QTL ANALYSIS FOR LITTER SIZE AND BODY WEIGHT IN MICE

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SUMMARY

Animals from two backcrosses were used to search for QTL controlling litter size and body weight in mice. These backcrosses were bred in an earlier study, using C57BL/6J (small body, small litters) and Inbred Quackenbush Swiss (large bodies, large litters) mice. Animals were genotyped for a total of seventeen informative microsatellite markers from chromosomes 1, 2, 4, 9, 10, 11 and an unknown chromosome, and preliminary analysis was performed for quantitative effects associated with these markers. Confirmatory evidence was obtained for the presence of litter size and body weight QTL near D4MIT37 which has been reported in an earlier study. Several other markers showed significant effects in only one backcross. Comparative mapping knowledge suggests that the region containing D4MIT37 is homologous with human chromosome 1p and cattle chromosome 3. These regions in humans and cattle may contain similar growth and reproduction QTL.

Keywords: Microsatellite markers, QTL, body weight, litter size, mice

INTRODUCTION

The discovery of polymorphic DNA markers and their applications in molecular genetics has opened new opportunities for animal breeding arising from identification of QTL from genome-wide scans. Understanding of the inheritance of growth and reproductive traits is vital to continued improvements in the efficiency of animal production. The availability of a very large number of hyperpolymorphic DNA markers on the genetic map of the mouse (Dietrich *et al.,* 1996) has facilitated the identification of QTL (Quantitative Trait Loci) in this species. Furthermore, the continuing development of good comparative maps will enable transfer of this information to farm animals (Williams, 1994). Litter size and body weight have been studied very extensively in mice (Bradford, 1971; Collins *et al.*, 1993; Silva, 1994; Pomp *et al.*, 1995; Horvat and Medrano, 1995 and Keightley *et al.*, 1996). The present study is a continuation of work started by Silva (1994), using two genetically divergent strains of inbred mice, namely C57BL/6J and Inbred Quackenbush Swiss (IQS) mice, with the aim of identifying regions of mouse chromosomes controlling litter size and body weight.

MATERIALS AND METHODS

Animal resources. Silva (1994) bred backcrosses using two strains of mice, namely C57BL/6J with an average body weight of 33.5 gm, litter size of 7.0 pups/litter; and IQ5 (developed at The University of Sydney from Quackenbush Swiss mice), with an average body weight of 43.1 gm and a litter size of 15.5. F_1 females from a cross involving these two grand-parents (IQ female and C57BL/6J male) were backcrossed to C57BL/6J males to give rise to BC1, and to IQ5 males to

give rise to BC2 families respectively. Females from BC1 and BC2 were recorded for body weights at different ages, namely Initial Mating Weight (IMW) at 50 days of age, and after the first four parturitions; and for litter size (number born alive) in the first four litters. DNA samples from all 53 BC1 animals and all 49 BC2 animals were obtained, adjusted to 100ng/μl and stored at -20°C.

Screening for Microsatellites markers. Markers were selected from chromosome 10 as part of a comparative positional candidate gene approach: Rothschild *et al.* (1996) showed that the Estrogen receptor (*ESR*) locus has a large and significant effect on litter size in pigs, and *ESR* maps to mouse Chromosome 10. D10MIT37, D10MIT44 and D10MIT53 markers were selected since they flank the ESR locus. Subsequently markers from Chromosome 1, namely D1MIT58, D1MIT66, D1MIT169, D1MIT211 and D1MIT294 were selected from a region where preliminary evidence had suggested the existence of a QTL. In addition, D10MIT88, D10MIT161 and D10MIT176 were selected from the high-growth region reported by Horvat and Medrano (1995). Finally it was decided to screen markers from positions on chromosomes 2, 4, 9 and 11 where Kirkpatrick *et al.* (1996) reported the presence of QTL for growth and litter size from a similar mouse cross. These were D2MIT7, D2MIT92, D4MIT9, D4MIT37, D4MIT219, D9MIT64, D9MIT285, D11MIT81, D11MIT231 and D11MIT271. Primers were obtained from Research Genetics (Huntsville AL) and a PCR protocol detailed by Silva (1994) was followed. Markers D10MIT37, D10MIT53, D11MIT231 and D11MIT271 were uninformative in the backcrosses, leaving 17 informative markers. Polyacrylamide gel electrophoresis and radioactive labelling were used to genotype all the 102 animals for the first nine informative markers, while the last eight informative markers were scored using an ABI 373 automatic genotyping system. This method uses direct incorporation of fluorescent dUTPs in the PCR products and multiple loadings of different markers.

