy between sets of characters are explained in the discussion below). The percentage of the total variation explained by each QTL was estimated by 100 times the squared partial multiple correlation value associated with each multiple regression solution.

To test whether the *a* values for any QTL significantly differed between the two sexes, multiple regressions for each of the characters were run as before, but with sex and the interaction of sex with the genotypic index values included in the model. Where a significant interaction occurred, multiple regressions were run for the separate sexes to obtain estimates of *a* (and their tests of significance) for both males and females.

RESULTS

For purposes of reference, Table 1 gives the mean and standard deviation for each of the nine characters in all mice. The means of these characters are given for the separate and combined sexes and show that males have larger body and organ weights, as well as longer limb lengths, than females. The four limb lengths have coefficients of variation (CVs) (not shown) that range from 3.6 to 4.4 and are relatively less variable than the organ weights whose CVs range from 10.8 (WT12) to 24.5 (spleen).

Table 2 shows the correlations between each pair of the nine characters, with those between each of the four limb lengths all being positive and high (mean = 0.71) in magnitude. Correlations of these four limb lengths with body, heart, liver, and kidney weights are significant and positive in sign, but there is no significant association between limb lengths and spleen weight. Body weight is significantly positively correlated with all other characters, espe-

Table 2. *Correlations among the 9 characters as well as the loadings on the first three principal components derived from a component analysis of the correlation matrix*

	Principal Component			Tibia	Humerus	Ulna	WT12	Heart	Liver	Spleen	Kidney
	I	II	III								
Femur	0.39	-0.30	-0.13	0.82†	0.77†	0.62†	0.52†	0.24†	0.34†	0.02	0.52†
Tibia	0.38	-0.31	-0.11		0.78†	0.63†	0.49†	0.25†	0.31†	0.04	0.51†
Humerus	0.39	-0.27	-0.01			0.64†	0.52†	0.32†	0.34†	0.05	0.56†
Ulna	0.34	-0.21	-0.14				0.45†	0.24†	0.30†	0.13	0.47†
WT12	0.37	0.28	-0.21					0.36†	0.79†	0.39†	0.52†
Heart	0.24	0.24	0.81						0.37†	0.20†	0.48†
Liver	0.31	0.47	-0.17							0.49†	0.46†
Spleen	0.14	0.58	-0.31								0.20†
Kidney	0.35	0.06	0.36								
Contributed variance, %	50.6	17.7	9.4								

† *P* < 0.01 in sequential Bonferroni tests.

cially liver weight. Heart, liver, spleen, and kidney weights are all significantly positively correlated, but the level of their association (mean correlation = 0.37) is much less than that (0.71) for the limb length characters.

The patterns of these associations among the nine characters are more readily seen in the results of the principal components of these correlations (Table 2). The first three principal components (I, II, and III) account for over 77% of the total variance. The first component clearly is an overall size vector that loads highly on most characters except spleen weight. The second component contrasts the limb lengths with body weight and organ weights, and the third component is mostly due to variation in heart weight. Thus this analysis suggests that the organ weights and limb lengths do cluster separately. This could be due to large numbers of genes with effects exclusive to either limb lengths or organ weights or to antagonistic pleiotropy at genes affecting both limb bones and organs.

The locations and confidence intervals for all QTLs significantly affecting the four organ weight characters are summarized in Table 3 (and are depicted in Fig. 1). All QTLs whose LOD scores reached significance at the 5% (\*) or 1% (†) chromosome-wise level are listed, since only 1 in 20 (or at the 1% level, 1 in 100) false-positive results might be expected at this level and thus nearly all of these should represent true QTLs. However, those QTLs whose LOD scores reached significance at the 5% experiment-wise level (LOD = 3.297) are specially differentiated in Table 3 with the designation “*Orgwq*.” In generating these results from the canonical correlation runs, conditioning markers were used on all autosomes except numbers 7, 8, 12, 13, and 14. Results of these analyses showed that 14 different QTLs on 12 different autosomes, including 2 each on chromosomes 2 and 3, appear to affect weights of one or more of the four organs. LOD scores for 11 of the 14 QTLs exceed the suggestive critical values at the 1%

level derived from the permutation runs (mean = 2.63, range 2.20–3.28), and those for 9 QTLs (*Orgwq1*–*Orgwq9*) exceed the 5% experiment-wise threshold value of 3.30. Confidence intervals for the sites of these QTLs vary considerably, averaging about 31 cM.

