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Mapping quantitative trait loci for body weight on the X chromosome in mice. I. Analysis of a reciprocal F_2 population

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Summary

Evidence of a large sex-linked effect accounting for 25% of the divergence between mouse lines selected for body weight has been described previously. A marker-based study was undertaken to determine the number and map positions of the putative X-linked quantitative trait loci (QTLs). An F_2 population was generated from a reciprocal F_1 between an inbred low line derived from the low selection line and the high selection line. To enable inference of marker-associated QTL effects on the X chromosome, an analytical technique was developed based on the multiple regression method of Haley and Knott. The analysis of data on 10 week weight indicated a single QTL of large effect situated at about 23 cM from the proximal end of the chromosome, with a peak LOD score of 24·4. The likelihood curve showed a single well-defined peak, and gave a 95% confidence interval for the QTL location of 8 cM. The estimates for the additive genotypic effects in males and females (half the differences between hemizygous males and between homozygous females) were 2·6 g in both cases, or 17% and 20% of the 10 week body weight in males and females respectively. Dominance effects in the females were found to be non-significant. No significant X-linked effect on carcass fat percentage was detected, but a single X-linked QTL appears to explain almost the entire X-linked body weight effect.

1. Introduction

Selection for quantitative traits has been performed effectively since domestication with little knowledge of the actions of individual genes and their interactions. Although loci with relatively large effects may be present, approaches to the utilization of quantitative genetic variation, usually by mass selection, have been effective as a result of the relative insensitivity of the methodology to failures in assumptions of the models. However, identification of individual quantitative trait loci (QTLs) may lead to improvements in the methodologies used in selective breeding. The discovery of an abundance of molecular genetic markers, for example microsatellites, has provided the opportunity to resolve quantitative genetic variation into individual loci and to understand the genetic basis of segregating variation in nature and artificial populations.

The selection lines investigated in this study have been divergently selected for body weight for more than 50 generations. Evidence from reciprocal crossing experiments between the high and low selection lines has strongly suggested that a large additive X-linked effect accounts for approximately 25% of the total divergence (Hastings, 1990; Hastings & Veerkamp, 1993; Veerkamp *et al.*, 1993; Rance *et al.*, 1994). In a reciprocal cross (e.g. H × L and L × H, female parent first), a difference in mean performance between the reciprocal crosses in males which is not present in females is indicative of the presence of X-linked factors. A reciprocal cross is a powerful method for detecting the effects of X-linked genes because male progeny inherit their X chromosome from their mother, whereas female progeny inherit one X from each parent. For example, in an experiment by Hastings & Veerkamp (1993), the mean differences in 10 week body weight between reciprocal crosses were 7·5 g and 0·9 g in males and females, respectively. The purpose of the present study is to map QTLs influencing body weight on the X chromosome in these mouse lines, with the objective of estimating the number of X-linked QTLs, the sizes of their effects and their map position(s). The analysis of data from

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an F_2 population was carried out using the multiple regression QTL mapping procedure (Haley & Knott, 1992) modified to account for X-linkage and for partially informative markers.

2. Materials and methods

(i) *The mapping population*

The origins of the mouse lines used in this study (the P lines), which have been divergently selected for body weight for more than 50 generations, have been described previously (Sharp *et al.*, 1984; Beniwal *et al.*, 1992). In brief, the base population was an F_1 derived from two inbred lines (JU and CBA) crossed to an outbred (CFLP). There were initially three replicates in each direction of selection, and males were selected at 10 weeks of age on an index of lean mass. After 20 generations of selection the three replicates within each selection direction were crossed to form the P6 lines, and the selection criterion changed to body weight at 10 weeks in both sexes. The mapping population consisted of a reciprocal F_1 population between the P6 high selection line at generation 52 of selection and a P6 inbred low line at generation 7 of inbreeding (established at generation 45 of selection and maintained by brother–sister mating). Attempts to produce a P6 high inbred line had been unsuccessful.

Fourteen matings between the high selection line and inbred low line were set up, with a total of seven families in each reciprocal half (female high (H) × male low (L), and female L × male H). An F_2 population was bred comprising 18 families, with parents of each mating from different halves of the F_1. Matings were maintained for up to three parities, yielding a total of 340 F_2 offspring surviving to 10 weeks. Male and female progeny were separated at weaning (3 weeks of age), and maintained in cages with up to 10 individuals per cage.