Statistical Procedures. Using GLM in MINITAB, separate analyses were conducted for each marker/backcross combination with each of the three following traits: mean litter size (MLS; mean of number born alive from the first four litters), initial mating weight (IMW) and mean post-parturient body weight (MPW; mean of the four post-parturient body weights). The two backcrosses were analysed separately because background genotype effect was confounded with marker-genotype contrast.

RESULTS AND DISCUSSION

The P values for all the informative markers for BC1 and BC2 are presented in Table 1.

Lander and Kryglyak (1995) have described stringent standards which should be followed in order to avoid false positives in QTL studies. In particular, putative QTL should be independently identified in separate studies. D4MIT37, reported by Kirkpatrick *et al.* (1996) to be linked with body weight and litter size QTL, also showed association with these traits in the present study. However, only the association with IMW in BC1 satisfies the $P<0.01$ criteria for confirmed linkage suggested by Lander and Kryglyak (1995). Thus the existence of QTL in this region is tentatively supported. The small sample size of this study (102) limits our ability to detect more subtle QTL effects, which may explain why we have not confirmed the other QTL observed by Kirkpatrick *et al.* (1996) whose sample size was 497. Despite being listed by the Whitehead

Table 1. Results of the marker analyses (P values)

		BC1			BC2		
Chromosome	Marker	MLS[a]	IMW[b]	MPW[c]	MLS[a]	IMW[b]	MPW[c]
1	D1MIT58	0.418	0.173	0.006**	0.637	0.633	0.768
	D1MIT66	0.520	0.142	0.001**	0.951	0.394	0.493
	D1MIT169	0.806	0.168	0.031*	0.267	0.554	0.315
	D1MIT211	0.806	0.168	0.031*	0.256	0.554	0.315
	D1MIT294	0.418	0.173	0.006**	0.686	0.477	0.813
2	D2MIT7	0.836	0.769	0.825	0.147	0.366	0.569
	D2MIT92	0.622	0.299	0.259	0.545	0.132	0.582
4	D4MIT9	0.524	0.019*	0.658	0.377	0.116	0.242
	D4MIT37	0.086	0.007**	0.698	0.049*	0.041*	0.172
	D4MIT219	0.765	0.012*	0.580	0.270	0.134	0.250
9	D9MIT64	0.078	0.588	0.260	0.173	0.052	0.058
	D9MIT285	0.187	0.929	0.469	0.017*	0.824	0.167
10	D10MIT88	0.610	0.216	0.550	0.310	0.302	0.712
	D10MIT161	0.682	0.557	0.638	0.639	0.569	0.188
	D10MIT176	0.739	0.977	0.808	0.694	0.614	0.429
11	D11MIT81	0.368	0.643	0.600	0.603	0.985	0.617
Unassigned	D10MIT44	0.877	0.747	0.021*	0.046*	0.004**	0.055

[a] Mean Litter Size
[b] Initial Mating Weight
[c] Mean Post-parturient Weight
* Significant at 5% level
** Significant at 1% level

Institute as a chromosome-10 marker, D10MIT44 showed independent segregation from the other three chromosome-10 markers. It also showed independent segregation from all other markers used in this study. The Whitehead Institute have since confirmed that this marker is now regarded as unassigned (Dietrich, pers. comm.). This is unfortunate, since it shows a strong association with IMW in BC2, and a weak association with MPW in BC1, and with MLS and MPW in BC2. D1MIT58, D1MIT66 and D1MIT294 all show a strong association with MPW, but only in BC1. The inconsistency between the two backcrosses for these and other markers has no obvious explanation (Silva, 1994), and is the subject of current investigations. D10MIT161 and D10MIT176, selected from the high-growth region reported by Horvat and Medrano (1995), failed to identify any effect on body weight in this study. This may simply be because we are dealing with a different populations of mice. However, in relation to the study of Kirkpatrick *et al.* (1996), we are dealing with a cross between very similar grand-parental stocks, and have produced tentative confirmatory evidence for at least two QTL (one each for body weight and litter size in the vicinity of D4MIT37, which is located in the middle of the long arm of chromosome 4. This region is homologous with human chromosome 1p and cattle chromosome 3 (BovGbase). These regions in humans and cattle may contain similar growth and reproduction QTL.

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