Table 4 summarizes the effects of each of the 14 QTLs on the 4 organ characters by listing the percentage of variation they accounted for as well as the difference they generated between the homozygote and heterozygote (*a*). Nine of the 14 total QTLs (and 6 of the 9 QTLs reaching significance at the experiment-wise level) affect more than one organ weight character, so pleiotropy for organ weights is prevalent. The direction of the effect for 12 of the 14 QTLs is positive, suggesting that the M16i allele generally tends to increase organ weights. Of the remaining two QTLs, one (*Orgwq5*) shows negative *a* values for both characters (heart and kidney weight) it affects, whereas the other QTL, *Orgwq1*, shows negative *a* values for heart and kidney weight but a positive *a* value for spleen weight. None of the *a* values for any of QTLs affecting the organ weights differed between the two sexes. The percentage of the total variation accounted for by individual QTLs affecting each character ranges from about 1% to over 12%, and over all QTLs, from 20.7% for heart weight to 32.9% for spleen weight.

Table 5 provides a listing of all QTLs found to significantly affect the limb length characters. Preliminary canonical correlation analyses indicated that at least one marker on chromosome 1, 2, 3, 5, 7, 8, 10, 11, 12, 14, 15, 17, 18, and 19 was significant, so the analyses that generated these results for each chromosome made use of a maximum of 14 conditioning markers. As may be seen, 12 QTLs on 12 different autosomes were identified as significantly affecting the limb bone lengths. LOD scores for nine of these QTLs exceed the suggestive critical values at the 1% level determined from permutation runs (mean = 2.55, range 2.22–3.08), and those for six of these QTLs (designated

Table 3. Chromosomes, locations, LOD scores, and confidence intervals of QTLs significantly affecting organ weights in the combined sexes

Chr	QTL	LOD	Proximal Marker	Marker Distance	Centromere Distance	Marker CI	Centromere CI
1	<i>Orgwq1</i>	14.51†	<i>D1Mit140</i>	8	63	<i>D1Mit9+8–D1Mit140+16</i>	53–71
2	<i>Orgwq2</i>	26.10†	<i>D2Mit61</i>	0	34	<i>D2Mit1+0–D2Nds1+2</i>	1–55
2	<i>Orgwq3</i>		<i>D2Mit164</i>	2	65	<i>D2Mit133+0–D2Mit224+4</i>	60–69
3	<i>Orgwq4</i>	5.38†	<i>D3Mit46</i>	20	34	<i>D3Mit46+12–D3Mit31+0</i>	26–75
3	<i>Orgwq5</i>		<i>D3Mit31</i>	0	75	<i>D3Mit10+6–D3Mit31+0</i>	41–75
4	<i>Orgwq6</i>	4.46†	<i>D4Mit27</i>	24	60	<i>D4Mit27+10–D4Mit27+40</i>	46–76
5		2.30*	<i>D5Mit48</i>	0	1	<i>D5Mit48+0–D5Mit48+38</i>	1–39
6		2.18*	<i>D6Nds5</i>	10	46	<i>D6Mit50+0–D6Nds5+30</i>	3–66
9	<i>Orgwq7</i>	7.55†	<i>D9Mit2</i>	12	29	<i>D9Mit2+4–D9Mit2+22</i>	21–39
10		3.18†	<i>D10Mit31</i>	4	33	<i>D10Mit16+2–IGF+6</i>	18–48
11	<i>Orgwq8</i>	6.27†	<i>D11Mit63</i>	22	24	<i>D11Mit63+12–D11Mit5+12</i>	14–49
15		3.11†	<i>D15Mit29</i>	4	43	<i>D15Mit121+0–D15Mit107+4</i>	23–48
18	<i>Orgwq9</i>	10.31†	<i>D18Nds1</i>	0	73	<i>D18Mit51+36–D18Nds1+0</i>	63–73
19		2.36*	<i>D19Mit29</i>	0	4	<i>D19Mit29+0–D9Mit29+18</i>	4–22

Locations and confidence intervals (CI) are given as distances in cM from the nearest proximal marker and from the centromere. QTLs designated “*Orgwq*–” had LOD scores that exceeded the 5% experimentwise significance value of 3.297. \**P* < 0.05 and †*P* < 0.01 for chromosomewise significance.

