

# Multiple-trait QTL mapping for body and organ weights in a cross between NMRI8 and DBA/2 mice

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## Summary

Multiple-trait analyses have been shown to improve the detection of quantitative trait loci (QTLs) with multiple effects. Here we applied a multiple-trait approach on obesity- and growth-related traits that were surveyed in 275 F<sub>2</sub> mice generated from an intercross between the high body weight selected line NMRI8 and DBA/2 as lean control. The parental lines differed 2.5-fold in body weight at the age of 6 weeks. Within the F<sub>2</sub> population, the correlations between body weight and weights of abdominal fat weight, muscle, liver and kidney at the age of 6 weeks were about 0.8. A least squares multiple-trait QTL analysis was performed on these data to understand more precisely the cause of the genetic correlation between body weight, body composition traits and weights of inner organs. Regions on Chr 1, 2, 7 and 14 for body weights at different early ages and regions on Chr 1, 2, 4, 7, 14, 17 and 19 for organ weights at 6 weeks were found to have significant multiple effects at the genome-wide level.

## 1. Introduction

The phenotypic variation of quantitative traits such as growth, body weight and obesity is controlled by genetic effects and modified by environmental factors. Knowledge about the quantitative inheritance of these genetic factors and the clarification of physiological changes resulting from genetic differences are fundamental in the determination of relationships between the gene pool and growth rate, body composition, nutrient turnover and fat storage in humans and livestock.

Various mouse strains, having undergone long-term selection for high and low body weights, have been used successfully to gain further understanding about the complex genetic architecture underlying extreme variation in body weight and growth (Brockmann & Bevova, 2002; Bunger *et al.*, 2001). The generation of inbred lines derived from divergently selected lines for the trait of interest is a valuable and powerful tool for the detection of individual genes underlying

quantitative trait loci (QTLs). One of the main advantages of inbred strains selected for high body weight is the accumulation and fixation of alleles contributing to the selection response and, therefore, the increase in the power of QTL detection. Furthermore, due to the relatively small numbers of individuals that have to be analysed, such strains provide the possibility of giving high statistical precision in QTL location without great expense (whereas the improvement in precision is limited by the lack of recombination events, as with small F<sub>2</sub> studies).

In the past, most QTL mapping experiments considering body weight, obesity, body composition and heat loss in mice focused on mice aged between 6 weeks and 8 months (reviewed by Brockmann & Bevova, 2002). There has been little investigation of growth regulation during the suckling period (1–3 weeks of age) or after weaning (3–6 weeks of age). To date, the genetic determination of murine growth at the age of 1–10 weeks has seldom been examined, for example in crosses between the mouse line LG/J selected for large body size and fatness and SM/J selected for small body size (Cheverud *et al.*, 1996, 2001; Vaughn *et al.*, 1999) and in a backcross population of wild mice and C57BL/6J (Ishikawa *et al.*, 2005).

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Potential interactions or dependencies between detected putative QTLs remain unclear. Currently little is known about the extent to which multiple-trait QTLs affect different traits such as body weights at several stages of early development or various organ weights (Leamy *et al.*, 2002; Yi *et al.*, 2006), although pleiotropy is one of the most commonly observed attributes of genes (Falconer, 1996; Otto, 2004) and is assumed to exist due to significant correlations between these traits. If traits are analysed singly, multiple effects accounting for their phenotypic variation are very likely to be detected in overlapping chromosomal regions for the different traits.

Several approaches for multiple-trait analyses have been proposed in the literature, for example by using maximum likelihood (Jiang & Zeng, 1995), logistic regression (Henshall & Goddard, 1999) or canonical transformation (Weller *et al.*, 1996; Mangin *et al.*, 1998). Knott & Haley (2000) showed that the implementation of a multiple-trait least squares method including a model for discriminating between linked QTLs and a multiple-trait QTL is straightforward and increases the power of QTL detection and location in a cross between inbred lines.

This article reports the results of a multiple-trait QTL search for age-dependent body weight during the suckling period until 3 weeks of age and the post-weaning period until 6 weeks of age, and for organ weights and body composition traits at the age of 6 weeks. The data used were previously presented in a study by Brockmann *et al.* (2004), and the method employed was multiple-trait least squares regression as proposed by Knott & Haley (2000).

## 2. Methods

### (i) Mouse lines

Mouse lines used in this study are phenotypically and genetically very different. The high body weight selected mouse line NMRI8 has been generated from the outbred strain NMRI8 at the Technical University of Munich-Weihenstephan, Freising, Germany (Butler & Pirchner, 1983). Selection has been carried out in a small population of eight breeding pairs following the strategy of mass selection at the age of 8 weeks. This age corresponds to the beginning of the adult phase of development when mice have finished their period of fast growth and are fertile. In 1995, the selection strain NMRI8 was transferred to the Research Institute for the Biology of Farm Animals, Dummerstorf, Germany. The inbred line DBA/2 is commercially available and was bought from Harlan Nederland, Horst, The Netherlands (DBA/2OlaHsd), at the age of 4 weeks. NMRI8 mice are approximately 2.5-fold heavier than animals from the unselected control line DBA/2.

### (ii) Pedigree structure

Using an F<sub>2</sub> intercross design by crossing one female of the high growth line NMRI8 to one male of the lean control inbred strain DBA/2 a pedigree was generated with a total number of 275 F<sub>2</sub> offspring by repeated mating of the parents and subsequently by repeated mating within subfamilies of seven pairs of F<sub>1</sub> offspring. The initial mating between F<sub>1</sub> individuals was performed at the age of 10 weeks and repeated after a further 6 weeks.

### (iii) Animal husbandry

Animals were reared in Macrolon cages type II with a 350 cm<sup>2</sup> floor space. All individuals were fed *ad libitum* with a breeding diet which contained 22.5% crude protein, 5.0% crude fat, 4.5% crude fibre, 6.5% crude ash, 13.5% water, 48.0% nitrogen free extract, vitamins, trace elements, amino acids and minerals (12.5 MJ/kg metabolic energy) (Altromin diet 1314, Germany). Mice had free access to water throughout the experimental period. They were maintained under conventional conditions with controlled lighting (12 h light) at a temperature of approximately 23 °C.