Body weights at 3, 6 and 10 weeks of age were recorded in the F_2 generation. To obtain information on fatness, animals were killed, and gonadal fat pads (GFP) removed from all F_2 males, weighed and then replaced in the body cavity. These were used to produce estimates of carcass fat percentage (Sharp *et al.*, 1984) according to

$$\text{GFP\%} = \left[\left(\frac{\text{GFP weight}}{\text{dead weight}}\right) \times 8\right] \times 100.$$

Individuals were freeze-dried, and the dry matter content used to provide a second estimate of carcass fat percentage as described by Hastings & Hill (1989):

$$\text{FDW\%} = \left[\left(\frac{\text{freeze-dried weight}}{\text{dead weight}}\right) \times 1{\cdot}13 - 0{\cdot}32\right] \times 100.$$

Full records (genotypic and phenotypic data) were available on a total of 334 animals. Genotypes were available for all grandparental and parental (F_1) individuals which contributed to the F_2.

(ii) *Microsatellite genotyping*

Genomic DNA was extracted from spleen tissue by phenol:chloroform extraction. Individuals were genotyped at *Mit* microsatellite markers (Research Genetics, USA) by polymerase chain reaction (PCR) based on the protocol described by Dietrich *et al.* (1992). Products were run on 20 cm long, vertical running 6% polyarcylamide gels for 3–4 h at 155 V. Gels were photographed under ultraviolet light after ethidium bromide staining.

The high selection line and inbred low line parents were genotyped at a total of 61 marker loci, yielding 13 polymorphic marker loci. All individuals in the pedigree were genotyped at the polymorphic marker loci. Genotypes were scored on two separate occasions to minimize incorrect genotypes.

(iii) *Regression analysis to estimate QTL effects in F_2*

To date, statistical methods for mapping QTLs in segregating populations have been developed almost exclusively for autosomal data (Lander & Botstein, 1989; Haley & Knott, 1992; Martinez & Curnow, 1992; Jansen, 1993, 1996; Zeng, 1994). The X chromosome is hemizygous in males, so specific modifications to the methodology are needed for mapping X-linked QTLs.

To estimate effects of X-linked QTLs, marker data and phenotypic data from the F_2 population were analysed using a modified version of the multiple regression method of Haley & Knott (1992). Initially, a cross between two inbred lines was considered, but the method was further developed for a cross between two non-inbred lines. The QTL was assumed to be fixed for different alleles in the high and low line throughout. The basic method considers a reciprocal cross between two inbred lines, with a QTL (Q) positioned between two co-dominant flanking markers (A and B). The two inbred lines were assumed to carry different alleles at all three loci. A reciprocal f_2 cross produces five possible QTL genotypes: three female genotypes (Q_2Q_2, Q_1Q_2 and Q_1Q_1) and two male hemizygous genotypes (Q_2 and Q_1). The phenotypes of the five QTL classes were assumed to have the same variance and to be normally distributed or could be transformed to be so. The mean genotypic effects in the F_2 (with additive terms estimated in males and females separately) were assumed to be $m_f + a_f$ for Q_1Q_1, $m_f + d$ for Q_1Q_2, $m_f - a_f$ for Q_2Q_2, $m_m + a_m$ for Q_1, and $m_m - a_m$ for Q_2, where m_f and m_m are population means in females and males respectively, a_f is the additive deviation in females (half the difference between the homozygotes), a_m is

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Table 1. *Expectations of mean genotypic effects of an X-linked QTL for all possible marker genotypes in a reciprocal F_2 population*