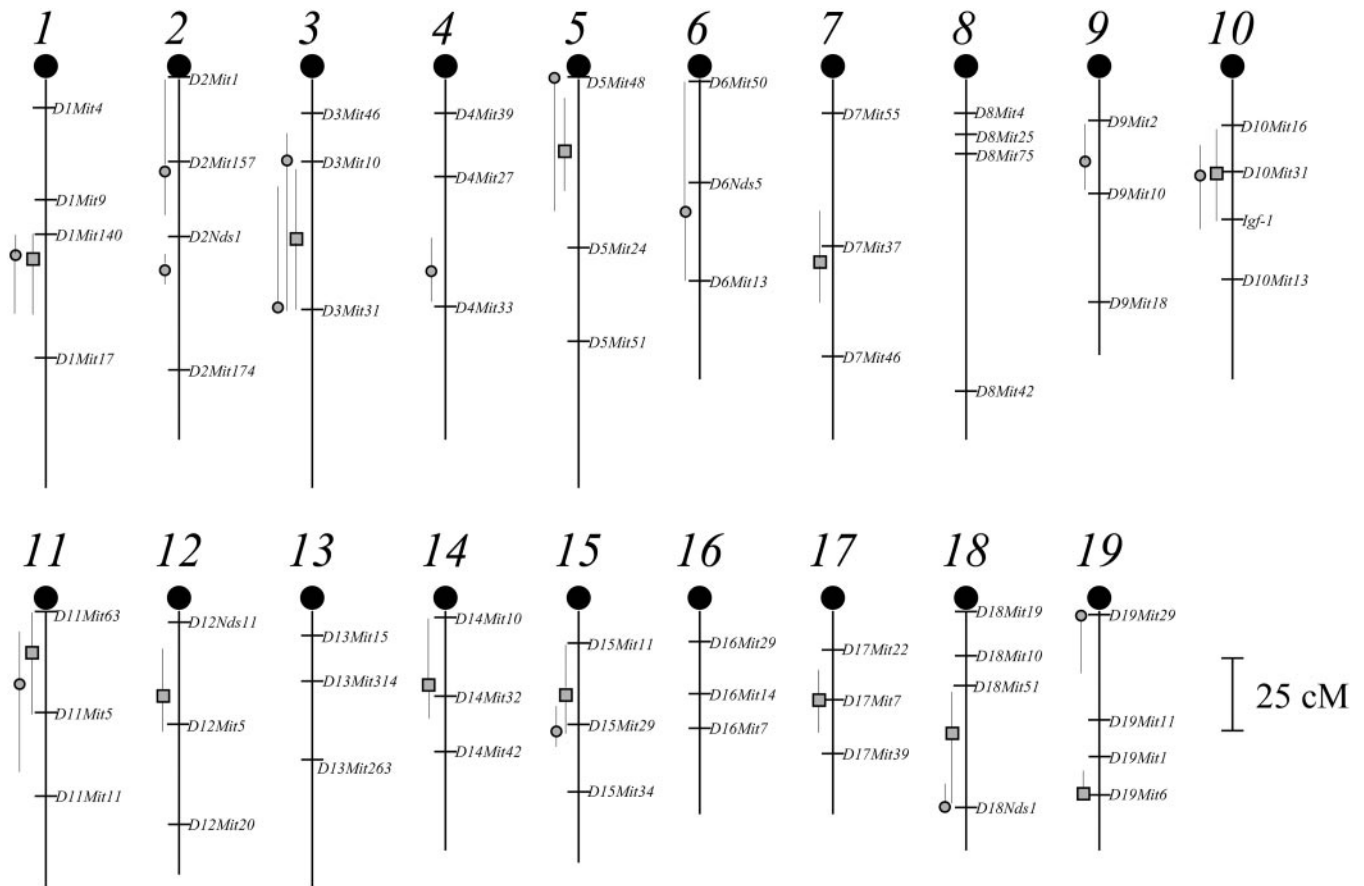


Fig. 1. Relative positions and confidence intervals of QTLs significant for organ weights (gray circles), and limb bone lengths (gray squares). The microsatellite molecular markers on each chromosome (four representatives only on chromosomes 2, 13, and 15) also are shown.