### (iv) Phenotypic characterization

Total body weights (bw) were measured weekly in the 275 non-fasted F<sub>2</sub> animals between the ages of 2 and 6 weeks. Organ weights and body composition traits were analysed in 6-week-old mice between 0900 and 1200 hours. After decapitation, abdominal fat weight (afw) was determined by measuring testicular fat pads in males and perimetrial fat pads in females. The abdominal fat percentage (afp) was obtained by the ratio of abdominal fat weight to total body weight. Furthermore, weights of quadriceps (musculus rectus femoris, m. vastus intermedius, m. vastus lateralis and m. vastus medialis) (muscle, mw), liver (liv), kidney (kid) and spleen (spl) were recorded. A small piece from the tail of every mouse was collected and frozen for later DNA extraction. For the characterization of the two parental lines, 14 or 15 NMRI8 mice and 9 to 18 DBA/2 individuals were analysed for their body weights at different ages and for body composition traits at 6 weeks of age as described before.

### (v) Genotypic characterization

DNA was extracted from tail tips using the QIAamp Tissue Kit (Qiagen, Hilden, Germany). The F<sub>2</sub> population of the intercross NMRI8 × DBA/2 were all genotyped for a set of 93 microsatellite markers covering all chromosomes at an average spacing of 14.1 cM (see table 1 in Brockmann *et al.*, 2004).

These markers had been screened in the parental inbred strains NMRI8 and DBA/2 and found to be

polymorphic in this intercross. The marker order was checked and a pedigree-specific marker map was built with CRIMAP (Lander & Green, 1987). Marker *D8Mit112* was reassigned to Chr 9 at 21 cM and marker *D13Mit221* to Chr 19 at 44 cM (Brockmann *et al.*, 2004) and incorporated into the map. As the map generated was consistent with the published consensus map, marker distances of the mouse genome consensus map (Dietrich *et al.*, 1996) were used for QTL mapping in the NMR18 × DBA/2 F<sub>2</sub> population. This method allowed the direct incorporation of detected QTL locations in the consensus map in The Mouse Genome Database (Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine, URL: <http://www.informatics.jax.org/>). Using the consensus map rather than the pedigree-specific map might slightly change test statistics and estimates but it does not change the results in principle.

#### (vi) Statistical methods

Animals of the mouse lines NMR18 and DBA/2, F<sub>1</sub> and F<sub>2</sub> animals were analysed for basic statistics and *t*-tests were performed to compare F<sub>1</sub> and F<sub>2</sub> animals. For a general description of the variation in the whole pedigree, means and standard deviations for all recorded traits were calculated prior to the QTL analyses. Body weight, abdominal fat weight and percentage, muscle weight and the weights of the inner organs were found to be normally distributed. Differences between sex, subfamilies (i.e. F<sub>2</sub> animals from the same pair of F<sub>1</sub> parents – seven classes), and groups of different parities (five classes) and pup sizes (number of offspring per litter categorized into three classes) were tested for every trait via two-way ANOVA with the Statistical Analysis System (SAS version 8.1). All factors were found to be significant and thus included in the multiple-trait analysis as fixed effects. Furthermore, pairwise Pearson's correlation coefficients among characters were estimated and tested for significance using the statistical package MINITAB (release 13.1).

The presence and locations of potential multiple-trait QTLs affecting body weights at different ages and for body composition and organ weights were determined using multiple-trait least squares regression (Knott & Haley, 2000). The basis of this approach is the single-trait analysis described by Haley & Knott (1992) and Haley *et al.* (1994). Briefly, the standard interval mapping model with a single multiple-trait QTL on a linkage group, including both additive and dominance genotypic effects, was fitted to the data. A likelihood ratio (LR, defined as  $2 \ln(\text{LR})$ ) was calculated for the test of a single QTL affecting at least one of the traits versus no QTL at 1 cM locations throughout the genome. If the calculated likelihood ratio

exceeded a specific threshold, then a multiple-trait QTL was inferred to be present with the most likely position being that with the highest LR.

The estimated QTL positions are given as the distance in centimorgans from the centromere. The X chromosome was analysed as a pseudo-autosomal chromosome in all analyses as all markers were located in that region.

Additionally, a scan fitting two multiple-trait QTLs was performed by a grid search at 1 cM intervals. A two-QTL model was accepted if the LR test indicated a statistically significant improvement in fit at the 5% significance level relative to the best fitting one-QTL model.

QTL estimates were revised by fitting all QTLs detected at the chromosome-wide 5% level as co-factors, i.e. considering the effect of the explanatory variables on all traits simultaneously (Zeng, 1993; Jansen, 1993).

Furthermore, if there was evidence for a QTL affecting at least two of the traits a likelihood ratio test for close linkage (one QTL affecting each trait with their locations fixed at the estimated locations from the single-trait results) versus 'pleiotropy' (one multiple-trait QTL) was carried out. The underlying question was whether a single QTL at one location accounts for the observed effects on the analysed traits, or whether there are closely linked QTLs specific for each trait.

The analysis of multiple-trait QTLs for abdominal fat weight (afw) and percentage (afp), muscle weight (mw), and the weights of liver (liv), kidney (kid) and spleen (spl) was carried out with three different approaches: first, only these six traits together; second, these six traits plus body weight at 6 weeks (bw6) as an additional trait; and third, these six traits with bw6 fitted as a covariate to examine the dependence between these traits.

To decide how many traits are affected by a given multiple-trait QTL the nominal 5% level was used (e.g. to generate Tables 3 and 4). This chromosomal threshold for the single-trait QTL may not be stringent enough but the genome-wide ones accounted for the complete genome being analysed and the multiple-trait ones accounted for all the traits (unlike single-trait analyses where one might argue for a more stringent threshold). All subsequent tests (e.g. which traits the multiple-trait QTL affected, linked QTLs, or two multiple-trait QTLs) relied on the fact that we had already found something at this stringent level.