Sex	Marker genotype	Coefficients of: *a* (additive effect)	Coefficients of: *d* (dominance deviation)
Male	A_1B_1	$[(1-r_A)(1-r_B)-r_A r_B]/(1-r)$	—
	A_1B_2	$[(1-r_A)r_B-r_A(1-r_B)]/r$	—
	A_2B_1	$-[(1-r_A)r_B-r_A(1-r_B)]/r$	—
	A_2B_2	$-[(1-r_A)(1-r_B)-(r_A r_B)]/(1-r)$	—
Female (A_1B_1 male F_1 parent)	$A_1A_1B_1B_1$	$[(1-r_A)(1-r_B)]/(1-r)$	$r_A r_B/(1-r)$
	$A_1A_1B_1B_2$	$[(1-r_A)r_B]/r$	$[r_A(1-r_B)]/r$
	$A_1A_2B_1B_1$	$[r_A(1-r_B)]/r$	$[(1-r_A)r_B]/r$
	$A_1A_2B_1B_2$	$r_A r_B/(1-r)$	$[(1-r_A)(1-r_B)]/(1-r)$
Female (A_2B_2 male F_1 parent)	$A_1A_2B_1B_2$	$-r_A r_B/(1-r)$	$[(1-r_A)(1-r_B)]/(1-r)$
	$A_1A_2B_2B_2$	$-[r_A(1-r_B)]/r$	$[(1-r_A)r_B]/r$
	$A_2A_2B_1B_2$	$-[(1-r_A)r_B]/r$	$[r_A(1-r_B)]/r$
	$A_2A_2B_2B_2$	$-[(1-r_A)(1-r_B)]/(1-r)$	$r_A r_B/(1-r)$

the additive deviation in males (half the difference between the hemizygotes), and d is the dominance deviation in females only. The recombination distance between A and Q was r_A, and between Q and B was r_B. The recombination fractions between the two markers was r, which was assumed to be known, or could be calculated from marker data prior to the analysis. Haldane's (1919) mapping function, which assumes no crossover interference, $r = 0{\cdot}5(1-\exp(-2x))$, was used to convert distances (x) in morgans to recombination fractions, and $r = r_A + r_B - 2r_A r_B$. The expected means of the F_2 individuals for each flanking marker genotype can be derived in terms of recombination fractions between markers and putative QTL genotypes. The calculations of expected marker genotype means for the putative QTL were considered for male and female parents separately, in order to account for the hemizygous state of the X chromosome. In the case of females, the two types of the reciprocal cross also require to be considered separately. The expected mean performance of a given F_2 marker genotype was obtained by summing over the QTL genotypes, and scaling by the total frequency. The coefficients of a_m, a_f and d in terms of recombination fractions for each of the 12 possible flanking marker combinations (4 male, and two sets of 4 female) are shown in Table 1. In analysing the F_2 males the statistical model is the same as for a backcross. The numerical values of the coefficients of a_f, a_m and d were calculated for each flanking marker genotype. For a given marker interval, regressions of the phenotypic value onto the numerical values of the coefficients of the additive and dominance terms were carried out at putative QTL positions (i.e. 1 cM intervals all along the chromosome), to obtain estimates of additive and dominance genetic deviations. The regression calculations were made using Genstat 5.3 (Genstat 5 Committee, 1993). Effects of sex, litter and number born, and a litter size covariate, were included in the model. A test statistic (TS) was used to obtain a likelihood curve for the QTL position along the chromosome. The test statistic used has been shown to provide a very close approximation to the likelihood ratio test (Haley & Knott, 1992):

$$\mathrm{TS} = n\log_e(RSS_{\mathrm{reduced}}/RSS_{\mathrm{full}}),$$

where n is the number of individuals in the analysis, RSS_{reduced} is the residual sum of squares of the regression with no QTL parameters fitted, and RSS_{full} is the RSS with the QTL parameters fitted. This test statistic is asymptotically distributed as χ^2 with degrees of freedom (d.f.) equal to the d.f. full model minus d.f. reduced model. In the model described above, in which the numerical values of the coefficients of a_m, a_f and d are fitted, the number of d.f. is 3. The null hypothesis used in the analysis was that there was no QTL in the marker interval tested. Simulation studies have shown that the X-linked regression analysis provides mean estimates of QTL effects and positions close to those simulated (Rance, 1996).