*Lmblgq1–Lmblgq6*) also exceed the 5% experiment-wise threshold value of 3.61. Locations of these QTLs are given as before (see also Fig. 1), as well as their confidence intervals, which vary considerably, averaging about 28.5 cM.

Table 6 summarizes the effects of each of the 12 QTLs on the individual limb length characters. Four of these QTLs (*Lmblgq1*, *Lmblgq3*, *Lmblgq6*, and a QTL on chromosome 17) significantly affect all four limb lengths, and the remaining eight QTLs affect either two or three limb lengths; no QTLs affect only one limb length. Lengths of the femur, tibia, humerus, and ulna are affected by 7, 11, 9, and 10 QTLs that account for 28.0%, 26.4%, 35.6%, and 22.2%, respectively, of the total variation in these characters. These QTLs individually contribute on average 3.0% of the variation in these characters, although range as high as 15% as seen in the effect of *Lmblgq1* on humerus length. The sign of these *a* values is the same for all limb lengths affected by individual QTLs (all positive for *Lmblgq3*, all negative for *Lmblgq4*, etc.). Six QTLs have negative *a* values for all affected characters, suggesting that the M16i allele consistently acts to decrease the lengths of all limb bones, whereas the opposite is true for the other six QTLs. It should also be noted that there is

sexual dimorphism in the effects of the QTL on chromosome 14 on ulna length, but especially for the QTL on chromosome 17, whose effects are significant for all 4 characters only in males.

DISCUSSION

We have identified a number of putative QTLs for organ weights and limb bone lengths in the backcross population of mice. This was expected, since the mean body weight for mice in the M16i and CAST inbred strains differed considerably and thus should have optimized the chance of discovering QTLs for these characters (33). But as is true in all QTL studies, this also means that the QTLs discovered here are initially limited to those whose alleles differ between these two strains. Furthermore, some QTLs even with allelic differences between these strains may have gone undetected for a variety of reasons, including dominance effects that might have resulted in little difference between the homozygous (M16i/M16i) and heterozygous (M16i/CAST) genotypes (33). Nonetheless, a sufficient number of putative QTLs was uncovered here for us to be able to test whether these loci affected only the organ weights or the limb length sets of characters

Table 4. *Descriptive data for all QTLs significantly affecting the organ weight characters in the combined sexes*

Chr	QTL	Character	Total Variation Explained, %	$\alpha$
1	<i>Orgwq1</i>	Heart	6.05	$-0.017 \pm 0.0034^\dagger$
		Spleen	1.28	$0.012 \pm 0.0054^*$
		Kidney	12.19	$-0.040 \pm 0.0054^\dagger$
2	<i>Orgwq2</i>	Spleen	1.92	$0.014 \pm 0.0051^\dagger$
		Liver	12.50	$0.322 \pm 0.0425^\dagger$
3	<i>Orgwq4</i>	Heart	2.08	$0.010 \pm 0.0033^\dagger$
		Liver	1.39	$0.100 \pm 0.0420^*$
3	<i>Orgwq5</i>	Heart	5.97	$-0.016 \pm 0.0032^\dagger$
		Kidney	3.72	$-0.020 \pm 0.0052^\dagger$
4	<i>Orgwq6</i>	Spleen	5.39	$0.028 \pm 0.0058^\dagger$
		Heart	1.23	$0.007 \pm 0.0029^*$
5		Liver	2.03	$0.108 \pm 0.0375^\dagger$
		Spleen	2.45	$0.015 \pm 0.0046^\dagger$
		Kidney	1.49	$0.011 \pm 0.0046^*$
		Spleen	1.44	$0.013 \pm 0.0054^*$
6	<i>Orgwq7</i>	Liver	2.01	$0.126 \pm 0.0437^\dagger$
		Spleen	8.85	$0.033 \pm 0.0053^\dagger$
		Kidney	1.65	$0.014 \pm 0.0054^\dagger$
10		Heart	1.62	$0.008 \pm 0.0031^*$
		Spleen	3.79	$0.019 \pm 0.0048^\dagger$
11	<i>Orgwq8</i>	Liver	7.76	$0.261 \pm 0.0448^\dagger$
		Spleen	3.09	$0.020 \pm 0.0055^\dagger$
		Kidney	1.72	$0.015 \pm 0.0082^\dagger$
15		Heart	1.48	$0.007 \pm 0.0030^*$
		Liver	1.99	$0.108 \pm 0.0379^\dagger$
		Spleen	2.94	$0.016 \pm 0.0047^\dagger$
18	<i>Orgwq9</i>	Heart	2.29	$0.009 \pm 0.0029^\dagger$
		Kidney	10.43	$0.031 \pm 0.0046^\dagger$
19		Spleen	1.78	$0.012 \pm 0.0046^\dagger$