The empirically derived significance thresholds for the one-QTL versus no-QTL test statistics were estimated with the permutation test proposed by Churchill & Doerge (1994). One thousand permutations of the data were analysed as for the unpermuted data and the following thresholds obtained: genome-wide highly significant ( $P=0.01$ ) and significant

Table 1. Phenotypic characterization of the mouse lines NMRI8 and DBA/2 and the cross population NMRI8 × DBA/2

	bw2	bw3	bw4	bw5	bw6	afw	afp	mw	liv	kid	spl
NMRI8*											
Mean	n.d.	13.50	26.00	34.64	39.39	0.93	2.26	0.44	2.78	0.62	0.20
SD	n.d.	4.52	5.22	4.19	4.98	0.32	0.54	0.05	0.29	0.06	0.03
DBA/2*											
Mean	n.d.	6.37	9.41	13.04	15.48	0.22	1.11	0.24	1.06	0.29	0.10
SD	n.d.	0.77	1.04	1.23	1.39	0.05	0.26	0.02	0.32	0.02	0.01
F <sub>1</sub> *											
Mean	5.93	10.32	19.53	27.04	31.06	0.44	1.40	0.32	1.94	0.46	0.11
SD	0.56	2.36	2.18	3.14	2.15	0.10	0.56	0.05	0.33	0.06	0.01
F <sub>2</sub> males											
Mean	5.95	8.44	15.76	23.54	28.02	0.41	1.42	0.28	1.72	0.38	0.14
SD	1.04	2.14	3.80	3.92	3.75	0.16	0.43	0.05	0.29	0.07	0.04
F <sub>2</sub> females											
Mean	6.16	8.88	14.93	20.42	22.85	0.27	1.16	0.23	1.21	0.26	0.11
SD	1.15	2.24	3.10	3.07	3.02	0.12	0.45	0.04	0.26	0.04	0.03

Trait abbreviations: bw2, bw3, bw4, bw5, bw6, body weight (g) at 2, 3, 4, 5, 6 weeks respectively; afw, abdominal fat weight (g); afp, abdominal fat percentage (%); mw, muscle weight (g); liv, liver weight (g); kid, kidney weight (g); spl, spleen weight (g).

n.d., not determined.

\* Males only.

All traits other than body weight were recorded at the age of 6 weeks.

( $P=0.05$ ) thresholds and the chromosome-wide significant ( $P=0.05$ ) levels as suggested by Lander & Kruglyak (1995). The chromosome-wide 5% significance levels were taken as genome-wide thresholds for suggestive multiple-trait QTLs. The equivalent of 1.0 LOD support intervals were obtained as the chromosomal region bounded by locations where the LR was 4.6 lower than the peak LR at the QTL position (Note that  $\text{LOD} = \text{LR}/2 \ln(10)$ ). Under asymptotic conditions this should give an approximately 97% confidence interval, although under more realistic conditions it tends to be an underestimate (Van Ooijen, 1999). For the test of two QTLs versus one QTL the significance thresholds were approximated by those obtained by a permutation test fitting one QTL versus no QTL. A parametric bootstrap as described by Knott & Haley (2000) was performed to obtain the empirical distribution for testing closely linked QTLs, each affecting a single trait, with the model of a single multiple-trait QTL. Briefly, replicate simulations were performed using the estimates for QTL effect and locations from the multiple-trait QTL model as parameters, the resulting data sets were analysed and the test statistics for linked QTLs versus a multiple-trait QTL calculated. As for the permutation test the appropriate thresholds were taken from the resulting test statistic distribution.

The proportion of variance in the F<sub>2</sub> population explained by the multiple-trait QTL was obtained for each trait separately by a comparison of the residual mean square fitting the QTL with the residual mean

square without the QTL in the presence of all the other QTLs.

### 3. Results

#### (i) Characterization of mouse lines and the cross-bred population

The phenotypic characteristics of the two parental lines and the cross-bred population are shown in Table 1. More detail is given in table 2 of Brockmann *et al.* (2004).

The high body weight selected mouse line NMRI8 was 2.5 times heavier (39 g versus 15 g) than the unselected control line DBA/2 at the age of 6 weeks. The two lines differed in body weight by 1.5, 3.2, 5.2 and 4.8 standard deviations at the ages of 3, 4, 5 and 6 weeks, respectively. The abdominal fat weight was 4.2 times higher in NMRI8 mice than DBA/2 mice at the age of 6 weeks, corresponding to a 100% increase of the abdominal fat percentage in selected mice. The weights of muscle and inner organs were about twice as high in selected than in control mice, which is consistent with the increase in body weight.

F<sub>1</sub> males were significantly heavier than F<sub>2</sub> males at all ages except at 2 weeks. In accordance with this difference in body weight at 6 weeks, the weights of muscle, liver and kidney were significantly heavier in F<sub>1</sub> than F<sub>2</sub> males, whereas the opposite relationship was found for spleen weight. Compared with the mid-parental value, the F<sub>1</sub> males had higher mean body

Table 2. Pearson's correlation coefficients for all traits analysed in the  $F_2$  population of the cross NMRI8  $\times$  DBA/2

	bw2	bw3	bw4	bw5	bw6	afw	afp	mw	liv	kid	spl
bw2	1	0.83	0.78	0.67	0.49	0.50	0.47	0.54	0.24	0.31	n.s.
bw3		1	0.80	0.67	0.48	0.50	0.47	0.57	0.22***	0.30	n.s.
bw4			1	0.87	0.67	0.65	0.57	0.75	0.45	0.55	0.15*
bw5				1	0.88	0.79	0.66	0.86	0.69	0.78	0.32
bw6					1	0.79	0.59	0.80	0.80	0.86	0.53
afw						1	0.95	0.68	0.66	0.66	0.33
afp							1	0.55	0.51	0.48	0.20***
mw								1	0.65	0.78	0.34
liv									1	0.81	0.47
kid										1	0.47
spl											1

Trait abbreviations as in Table 1.

Pearson's correlation coefficients are given for all animals independent of sex.

Significance levels are all  $P < 0.0001$  unless shown otherwise: \* $P < 0.05$ , \*\*\* $P < 0.001$ , n.s.,  $P > 0.05$ .

weights at all ages between 3 and 6 weeks, and reduced abdominal fat weights. The shifts of mean values towards the body weight of the selection line NMRI8 and towards the lower fat deposition in line DBA/2 suggest the presence of heterosis.

Correlation coefficients between all measured growth traits in the  $F_2$  population are given in Table 2. Within the  $F_2$  population, the correlations between body weights recorded at intervals of 1 week were generally 0.80 or higher. The correlation between body weight at 2 weeks and body weights at later ages continuously decreased until the age of 6 weeks, when the Pearson's correlation coefficient was 0.49. The coefficients for the correlation between body weight at 6 weeks and the weights of abdominal fat pads, muscle, liver and kidney at the same age were around 0.80. The lowest correlations were seen between spleen weight and any other trait. Generally, the mean correlation among body weights was 0.71; this was higher than the mean correlation among organ weights, which was 0.58.