The mouse lines investigated in this study show considerable segregation within lines at marker loci (Table 2), so the analytical method described above was adapted to enable analysis of an F_2 population where there was segregation at marker loci within the grandparental lines. An F_1 male whose female parent is heterozygous for a marker produces fully informative F_2 genotypes, because the F_1 male transmits its X chromosome intact. However, non-informative marker genotypes in F_2 male or female progeny occur in cases where an F_1 female marker allele could have come from either of the F_2 grandparents. Consider a point x on the chromosome. For each F_2 individual, the closest informative proximal and distal markers to x were used to calculate coefficients of a_m or a_f and d based on map distances from the markers and x (Table 1). Because markers with 100% information on all individuals were available from position 12·2 cM and to 60·9 cM (Table 2), for x lying between these positions it was always possible to find fully in-

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Table 2. *Proportion of individuals with full records (genotypic and phenotypic data) in the F_2 population for segregating* DXMit *marker loci*

Marker	Map position[a]	Percentage of informative individuals
DXMit55	0·0	25·1
DXMit187	12·2	100
DXMit50	19·8	100
DXMit46	30·4	53·6
DXMit25	33·7	40·4
DXMit62	34·6	35·9
DXMit16	41·9	28·4
DXMit64	48·9	47·3
DXMit79	52·6	28·7
DXMit38	56·0	44·3
DXMit13	60·9	100
DXMit121	71·4	15·6
DXMit31	72·0	15·6

[a] Map positions of markers were estimated using CRIMAP.

formative flanking markers. Outside the region bracketed by fully informative markers, i.e. at the ends of the chromosome, it was necessary for some individuals to use the nearest informative marker to calculate coefficients appropriate for a single marker regression analysis. Thus, information from all the F_2 individuals was used at each point on the chromosome. The marker map positions were estimated from the F_2 marker data, using CRIMAP, accounting for X-linkage.

Empirical significance thresholds for QTL detection were obtained by simulating 1000 replicates of trait data with no QTL segregating, with phenotypic data sampled from a normal distribution with mean of zero and variance of one, and with the pedigree and marker genotypes from the F_2 population (as discussed by Andersson *et al.*, 1994). By ordering the maximum TS obtained from each replicate analysis and taking the 99, 95 and 90 percentiles, the thresholds for $P < 0{\cdot}01$, $P < 0{\cdot}05$ and $P < 0{\cdot}10$ were obtained under the null hypothesis of no QTL segregating in the population. The estimated empirical LOD score thresholds for the one QTL model were: 3·3 for $P < 0{\cdot}01$, 2·5 for $P < 0{\cdot}05$ and 2·1 for $P < 0{\cdot}10$.

3. Results

(i) *Polymorphic markers in the F_2*

All animals (with full records) in the F_2 pedigree were genotyped at the 13 polymorphic markers. Table 2 summarizes the distribution of fully informative markers along the chromosome. The percentage information at each marker is given (determined by the percentage of individuals in the F_2 generation where marker information could be traced unequivocally to the base population). Markers *DXMit187*, *DXMit50* and *DXMit13* were fully informative; otherwise percentage information ranges between 16% and 54% in the remainder.

(ii) *The F_2 phenotypic data set*

Summary statistics for the phenotypic data are shown in Table 3. Coefficient of variation (CV) for body weight declined with age, presumably due to reduced maternal influence. The estimates of the total carcass fat percentage from GFP were slightly higher than estimates based on freeze-dried weights. The correlation between fat percentage calculated using freeze-dried weight in males and fat percentage calculated using GFP weight was 0·89.

(iii) *QTL mapping using the X-linked regression analysis*

Using marker map positions estimated by CRIMAP (Table 2), QTLs for body weight at 3, 6 and 10 weeks were tested by interval mapping with the X-linked multiple regression method. The likelihood curves (Fig. 1) for all body weight measurements show peaks with maximum LOD scores of 7·8, 18·7 and 24·4 for 3, 6 and 10 week weight respectively. The maximum TS were found at about 23 cM for all body weight measurements, with all the likelihood curves showing a single smooth peak. Using the equivalent of a one LOD drop-off to obtain an approximate 95% confidence interval for QTL position (a drop of 4·6 in the TS), the confidence intervals (CIs) obtained were of length 12 cM, 9 cM and 7 cM for 3 week, 6 week and 10 week weight respectively. A summary of the estimates is shown in Table 4.