Values are means  $\pm$  SE, as indicated;  $\alpha$ , effect ( $\pm$  SE) of replacing one CAST allele (in the heterozygote) with an M16 allele (in the homozygote). \* $P < 0.05$ .  $^\dagger P < 0.01$ .

and to discover their level of pleiotropy within either of these sets.

**QTLs for organ weights.** We discovered 14 separate QTLs that affected absolute organ weights, including 9 (on chromosomes 1, 2, 3, 4, 9, 11, and 18) that reached

significance at the 5% experiment-wise level. Of these nine QTLs, four, five, five, and four QTLs affected liver, spleen, kidney, and heart weights, respectively, results comparable to those found by Brockmann et al. (1, 2) for several organ weights in two QTL studies using mice selected for high growth. Our joint QTL analysis showed that six of these nine QTLs affected more than one organ weight (Table 4), and thus that pleiotropy was fairly prevalent, as originally hypothesized. This pleiotropy did not generally extend to all characters, however, since only one QTL on chromosome 5 that was weakly supported statistically showed significant effects on all four organ weights. Probably this is a reflection of the low-to-moderate level of associations among the organ characters.

The patterns of pleiotropy shown by these six QTLs appear to reflect the correlation patterns shown earlier (Table 2). As an example, heart weight is more strongly correlated with kidney weight than with spleen weight, and although three QTLs show joint effects on heart and kidney weight, only one QTL (*Orgwq1*) does so for heart and spleen weight. Furthermore, the direction of the effect for *Orgwq1* is negative for heart weight and positive for spleen weight, with this being the only example of antagonistic pleiotropy among these QTLs. In general, therefore, the QTLs we have discovered for organ weights often affect more than one organ weight in the same direction. Probably this is a result of shared growth patterns because instances of antagonistic pleiotropy increased in a preliminary analysis done with organ weights adjusted for body weight differences.

**QTLs for limb bone lengths.** The interval mapping analysis for the four limb lengths revealed 12 QTLs that significantly affected these characters, including 6 at the 5% experiment-wise threshold level. All of these QTLs affected two or more limb bone lengths, none affecting only a single limb length. Thus evidence of pleiotropy for QTLs affecting limb lengths is greater than for those affecting organ weights, presumably

Table 5. *Chromosomes, locations, LOD scores, and confidence intervals of QTLs significantly affecting limb lengths in the combined sexes*

Chr	QTL	LOD	Proximal Marker	Marker Distance	Centromere Distance	Marker CI	Centromere CI
1	<i>Lmblgq1</i>	13.62 $^\dagger$	<i>D1Mit140</i>	12	67	<i>D1Mit140+2—D1Mit140+20</i>	57-75
3		3.02 $^\dagger$	<i>D3Mit10</i>	24	59	<i>D3Mit10+6—D3Mit31+0</i>	41-75
5	<i>Lmblgq2</i>	7.47 $^\dagger$	<i>D5Mit48</i>	26	27	<i>D5Mit48+12—D5Mit48+40</i>	13-41
7		5.05 $^\dagger$	<i>D7Mit37</i>	8	65	<i>D7Mit55+28—D7Mit37+22</i>	43-79
10	<i>Lmblgq4</i>	3.94 $^\dagger$	<i>D10Mit31</i>	2	31	<i>D10Mit16+2—D10Mit31+10</i>	18-41
11		2.11 $^*$	<i>D11Mit63</i>	10	12	<i>D11Mit63+0—D11Mit5+2</i>	2-39
12	<i>Lmblgq5</i>	3.96 $^\dagger$	<i>D12Nds11</i>	26	32	<i>D12Nds11+10—D12Mit5+6</i>	16-47
14		2.77 $^\dagger$	<i>D14Mit10</i>	22	25	<i>D14Mit10+0—D14Mit32+8</i>	3-38
15		2.61 $^*$	<i>D15Mit121</i>	6	29	<i>D15Mit11+0—D15Mit107+2</i>	10-46
17		2.92 $^\dagger$	<i>D17Mit7</i>	0	33	<i>D17Mit22+4—D17Mit7+10</i>	23-43
18	<i>Lmblgq6</i>	5.13 $^\dagger$	<i>D18Mit51</i>	24	51	<i>D18Mit51+6—D18Mit51+42</i>	33-69
19		2.16 $^*$	<i>D19Mit6</i>	0	64	<i>D19Mit1+6—D19Mit6+0</i>	58-64