#### (ii) Multiple-trait QTL locations for body weights at different ages

The chosen multitrait QTL mapping method revealed two multiple-trait QTLs for body weights at several early ages that exceeded the 1% genome-wide level: one on Chr 1 and the other on Chr 14; two QTLs that exceeded the 5% genome-wide level: on Chr 2 and Chr 7; and four QTLs that exceeded the 5% chromosome-wide significance threshold: on Chr 3, Chr 12, Chr 13 and Chr 15 (Fig. 1). The multiple-trait and single-trait QTL locations are summarized in Table 3 and more detailed results including effects are given in Supplementary Table 1.

Despite reduced stringency for the single-trait analyses (with results reported if they reached the

chromosome-wide 5% threshold) the multiple-trait QTL model detected more QTL effects although, in general, the results were very similar. The observed differences in the single-trait location estimates, in most cases, were consistent with variation in estimation and the multiple-trait model detected only one QTL. The linked QTL model was never a significant improvement over the multiple-trait model (results not shown). From the single-trait results there was a suggestion of an additional QTL on Chr 2 affecting bw6 near the end of the chromosome, but this was not picked up with the multiple-trait analyses although the test statistic profile did indicate a second peak in this region.

Of the eight QTLs detected, three affected all the body weight traits (Chr 2, 7 and 14) although the one on Chr 14 had a greater effect on intermediate weights. The one on Chr 1 had a greater effect on the post-weaning rather than suckling body weights and those on Chr 3 and Chr 15 affected early weights. The QTL detected on Chr 13 did not appear to have multiple effects as it significantly affected only body weight at 3 weeks. Chr 12 was unusual, as although a borderline significant QTL was detected with the multiple-trait QTL model, on investigation its effect on the individual traits did not reach significance (using a 5% nominal level). The QTLs affecting fewer traits tended to be those only significant at the chromosomal level.

Considering the estimated effects of the QTLs (see Supplementary Table 1) the additive effects were generally positive, meaning that the allele derived from the selected line NMRI8 had an increasing effect on body weight. The QTL on Chr 14 was unusual in having a large additive effect on all traits with the increasing allele coming from the smaller control line DBA/2. Detected dominance effects were positive, meaning that the heterozygous genotype had an

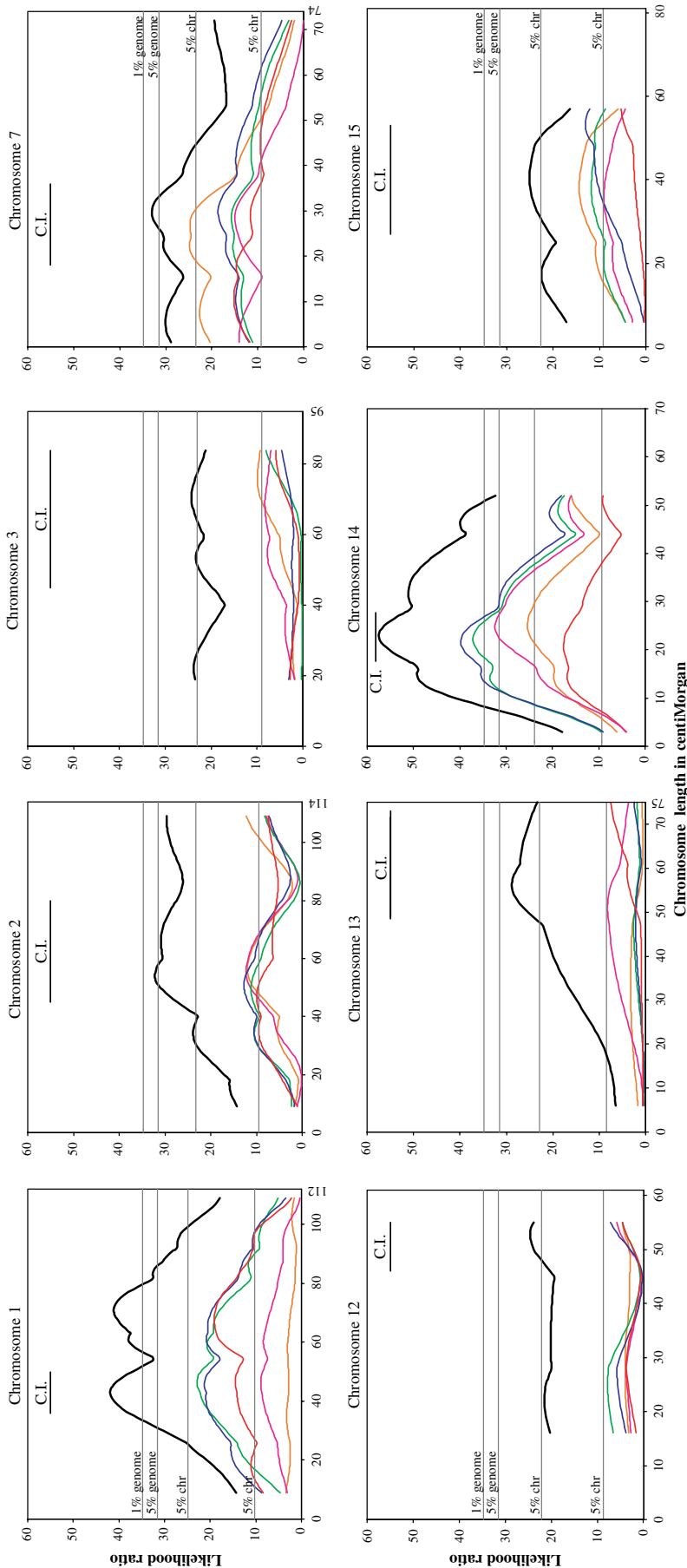


Fig. 1. Likelihood ratio curves determined by interval mapping for body weights between 2 and 6 weeks of age. Black curves indicate the likelihood ratio profiles of the multiple-trait QTLs for body weights at 2, 3, 4, 5 and 6 weeks (bw2, 3, 4, 5 and 6); orange, single QTL for bw2; green, single QTL for bw3; blue, single QTL for bw4; red, single QTL for bw5; magenta, single QTL for bw6. Top grey lines mark the genome-wide 1% significance threshold for the multiple-trait QTLs, followed by the genome-wide 5% significance threshold and the chromosome-wide 5% level. Bottom grey lines are the chromosome-wide 5% threshold for the single-trait QTLs. Black bars above the QTL peaks show the 1 LOD drop confidence interval (CI) of the multiple-trait QTLs. There was evidence for highly significant multiple-trait QTLs at the genome-wide 1% level for body weights between 2 and 6 weeks of age on Chr 1 and 14. Additional QTLs were mapped on Chr 2, 3, 7, 12, 13 and 15 at the 5% chromosome-wide significance level. The multiple-trait QTLs on Chr 7 and Chr 14 explain an equal proportion of the  $F_2$  variance in all traits.