The estimated additive effects in males (half the difference between the hemizygotes) tended to be slightly greater than the additive effects in females (half the difference between the homozygotes). In males and females the estimated additive effects for 10 week weight were both 2·6 g, corresponding to a total difference of 5·2 g between the two homozygote female genotypes and the two hemizygote male genotypes, or a change in body weight of 17% or 20% in males and females respectively. Smaller standard errors for the male estimates compared with the female estimates are due to the greater information coming from the hemizygous males. The dominance deviations estimated in females for the body weight traits were positive (i.e. increasing body weight), but not significantly different from zero.

The estimated QTL effects (Table 4) expressed as a percentage of mean body weights give an indication of the percentage contribution of the QTL at the three body weights measured. The percentage effect of the QTL in males ranges between approximately 7% and 8% of the corresponding mean body weight, increasing slightly with age. In females, estimated effect were 4%, 9% and 10% at 3, 6 and 10 weeks,

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Table 3. *Summary statistics for phenotypic data in the F_2 population*

	Male (n = 157)			Female (n = 177)		
Trait	Mean	SD	CV (%)	Mean	SD	CV (%)
3 week weight	10·4	1·6	15	10·1	1·5	15
6 week weight	25·1	3·4	14	21·0	2·8	13
10 week weight	31·5	4·0	13	25·9	3·2	12
FDW% at 10 weeks	12·4	4·1	33	8·2	2·9	35
GFP% at 10 weeks	13·3	4·4	33			

The estimates shown are the least square means for body weights (g), and estimated fat percentage from freeze-dried weight (FDW%), or from gonadal fat pad weight in males (GFP%).

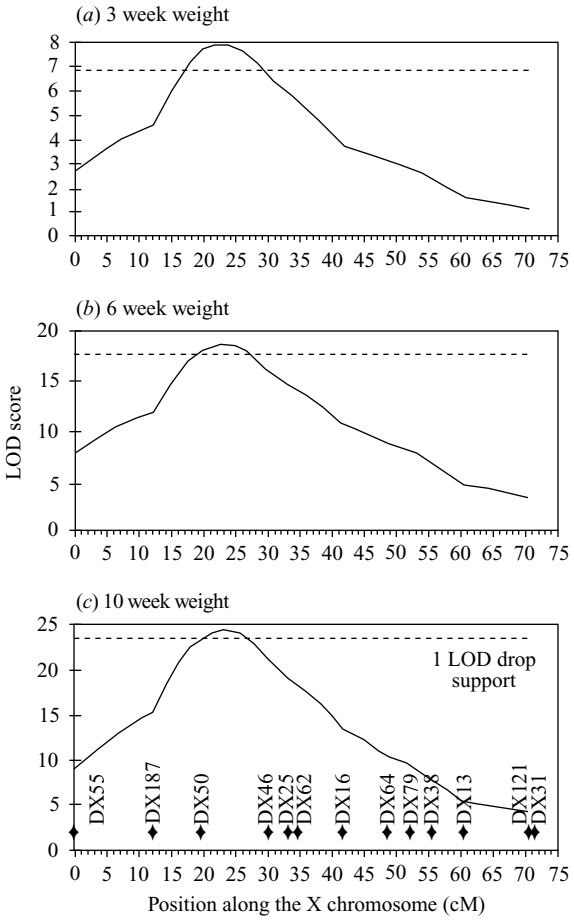

Fig. 1. Likelihood profiles for body weight traits obtained by the X-linked multiple regression method. Positions of the *DXMit* microsatellite markers are illustrated. (*a*) 3 week weight, (*b*) 6 week weight, (*c*) 10 week weight.