Locations and confidence intervals (CI) are given as distances in cM from the nearest proximal marker and from the centromere. QTLs designated "*Lmblgq*-" had LOD scores that exceeded the 5% experimentwise significance value of 3.61. \* $P < 0.05$  and  $^\dagger P < 0.01$  for chromosomewise significance.

Table 6. Descriptive data for all QTLs significantly affecting the limb length characters in the combined sexes (and where significant sex by a interactions were present, in the separate sexes)

Chr	QTL	Character	Total Variation Explained, %	$a$
1	<i>Lmblgq1</i>	Femur	9.67	$-0.54 \pm 0.082^\dagger$
		Tibia	7.43	$-0.46 \pm 0.082^\dagger$
		Humerus	15.49	$-0.46 \pm 0.054^\dagger$
		Ulna	6.00	$-0.31 \pm 0.062^\dagger$
3		Tibia	1.89	$-0.23 \pm 0.082^\dagger$
		Ulna	1.09	$-0.13 \pm 0.063^*$
5	<i>Lmblgq2</i>	Humerus	2.20	$0.19 \pm 0.063^\dagger$
		Ulna	4.23	$0.31 \pm 0.072^\dagger$
7	<i>Lmblgq3</i>	Femur	1.35	$-0.18 \pm 0.075^*$
		Tibia	1.91	$-0.21 \pm 0.075^\dagger$
		Humerus	5.75	$-0.25 \pm 0.050^\dagger$
		Ulna	2.47	$-0.18 \pm 0.057^\dagger$
10	<i>Lmblgq4</i>	Femur	3.43	$-0.26 \pm 0.069^\dagger$
		Tibia	1.16	$-0.15 \pm 0.069^*$
		Humerus	1.12	$-0.10 \pm 0.045^*$
11		Femur	3.02	$0.28 \pm 0.078^\dagger$
		Tibia	1.09	$0.17 \pm 0.078^*$
		Humerus	1.18	$0.11 \pm 0.052^*$
12	<i>Lmblgq5</i>	Femur	3.69	$-0.30 \pm 0.077^\dagger$
		Tibia	3.55	$-0.30 \pm 0.077^\dagger$
		Ulna	1.85	$-0.16 \pm 0.058^\dagger$
14		Tibia	2.62	$-0.24 \pm 0.072^\dagger$
		Humerus	1.88	$-0.13 \pm 0.047^\dagger$
		Ulna	0.43	$-0.73 \pm 0.055^\dagger$
		Males	0.20	$0.05 \pm 0.078$
15		Females	2.29	$-0.16 \pm 0.077^*$
		Tibia	2.28	$0.21 \pm 0.068^\dagger$
		Humerus	1.42	$0.11 \pm 0.045^*$
		Ulna	2.87	$0.18 \pm 0.052^\dagger$
17		Femur	0.39	$0.09 \pm 0.066$
		Males	2.54	$0.24 \pm 0.094^*$
		Females	0.37	$-0.08 \pm 0.096$
		Tibia	0.45	$0.09 \pm 0.067$
		Males	2.20	$0.20 \pm 0.094^*$
		Females	0.05	$-0.03 \pm 0.097$
		Humerus	3.12	$0.16 \pm 0.044^\dagger$
		Males	7.21	$0.25 \pm 0.063^\dagger$
		Females	0.61	$0.07 \pm 0.063$
		Ulna	0.54	$0.08 \pm 0.051$
18	<i>Lmblgq6</i>	Males	5.01	$0.23 \pm 0.071^\dagger$
		Females	0.81	$-0.09 \pm 0.072$
		Femur	6.42	$0.46 \pm 0.086^\dagger$
		Tibia	2.54	$0.29 \pm 0.088^\dagger$
		Humerus	3.42	$0.22 \pm 0.058^\dagger$
19		Ulna	1.54	$0.17 \pm 0.067^*$
		Tibia	1.47	$0.17 \pm 0.067^*$
		Ulna	1.17	$0.11 \pm 0.051^*$