Table 3. Summary of genomic locations of multiple-trait QTLs for body weights at different ages

Chr	Location in cM (CI)	Affected by multiple-trait QTL (cM location in single-trait analysis)				
		bw2	bw3	bw4	bw5	bw6
1	<b>43 (36–50)</b>		✓	✓ (46)	✓ (46)	✓ (68)
2	<i>54 (45–80)</i>	✓ (109)	✓ (55)	✓ (50)	✓ (51)	✓ (47)
3	70.5 (44.5–83.5)	✓ (75.5)	✓			
7	29.5 (17.5–35.5)	✓ (21.5)	✓ (27.5)	✓ (27.5)	✓ (29.5)	✓ (10.5)
12	53 (46–55)					
13	56 (49–73)		✓			
14	<b>23 (19–27)</b>	✓ (25)	✓ (25)	✓ (22)	✓ (22)	✓ (21)
15	40.4 (27.4–53.4)	✓ (38.4)	✓ (36.4)	✓ (38.4)	✓ (53.4)	

Trait abbreviations as in Table 1.

Chr, chromosome; cM, centimorgan; CI, confidence interval (1 LOD drop).

The most likely genomic locations are given as distances from the centromere. Highly significant QTLs at the 1% genome-wide level are given in **bold** type and significant QTLs at the 5% genome-wide level are given in *italic*; others are significant at the 5% chromosome-wide level. A tick indicates that the trait is significantly affected by the multiple-trait QTL. Values in parentheses are location estimates from the single-trait analyses where significance was attained at the 5% chromosome-wide threshold.

increasing effect on body weight compared with the mid-homozygote effect. The regions on Chr 1, 7 and 15 had predominantly additive effects, while the effects of the QTL on Chr 2 were mainly detected through the heterozygous individuals. QTLs with the largest additive effects of about 1 g on the later weight traits (bw4, bw5 and bw6) were found on Chr 1, 7, 14 and 15. QTLs on Chr 2 and 14 had the largest dominance effects of 1.4–2.3 g in the later traits.

### (iii) Multiple-trait QTL locations for body composition and organ weights at 6 weeks with body weight at 6 weeks as a trait

Multiple-trait QTLs for body composition and organ weights at the age of 6 weeks were analysed together with bw6 as a trait and those that exceeded the 1% genome-wide threshold were found on Chr 1, 2, 4, 7, 11, 14, 17 and 19; QTLs that exceeded the 5% genome-wide level on Chr 5, 12 and 13; and QTLs that exceeded the 5% chromosome-wide level on Chr 3, 9 and 15. Fig. 2 gives the test statistic profiles for these significant chromosomal regions and Table 4 summarizes the locations compared with the single-trait analyses (additional information can be found in Supplementary Table 2).

Compared with the body weight traits the pattern of effects over the traits of the QTLs affecting these body composition and organ weight traits varied to a greater extent. Of the 14 QTLs detected, five (on Chr 2, 4, 7, 14 and 17) showed multiple effects on both organ and body composition traits, whereas one (Chr 1) appeared to be organ-specific and two (Chr 9 and Chr 19) were body-composition-specific. QTLs on four of the chromosomes affected only one trait (Chr 3, 11, 12 and 13). The QTL on Chr 5 affected

bw6 as well as spleen weight. The multiple effect on Chr 15 was unusual in not affecting abdominal fat weight (afw) but only the ratio of fat weight to total body weight as well as kidney and spleen weights.

For Chr 12 and Chr 14, when the multiple-trait QTL model was tested against a model with each trait having a QTL at the location estimated from the single-trait analyses (i.e. a model minimizing the diagonal of the residual SS matrix), the simple multiple-trait QTL model was rejected, suggesting more than one QTL was present on the chromosome. Looking more closely at the estimates, the results for Chr 12 were still consistent with a QTL affecting only spleen weight. Chr 14 appeared to be more complicated, with perhaps a QTL affecting bw6, the fat traits, muscle weight and possibly kidney weight and liver, and another affecting spleen weight further along the chromosome which would be supported by the differing direction of effect for the QTL on spleen weight compared with the other traits (see Supplementary Table 2). A second QTL affecting spleen weight was picked up in the single-trait analyses on Chr 2, with a QTL in this region affecting bw6; however, a model with two multiple-trait QTLs was not significantly better than a single multiple-trait QTL.

In most cases the allele derived from the selected line NMRI8 increased the trait, as expected given the differences observed in the original lines. There were, however, a number of exceptions: the QTLs affecting spleen weight on Chr 5 and Chr 12, although the QTLs on Chr 5 affected bw6 in the expected direction; the QTLs affecting only muscle weight on Chr 13; and the multiple-trait QTLs on Chr 14, 15, 17 and 19. The observations for the QTLs on Chr 14 were consistent with those observed for body weights, except for the spleen which was affected in the opposite direction.