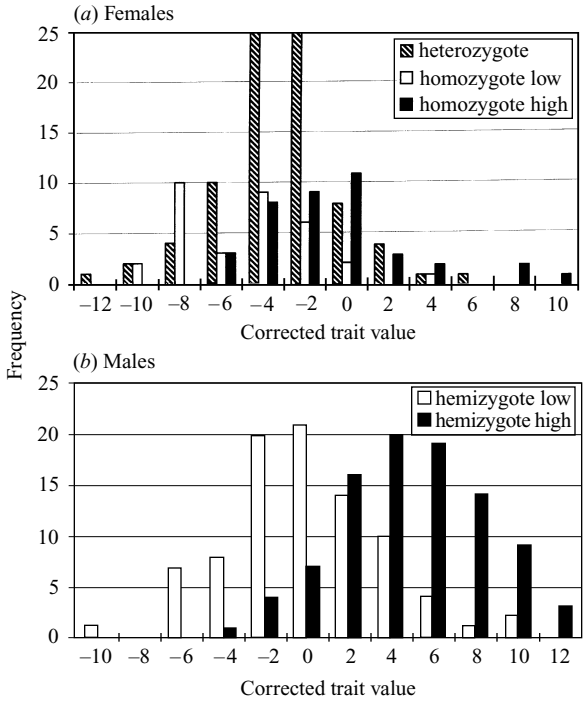

Fig. 2. Distribution of values for 10 week weight (g) corrected for fixed effects and the population mean within marker allele classes of *DXMit50*. (*a*) F_2 females, (*b*) F_2 males.

suggesting that the effect of the QTL increases with age.

The likelihood curves (Fig. 1) are smooth with single peaks, indicating the presence of a single locus or several very tightly linked loci. To further investigate the possibility of multiple QTLs, data were

Table 4. *Summary of QTL effect estimates* (g) *for the body weight traits obtained using X-linked multiple regression method*

Trait	Max. LOD	Male additive	Female additive	Dominance	% F_2 variance	Map position (cM)	95% CI (cM)
3 week weight	7·8	0·69 (0·12)	0·39 (0·22)	0·10 (0·27)	7·7	23	16–30
6 week weight	18·7	2·00 (0·24)	1·83 (0·42)	0·38 (0·53)	14·2	23	18–28
10 week weight	24·4	2·63 (0·27)	2·62 (0·48)	0·37 (0·60)	27·9	23	19–27

Standard errors of estimates are shown in parentheses.

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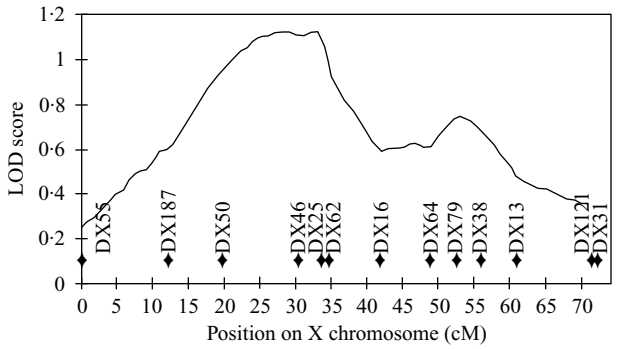

Fig. 3. Likelihood profile for fat content (estimated from freeze-dried body weight).

analysed with a two QTL model (Haley & Knott, 1992), but this did not lead to a significant change in the TS.

Analyses of body weight gains (3–6 weeks, 6–10 weeks, 3–10 weeks) show similar smooth curves to Fig. 1, with single peaks at 21 cM and 23 cM for 3–6 week and 3–10 week weight gain respectively. The peak LOD scores were 11·5 for 3–6 week body weight gain, and 16·7 for 3–10 week weight gain, which are lower than for the actual weights. Using a drop in LOD of 1, the CIs obtained for the QTL positions were 9 cM and 11 cM for 3–6 week and 3–10 week body weight gains respectively. In the QTL analysis of body weight gain between 6 and 10 weeks the maximum LOD reached was 5·2 at 23 cM, although a rise in TS was seen towards the distal end of the X chromosome. The second peak reached the threshold of $P < 0{\cdot}05$, suggesting the presence of a second QTL on the X chromosome. An analysis of the data to compare the fit of a two QTL versus one QTL model did not, however, show a significant change in the TS.