Values are means  $\pm$  SE, as indicated;  $a$ , effect ( $\pm$ SE) of replacing one CAST allele (in the heterozygote) with an M16 allele (in the homozygote). \* $P < 0.05$ ,  $^\dagger P < 0.01$ .

because of the greater level of integration (higher correlations) among the limb length characters. This apparent pleiotropy was entirely consistent in direction, so that if one QTL acted to increase (decrease) the length of one limb bone, it also acted to increase (decrease) the length of any other limb bone affected.

One or more of these QTLs affecting limb bone lengths may be among the many genes now known to affect the mouse skeleton and/or specifically its appen-

dicular components. As an example, there are at least three genes on chromosome 5 reasonably close to *Lmblgq2* that cause a reduction in various limb bone lengths (32): *Ssq* (16 cM), *lx* (22 cM), and *Hx* (16 cM). On the other hand, *Lmblgq2* affects only the length of the upper limb (humerus and ulna) and may be influenced by *tbx5*, a T-box gene also located on chromosome 5 (65 cM) that differentially affects the forelimbs of vertebrates (13). Still other genes could be responsible for differential growth among the various limb bones. One such gene may be *Lmblgq1*, because it showed a more pronounced effect on the proximal compared with the distal limb lengths (Table 6). This is precisely the kind of anterior-posterior patterning suggestive of the action of homeobox-containing (Hox) genes. In fact there are at least two such genes on chromosome 1 fairly close to *Lmblgq1*: *Gbx2* (gastrulation brain homeobox 2) at 65 cM and *En-1* (Engrailed 1) at 64.1 cM (32). *En-1* participates in the signaling pathway for limb development (29, 35), so it is a candidate for *Lmblgq1*. Another possibility for *Lmblgq1*, however, is *Myog* (72.3 cM; 32), a myogenic regulatory gene. The QTLs on chromosomes 3 and 19 are interesting in that they affect only the distal limb lengths and also may represent, or may be influenced by, one of the Hox genes.

*Pleiotropy of QTLs across character sets.* Six of the QTLs for limb bone lengths, specifically those on chromosomes 1, 3, 10, 11, 15, and 18, were determined to be on the same chromosomes in roughly the same region as QTLs for organ weights, so it seemed natural to ask whether some of these QTLs are the same for both groups of characters. This was accomplished by a pleiotropy test recently developed by Cheverud (3) in which a  $\chi^2$  value is calculated as the test statistic. A significant  $\chi^2$  value indicates that pleiotropy is not likely and that two separate QTLs probably are involved, whereas a nonsignificant  $\chi^2$  value suggests that a single QTL may be affecting both groups of characters (3). It should be emphasized that this test is designed to detect common effects of a gene in a specific interval on a chromosome, which is the conventional interpretation of pleiotropy in QTL studies (21). The test cannot distinguish pleiotropy in the strict sense (that due to common effects of a QTL at the nucleotide level), however, from effects potentially due to closely linked QTLs in that specific region.

In applying this test, one-QTL models only were used with no conditioning variables. Probabilities associated with the  $\chi^2$  values were evaluated with the sequential Bonferroni procedure (34) to ensure an experiment-wise error rate no greater than 5% among these six tests. Significance occurred only for the QTLs on chromosome 18, suggesting that QTLs on chromosomes 1, 3, 10, 11, and 15 may in fact be genes that have pleiotropic effects on both the limb bone length and organ weight sets of characters.