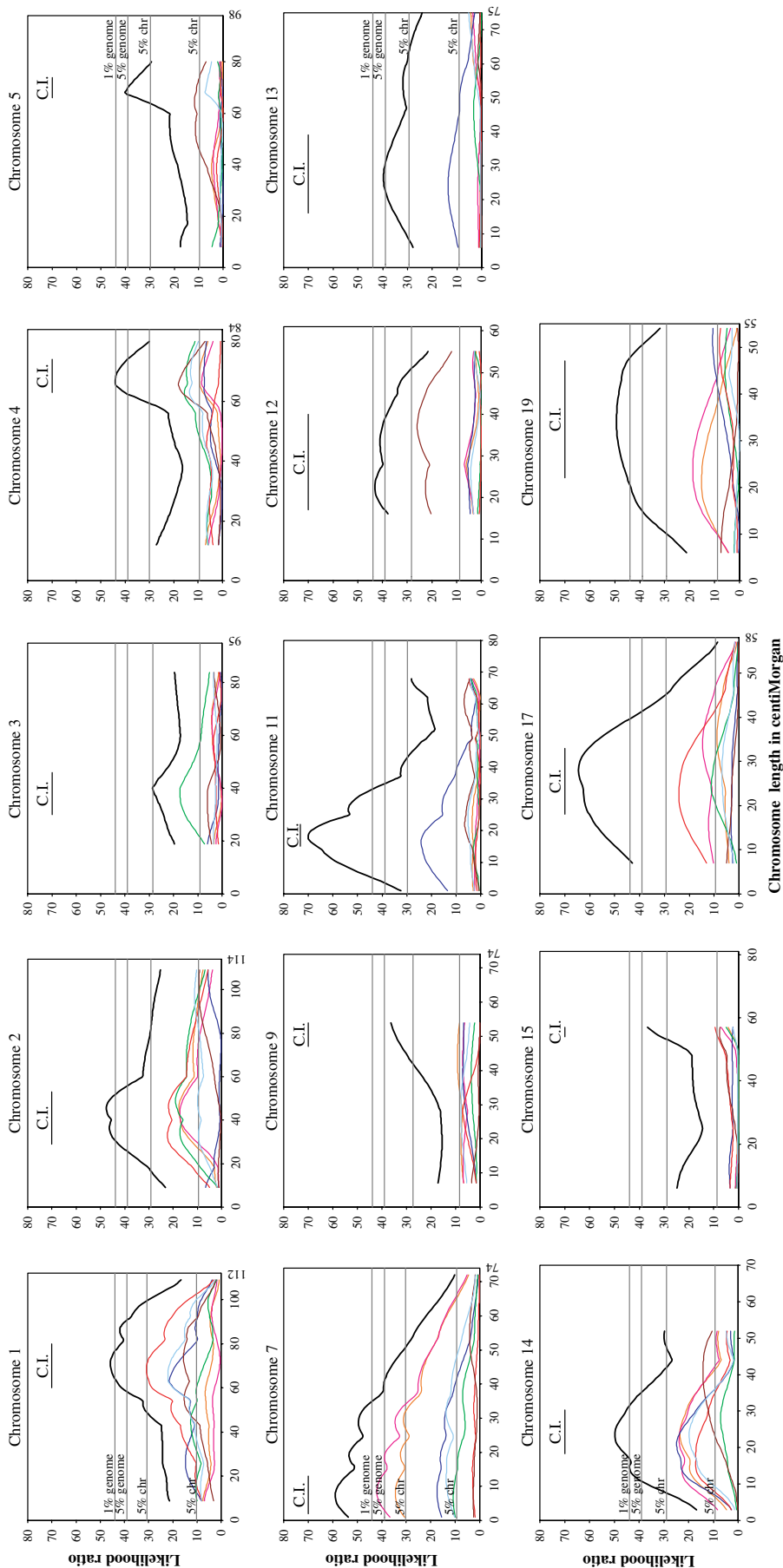


Fig. 2. Likelihood ratio curves determined by interval mapping for body composition traits, organ weights and body weight at 6 weeks of age. Black curves indicate the likelihood ratio profiles of the multiple-trait QTLs for body composition traits, organ weights and body weight; orange, single QTL for abdominal fat weight (afw); magenta, single QTL for abdominal fat percentage (afp); blue, single QTL for muscle weight (mw); green, single QTL for liver weight (liv); red, single QTL for kidney weight (kid); brown, single QTL for spleen weight (spl); light blue, single QTL for body weight at 6 weeks (bw6). Thresholds as in Fig. 1. Multiple-trait QTLs for at least two of the recorded traits were found on Chr 1, 2, 4, 7, 14, 17 and 19. The multiple-trait QTLs had effects of different magnitude on the traits.



Table 4. Summary of genomic locations of multiple-trait QTLs for body composition, organ weights and body weight at 6 weeks

Chr	Location in cM (CI)	Affected by multiple-trait QTL (cM location in single-trait analysis)						
		bw6	afw	afp	mw	liv	kid	spl
1	<b>71 (60–80)</b>	✓ (65)			✓	✓	✓	✓
2	<b>45 (29–53)</b>	✓ (97)	✓ (45)	✓ (43)		✓ (49)	✓ (33)	(100)
3	39.5 (29.5–45.5)					✓ (37.5)		
4	<b>67.1 (63.1–74.1)</b>	✓ (63.1)	✓	✓	✓	✓		✓
5	68 (66–74)	✓						✓ (65)
7	<b>6.5 (1.5–12.5)</b>	✓ (31.5)	✓ (7.5)	✓ (7.5)	✓ (7.5)	✓ (6.5)		
9	54 (47–54)		✓ (43)	✓	✓			
11	<b>18 (15–22)</b>				✓ (16)			
12	22 (17–40)							✓ (37)
13	27 (16–39)				✓ (24)			
14	<b>23 (18–30)</b>	✓ (23)	✓ (24)	✓ (22)	✓ (21)	✓	✓ (15)	✓ (41)
15	57.4 (54.4–57.4)			✓			✓ (57.4)	✓
17	<b>27.5 (17.5–32.5)</b>	✓	✓	✓ (33.5)		✓ (23.5)	✓ (21.5)	
19	<b>34 (22–47)</b>		✓ (20)	✓ (24)	✓ (51)			

Abbreviations as in Tables 1 and 3.

Explanations as in Table 3.

For Chr 15 and Chr 17, the affected body composition traits are also in the opposite direction to that expected whilst the effects on organ weights and body weight were as expected. For Chr 19 the QTL allele from NMRI8 increased the fat traits but decreased muscle weight, which could make physiological sense in terms of muscle degradation due to fatness, as fatter mice tend to be inactive and therefore their muscle mass may decrease.

As for the body weight traits, the majority of QTLs acted predominantly in an additive way. The QTL on Chr 15 also had a significant dominant effect on all the traits it affected and the QTLs on Chr 1, 14 and 17 had significant dominant effects on some of the traits. The QTL on Chr 2 was mostly additive in action whereas on the body weight traits it was dominant (with heterozygotes having a larger effect than homozygotes).