The interval mapping analyses of body weight traits and body weight gains indicate that the marker *DXMit50* is closely linked to the putative QTL. The distribution of 10 week weight corrected for the fixed effects fitted in the model within the marker classes of *DXMit50* (fully informative in the F_2 population) is illustrated in Fig. 2. In the female data set (Fig. 2*a*), the heterozygotes have intermediate marker-associated QTL effects compared with the two homozygote genotypes, and illustrate the predominantly additive action of the marker-associated QTL effect. For the two marker classes found in the males, there is a large difference in mean (Fig. 2*b*). Assuming complete linkage between marker *DXMit50* and the QTL, the two QTL genotypes cannot be distinguished visually by the residual phenotypic measurements alone, as there is still substantial overlap between the distributions (Fig. 2*b*).

(iv) *QTLs for fat percentage*

In addition to the body weight measurements taken on the F_2 generation, estimates of carcass fat percentage were obtained. To test for linkage between QTLs influencing fat deposition and markers elsewhere on the X chromosome, the predicted carcass fat percentage data were analysed by interval mapping. The maximum LOD score reached for GFP weight in males was 1·3, and for fat percentage from freeze-dried body weights (estimated in males and females) the maximum was 1·1 (Fig. 3). Fat percentage does not show a significant association with a QTL on the X chromosome ($P > 0{\cdot}10$).

4. Discussion

(i) *Distribution of polymorphic markers in the F_2*

The initial screen for polymorphic markers showed a low rate of X-linked microsatellite polymorphism in the P6 lines. Of the 61 markers investigated, 13 were polymorphic and produced consistently scorable PCR products (a further 4 of the 61 markers investigated were polymorphic but were not consistently scorable in the F_2 population). The percentage of X-linked polymorphic marker sites observed is lower than in the mouse autosomes in this cross (34% of markers polymorphic; P. Pignatelli, unpublished observations), and is consistent with previous studies in mice (Dietrich *et al.*, 1996) and humans (Hofker *et al.*, 1986; Dib *et al.*, 1996). As well as having low rates of polymorphism at individual markers (Dietrich *et al.*, 1996), microsatellite marker loci appear to be underrepresented on the X chromosome (Dietrich *et al.*, 1996).

(ii) *Detection of a major X-linked QTL influencing body weight*

The main objective of the investigation was to estimate the number of X-linked QTLs, their positions, and the magnitude of their effects on a variety of growth-related traits. The likelihood curves obtained from the QTL analysis show strong evidence for a single QTL with an effect of about 5 g at 10 weeks of age at approximately 23 cM (about 3 cM distal to marker *DXMit50*). The estimated QTL effect appears to explain the entire X-linked effect in the P6 lines (Hastings, 1990; Hastings & Veerkamp, 1993; Veerkamp *et al.*, 1993; Rance *et al.*, 1994), as the estimated effects derived from reciprocal crosses and a segregation analysis were also about 5 g. The LOD scores obtained are extremely high, well above any threshold for marker-based analysis of a single chromosome (Lander & Botstein, 1989). The shapes of the likelihood curves, which show single smooth peaks, are consistent with the presence of a single QTL. The analysis using the two QTL model did not result in a significant change ($P > 0{\cdot}10$) in the TS at any position other than the peak at 23 cM. However, we are unable to eliminate the possibility that the X-linked effect is controlled by two (or more) closely linked QTL close to the peak LOD score. The

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inability to distinguish between closely linked loci occurs because there are insufficient recombination events to break down the association between closely linked loci. The lack of recombination is a greater problem with the X chromosome, as it occurs only in the F_1 females. The estimated additive QTL effects at the different ages were similar in the two sexes. Dominance deviations in females were non-significantly different from zero at any age, a result consistent with previous work on the same lines by Veerkamp *et al*. (1993), in which a segregation analysis suggested an X-linked effect with additive action. The X-linked QTL explains approximately 8% of the phenotypic variance at 3 weeks, increasing to 14% at 6 weeks and 28% at 10 weeks, and therefore explains an increasing part of the variance with age. As discussed previously, much of the variation in early growth is attributable to the mother (Riska *et al*., 1984). The interval mapping analyses for the body weight gain traits show single peaks in the TS for the different traits (3–6 week, 3–10 week, and 6–10 week gains), and consistent with the analysis for body weight at specific ages (Tables 3, 4). Cheverud *et al*. (1996) estimated marker-associated QTL on age-specific body weights in an F_2 population of mice derived from divergently selected lines. In contrast with the action of the QTL in the present study, their linkage analysis indicated that many of the QTLs had significant effects at specific growth periods, but not for postnatal growth as a whole. The QTL effects on growth are not accompanied by changes in fatness, as no QTLs for fatness traits were detected anywhere on the X chromosome.