Of the five QTLs on chromosomes potentially common to both sets of characters, three (those on chromo-

APPENDIX. *Microsatellite markers genotyped and their chromosomal location in Haldane units*

Marker	Location, cM	Marker	Location, cM	Marker	Location, cM	Marker	Location, cM
D1Mit4	12	D2Mit200	105	D10Mit16	16	D15Mit131	12
D1Mit9	45	D3Mit46	14	D10Mit31	29	D15Mit86	19
D1Mit140	55	D3Mit10	35	Igf-1	41	D15Mit121	23
D1Mit17	97	D3Mit31	75	D10Mit13	57	D15Mit3	30
D2Mit1	1	D4Mit39	11	D11Mit63	2	D15Mit64	35
D2Mit79	13	D4Mit27	36	D11Mit5	37	D15Mit29	39
D2Mit120	15	D4Mit33	78	D11Mit11	67	D15Mit107	44
D2Mit157	30	D5Mit48	1	D12Nds11	6	Ppara	48
D2Mit61	34	D5Mit24	60	D12Mit5	41	D15Mit34	62
D2Mit37	43	D5Mit51	92	D12Mit20	75	D16Mit29	13
D2Nds1	53	D6Mit50	3	D13Mit15	10	D16Mit14	33
D2Mit103	58	D6Nds5	36	D13Mit181	16	D16Mit7	45
D2Mit133	60	D6Mit14	70	D13Mit311	20	D17Mit22	19
D2Mit164	63	D7Mit55	15	D13Mit314	29	D17Mit7	33
D2Mit224	65	D7Mit37	57	D13Mit160	31	D17Mit39	45
D2Mit166	70	D7Mit46	97	D13Mit36	37	D18Mit19	2
D2Mit22	73	D8Mit4	14	D13Mit51	41	D18Mit10	17
Agouti	75	D8Mit25	21	D13Mit53	50	D18Mit51	27
Ghrh	76	D8Mit75	26	D13Mit263	52	D18Nds1	73
D2Mit49	80	D8Mit42	110	D14Mit10	3	D19Mit29	4
D2Mit25	90	D9Mit2	17	D14Mit32	30	D19Mit11	38
D2Mit147	93	D9Mit10	43	D14Mit42	48	D19Mit1	52
D2Mit174	101	D9Mit18	75	D15Mit11	10	D19Mit6	64

Location of the first marker on each chromosome was taken from the Mouse Genome Database. Markers for *Agouti*, *Ghrh*, and *Ppara* were PCR-RFLP.

somes 3, 11, and 15) exhibited  $a$  values that were the same sign among all organ weight and limb length characters (Tables 4 and 6). This consistency was nearly true for the common QTL on chromosome 1 as well, since *Orgwq1* exhibited negative  $a$  values for two of three organ weights and *Lmb1gq1* showed negative  $a$  values for all four limb lengths. Only the common QTL on chromosome 10 exhibited a different pattern: positive  $a$  values for the organ weights but negative  $a$  values for the limb lengths. In general, therefore, this suggests that the effect of the M16i allele at these loci was either to increase or decrease organ weights as well as the length of the limbs. Only one QTL was found in this backcross population that increased all the characters in one group while decreasing all of the characters in the other.

Other studies (for example, see Refs. 4, 27) also have found little evidence for antagonistic pleiotropy across groups of characters that are functionally or developmentally distinct. Instead, there appears to be mounting evidence for phenotypic modularization (3, 36) in which different genes affect different sets of characters. The principal components analysis suggested that the limb lengths represent a different functional group than organ weights, and thus it was not surprising that the majority of QTLs affected characters in only one of these two functional groups. But the number of significant phenotypic correlations between individual organ weights and limb lengths (Table 2) also implies that these two groups of characters are not entirely independent, and therefore some genes common to both groups, such as the five identified, might be expected as well.

*Conclusions.* A number of QTLs was identified in this study that affected organ weights and limb bone lengths. As hypothesized, most QTLs affecting a given organ weight also affected various other organ weights, and most QTLs affecting a given limb length affected other limb lengths. Although five QTLs were identified as affecting both the organ weight and limb length characters, in general, most QTLs affected characters in only one group. It would appear that QTLs affecting adult organ weights and limb bone lengths probably represent a diversity of genes with various roles, but their precise nature will only be understood when finer-scale mapping studies are done that ultimately lead to tests of candidate loci. The patterns of pleiotropy revealed in this preliminary study should prove useful in guiding the selection of the most appropriate candidate loci for such eventual testing, by uncovering potentially shared pathways and mechanisms. In addition, greater understanding of pleiotropic effects will be useful in unraveling the underlying nature of genetic correlations and the overall genetic architecture of complex traits.

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