#### 4. Discussion

The aim of this study was to understand more precisely the cause of the genetic correlation between body weights at different early ages and organ weights at 6 weeks, and whether this is due to multiple-trait QTLs affecting these recorded traits. An additional objective was to explore whether the same genes controlling body weight also affect organ weights. Models were considered with one or two multiple-trait QTLs affecting the traits. In a cross between the high body weight selected mouse line NMRI8 and the lean inbred line DBA/2, chromosomal regions were determined with multiple effects on different linkage groups. In the analysis of body weights from 2 to

6 weeks of age regions on Chr 1 (36–50 cM), 2 (45–80 cM), 7 (17.5–35.5 cM) and 14 (19–27 cM) were found to be significant at the genome-wide level; for body composition traits and inner organ weights at 6 weeks together with bw6 as a trait on Chr 1 (60–80 cM), Chr 2 (29–53 cM), Chr 4 (63.1–74.1 cM), Chr 5 (66–74 cM), Chr 7 (1.5–12.5 cM), Chr 11 (15–22 cM), Chr 12 (17–40 cM), Chr 13 (16–39 cM), Chr 14 (18–30 cM), Chr 17 (17.5–32.5 cM) and Chr 19 (22–47 cM). The results obtained in this study were very consistent with the single QTL locations reported by Brockmann *et al.* (2004), which had been the impetus for this study looking for the presence of multiple-trait QTLs. As expected from the high phenotypic correlations between body weights at several early ages and between organ weights, many of the single QTLs identified were located in overlapping chromosomal regions in the Brockmann *et al.* (2004) study and, therefore, were likely to be due to a single QTL with effects on multiple traits.

*QTLs for body weights.* Body weight at different stages of development has been the subject of numerous QTL mapping studies because it is one of the easiest characters to measure. Therefore, many QTLs for growth-related traits are known (reviewed by Brockmann & Bevova, 2002 or Corva & Medrano, 2001; Rocha *et al.*, 2004a). However, in most studies mice at later ages than ours were examined or measurements were not made at weekly intervals, which makes a comparison with our data problematic. In contrast, Ishikawa *et al.* (2005) reported a single QTL on Chr 2 affecting the whole investigated growth phase of 3 to 10 weeks. This chromosomal region overlapped with the multiple-trait QTL which

affected all five body weights in our experiment. Chr 2 in general seems to play a major role in murine growth and fatness. Jerez-Timaure *et al.* (2005) were able to fine map a region on distal Chr 2 between 78.1 and 82.4 cM harbouring a QTL for bw9, bw12 and bw15. The multiple effect we detected lay in a support interval between 45 and 80 cM influencing bw2–6. Further concordance could be found with the findings of Cheverud *et al.* (1996) for a region on Chr 7 at approximately 30 cM affecting body weight at 1–10 weeks. Our multiple-trait QTL was located at 29.5 cM, explaining a high proportion of the observed phenotypic variance of all five recorded weights. These findings suggest a chromosomal region with a predominantly additive effect on the whole pre-adult growth phase in mice. In addition, we confirmed the observations of Ishikawa *et al.* (2005) and Vaughn *et al.* (1999) that postnatal and later growth are regulated in part by different genes. The multiple effect on Chr 1 was predominantly made up of body weight at 4, 5 and 6 weeks, while on Chr 3 only bw2 and bw3 contributed significantly to the detected effect.

In order to remove some of the dependence between traits due to the fact that, for example, bw5 is part of bw6, we analysed body weight gains between 2 and 3 weeks, 3 and 4 weeks, and so on. This approach, however, was not appropriate for our data due to the frequent measurements (i.e. small expected weight gains) combined with the imprecision of the weight measurement. In addition, environmental factors such as food intake or defecation shortly before the measurement could explain a very high proportion of the variation of body weight gains. Nevertheless, there are techniques for the genetic analysis of longitudinal traits (Ma *et al.*, 2002; Wu *et al.*, 2004; Macgregor *et al.*, 2005) that could be applied to our data in the future.

*QTLs for body composition and organ weights.* Because fat tissues, muscles and inner organs contribute to overall body weight, and body weight at 6 weeks and organ weights are significantly correlated (Table 2), we expected to find chromosomal regions that have multiple effects on body *and* organ weights at 6 weeks. QTL mapping results for all these single traits separately (determined by Brockmann *et al.*, 2004) led us to the hypothesis of co-segregation of loci that account for body weight variation and variance in fat weight, muscle weight, and weights of liver, kidney and spleen.

We chose to present the results of the analyses that jointly fitted the QTL to bw6 along with the organ and body composition traits. Knowledge of physiology and previous results would suggest that we may detect QTLs that have a generalized effect on growth – increasing both body weight and organ weights (although possibly to different extents). Additionally

we may detect QTLs that have a specific effect on one or two of the organs because of different patterns and control of development. We can identify both these types of QTL through our analyses, although we cannot determine causal relationships. Including bw6 in the analysis will provide more power to detect QTLs that have a multiple effect on bw6 and the other traits.

An alternative approach would have been an analysis with bw6 as a covariate. This would have the effect of standardizing for body weight such that we would be comparing the other traits at a constant body weight. As body weight is itself genetically controlled, however, this makes interpretation of the results more difficult: the covariate standardizes the phenotypes but not the genotypic effect at any QTL; hence, as the correlation between the effects at any QTL affecting body weight and the phenotypes is not 1 we may still pick up a QTL that affects body weight (i.e. we will detect QTLs which affect bw6 differently to the other traits in the analysis). For comparison, Table 5 contrasts the significant (at the 5% chromosome-wide level) results of different approaches, i.e. analysis of body composition traits and organ weights without bw6, with bw6 as a trait and with bw6 as covariate.

We would have hoped that including bw6 would increase the number of QTLs detected if they had a multiple effect on bw6 and the organ weights. However, this is not clearly the case. We assumed also that if a QTL affected bw6 as well as other traits when including it in the analysis we should have seen greater power and smaller confidence intervals (CIs). It is difficult to evaluate the greater power. It mostly caused a reduction in CIs (not Chr 1, 14 and 17) but the smaller CIs may have been affected by the problem of effects in different directions.