The X-linked effect was present in all three of the original P-replicate lines (Hastings & Veerkamp, 1993), so it must have been present in the base populations, rather than due to a mutation which occurred during the selection process. The P lines were derived from an F_1 cross between two inbred lines (JU and CBA) which was crossed to an outbred population (CFLP) from the Carnworth Laboratory (see Sharp *et al*., 1984). The two inbred lines JU and CBA are still available, and therefore if we assume the QTL originated from a single line, a reciprocal F_1 population made between these two inbred lines would provide a simple test for the presence of the X-linked QTL. Investigating whether the X-linked QTL originated for the outbred populations is more problematic as, if the population is still available, it is unlikely to resemble the original CFLP due to random drift. However, there are a number of inbred lines derived from the Carnworth Lab outbred population, e.g. CFW and CFCW. These lines could be used to test for the X-linked effect if the inbred lines failed to show the X-linked effect. Analysis of other mouse lines selected for body weight, derived from different base populations, has not revealed evidence for a substantial contribution of X-linked genes (White *et al*., 1970; Bakker *et al*. 1976; Keightley *et al*., 1996). In the present study, the finding that such a high proportion (about one-quarter) of the response in artificially selected lines can be attributed to a single locus (or very short region of chromosome) is surprising, but not without precedents from classical and molecular studies using *Drosophila* bristles as a model, and particularly in plants (Falconer & Mackay, 1996).

(iii) *The X-linked QTL for body weight and candidate loci*

In a study by Dragani *et al*. (1995), where two interspecific test-cross populations were made – HSB: female (C3H/He × *Mus spretus*) × male C57BL/6J; and ASB: female (A/J × *Mus spretus*) × male C57BL/6J – linkage analysis revealed an X-linked QTL for body weight at 40 weeks. Using composite interval mapping (Zeng, 1994), two complementary QTLs were found on the X chromosome in the HSB test-cross (*Bwt1* and *Bwt2*), together explaining 26% of the phenotypic variance in the cross. Linkage analysis of the ASB test-cross analysis suggested three X-linked QTLs (*Bwt1*, *Bwt2* and *Bwt3*), explaining 24% of the phenotypic variance, but effects were recessive, and detectable only in the hemizygous males. In the present study, the QTL has an additive action, with equal effects in males and females at 10 weeks. The apparently additive action observed in females could, however, conceal a different mode of action at the cellular level, with an overall additive action due to random X-inactivation (Lyon, 1992). The X-linked QTL in this study mapped to the marker interval *DXMit50* to *DXMit25*, while the QTL *Bwt1* mapped by Dragani *et al*. (1995) mapped close to *DXMit48*. *DXMit50* and *DXMit48* map to the same location on the X chromosome (at 22·5 cM: Mouse Genome Database). The estimated QTL effect at *Bwt1* is approximately 17% of the mean body weight in the HSB mice and 14% in the ASB mice. The origin of the body weight alleles in these experiments is unlikely to be from a common ancestor, as the X-linked QTLs in the Dragani *et al*. (1995) study were derived from the *M*. *spretus* and C3H parental strains. It is clear that further investigation is needed before any conclusion can be drawn as to whether these may be alleles at the same loci. Molecular investigation of the region associated with the X-linked QTLs in the two studies could determine whether the X-linked QTLs (from the two mouse lines), segregating on different genetic backgrounds, resulted in the recessive and additive QTL phenotypes.

In addition to examining evidence for an X-linked QTL influencing body weight isolated in other studies, the Mouse Genome Database was used to search for candidate loci. However, no obvious candidate loci were found in the marker interval *DXMit50* to *DXMit25* (MGD positions 12·5 cM and 28·0 cM respectively). The Mouse Genome Database is con-

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stantly updated; therefore future investigation may provide suitable candidate loci. It may also be possible to isolate candidate loci by using the human/mouse genome comparative map. However, as for the results presented above for the mouse genome search, no suitable loci have been isolated to date.

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