One might also expect the results with bw6 as a covariate to be most different as these may not pick up QTLs which have the same effect on the organ weights and bw6 because evidence is lost when fitting bw6 as a covariate. This model with adjustment for body size is effectively looking for QTLs which have differing magnitude of effects on the different traits. This may be a reasonable explanation for Chr 4 but not for Chr 3 where only liver is significant. Overall the results are surprisingly very similar.

Leamy *et al.* (2002) analysed organ weights at 12 weeks in the mouse cross M16i (rapid growth rate) × CAST/Ei (wild). They considered heart weight in addition to liver, kidney and spleen. Although a number of the same chromosomes were picked up as having significant QTLs (Chr 1, 2, 4, 5, 11 and 19), these QTLs are not necessarily being picked up as affecting the same traits. Some discrepancies could be due to the fact that different sets of traits were analysed, i.e. Leamy *et al.* (2002) included heart weight whereas in

Table 5. Comparison of genomic locations of significant multiple-trait QTLs for organ weights analysed with different approaches

Chr	Only organ weights			Organ weights with bw6 as trait			Organ weights with bw6 as covariate		
	cM	CI	LR	cM	CI	LR	cM	CI	LR
1	70	60;79	45.7	71	60;80	45.8	87	70;95	31.7
2	34	26;53	44.2	45	29;53	47.5	36	28;49	47.2
3	39.5	32.5;44.5	28.8	39.5	29.5;45.5	28.6	–	–	–
4	66.1	62.1;74.1	43.0	67.1	63.1;74.1	44.0	–	–	–
5	68	60;75	28.9	68	66;74	40.2	68	64;73	34.3
7	6.5	0.5;12.5	56.8	6.5	1.5;12.5	59.0	5.5	0.5;11.5	47.0
9	54	48;54	39.1	54	47;54	36.4	54	45;54	28.0
10	34	27;51	27.8	–	–	–	–	–	–
11	18	12;21	65.6	18	15;22	70.1	17	13;20	77.0
12	23	16;38	37.7	22	17;40	43.0	36	29;44	41.9
13	26	16;37	40.7	27	16;39	39.7	28	18;40	43.3
14	23	18;29	50.4	23	18;30	49.8	21	10;29	28.6
15	57.4	54.4;7.4	30.9	57.4	54.4;57.4	36.6	57.4	54.4;57.4	36.7
17	27.5	17.5;32.5	62.9	27.5	17.5;32.5	64.4	19.5	12.5;31.5	57.2
19	38	28;48	50.7	34	22;47	49.3	30	20;39	47.2

Abbreviations as in Tables 1 and 3.  
LR, likelihood ratio, defined as  $2 \ln(\text{LR})$ .

this study fat traits, body and muscle weight are included. For example the QTL detected by Leamy *et al.* (2002) on Chr 3 had largest effects on heart weight. Additionally the correlation structure between traits does affect the ability of the multiple-trait QTL model to detect QTLs, so the differing traits combinations may influence the results even for traits in common in the analyses.

Kenney-Hunt *et al.* (2006) presented another study on multiple-trait QTLs for body size components in an  $F_2$  population of Large (LG/J)  $\times$  Small (SM/J) mice. They identified chromosomal regions affecting four organ weights (liver, kidney, spleen, heart) and four long bone lengths. But again, due to the different set of analysed traits, we can hardly find concordance with our results. Only the QTL *BOD14.1* located at 22 cM (CI 6–32 cM) and affecting body weight, liver and kidney showed some similarities with our QTL on Chr 14. However, Kenney-Hunt *et al.* (2006) showed also that some of their pleiotropic loci affect only one single trait, a finding we can support (Chr 11, 12 and 13). They argue that a variety of genes with different functions could be expressed very specifically in single organs and thus lead to the different pleiotropic patterns.

A general observation we can make is that the majority of multiple-trait QTLs found had effects on only a few traits. In the analysis of body weights three cases (Chr 2, 7 and 14) were seen where all recorded characters were influenced by the same locus, but that was an exception rather than the rule. This not unexpected phenomenon presumably resulted from the

significant but not very high correlations, especially between organ weights. As suggested by Leamy *et al.* (2002) inner organ weights follow a common growth pattern, i.e. bigger animals have mostly bigger and thus heavier livers or kidneys. This would be likely to be picked up as a multiple-trait QTL, but it would still be anticipated that because of the distinct functions of these inner organs, genes could be detected that specifically affected one or more of them in the absence of an effect on growth, and vice versa. Nevertheless, although organs have diverse functions they are developmentally related and thus could be regulated together to some extent. For instance, skeletal muscle, kidney and spleen are formed of the germ layer mesoderm, whereas the liver originates from the endoderm. On the one hand, the effect predominantly on the weights of muscle, kidney and spleen we detected on Chr 1 and 14 could be explained in part by a common regulation of their development and growth (e.g. via transcription factors). On the other hand, the multiple effects on liver and kidney (and spleen) on Chr 2 and 17 (and Chr 4) could result from their joint role as storage and detoxication organs. These three inner organs are involved in protein catabolism and therefore belong to a functional unit.

But, for all that, generally we can infer that co-localization of detected QTLs for correlated characters is not a definitive indication for only one gene in the region controlling all these phenotypes. This is also the reason why we tried to avoid the term ‘pleiotropic QTL’ for what we have mapped with our approach. We used the term ‘multiple-trait QTL’ to indicate

that the detected QTL was affecting more than one trait in the analysis. It is therefore dependent on the traits measured. However, it is clear from these results that some QTLs have a more general effect on body composition over time whereas others are more specific in their effect. Multiple-trait QTLs that we identified but which have not been found in other studies (which due primarily to the situation that, to our knowledge, there are few studies on multiple-trait QTLs for organ weight) have to be confirmed in independent populations to determine that they are real effects and to investigate whether these loci are important in populations other than this special  $F_2$  of  $NMRI8 \times DBA/2$ .

In conclusion, this study investigating the extent of multiple-trait QTLs affecting body and organ weights was one of the first of its kind in mice. We have successfully identified a number of multiple-trait QTL regions that can affect both growth at all or some of the ages considered and organ weights. The chromosomal regions identified here have to be revised and fine-mapped in further analyses and, more importantly, in a population other than an  $F_2$  to get insights into the genetic architecture of these complex traits and to understand the genetic cause of their correlations.

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