

Epistasis affecting litter size in mice

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Abstract

Litter size is an important reproductive trait as it makes a major contribution to fitness. Generally, traits closely related to fitness show low heritability perhaps because of the corrosive effects of directional natural selection on the additive genetic variance. Nonetheless, low heritability does not imply, necessarily, a complete absence of genetic variation because genetic interactions (epistasis and dominance) contribute to variation in traits displaying strong heterosis in crosses, such as litter size. In our study, we investigated the genetic architecture of litter size in 166 females from an F_2 intercross of the SM/J and LG/J inbred mouse strains. Litter size had a low heritability ($h^2 = 12\%$) and a low repeatability ($r = 33\%$). Using interval-mapping methods, we located two quantitative trait loci (QTL) affecting litter size at locations $D7Mit21 + 0$ cM and $D12Mit6 + 8$ cM, on chromosomes 7 and 12 respectively. These QTL accounted for 12.6% of the variance in litter size. In a two-way genome-wide epistasis scan we found eight QTL interacting epistatically involving chromosomes 2, 4, 5, 11, 14, 15 and 18. Taken together, the QTL and their interactions explain nearly 49% (39.5% adjusted multiple r^2) of the phenotypic variation for litter size in this cross, an increase of 36% over the direct effects of the QTL. This indicates the importance of epistasis as a component of the genetic architecture of litter size and fitness in our intercross population.

Introduction

Litter size is an important aspect of fitness in mammals. Variation in litter size along with the timing of reproduction over the lifespan determines the intrinsic rate of increase (Charlesworth, 1980), which in its relationship with phenotypes of interest defines selection. The genetic basis of fitness-related traits has been widely discussed (Charlesworth, 1987; Mousseau & Roff, 1987; Price & Schlutter, 1991; Crnokrak & Roff, 1995; Merilä & Sheldon, 1999, 2000; Stirling *et al.*, 2002). The principal focus of the discussion has been the interpretation of Fisher's Fundamental Theorem that says the response of fitness to natural selection is proportional to the additive genetic variance for fitness in the population (Fisher,

1958). Based on this theorem, some suggest that the genetic variance of fitness-related traits should be close to zero due to the rapid fixation of alleles conferring higher fitness. Others contend that this interpretation is only appropriate under certain conditions, such as weak selection, constancy of genotypic fitnesses over time and the absence of migration (Charlesworth, 1987).

In contrast to other traits, fitness-related traits usually show low heritability (Mousseau & Roff, 1987; Falconer & Mackay, 1996). For instance, litter size heritability ranges from 5 to 20% in various mammalian populations (Falconer, 1960; Avalos & Smith, 1987; Falconer & Mackay, 1996; Johnson *et al.*, 1999). To some, this suggests the presence of only a few segregating genes with additive effects responsible for variation in fitness. Most genes affecting fitness would be fixed. Alternatively, heritability may be low not because heritable variance is low but because nonheritable variance is relatively high for fitness-related traits (Houle, 1992). The nonheritable variance is composed of the random

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environmental variance and the dominance and epistatic, nonheritable, genetic variances. Price & Schluter (1991) argue that life-history traits have relatively high environmental variance because additional environmental factors beyond those affecting underlying morphology and behaviour affect survivorship and fecundity. Some expect that the total genetic variation for fitness should be high because of the large number of loci potentially affecting this trait (Merilä & Sheldon, 2000). Nonadditive genetic effects could be responsible for observations of low heritability combined with high total genetic variance. Indeed, nonadditive genetic variance may have an important evolutionary role because it can become heritable in a population as allele frequencies change due to either selection or random evolutionary processes, like founder effects or genetic drift (Cheverud & Routman, 1995, 1996; Wade & Goodnight, 1998). In such cases, with two epistatically interacting loci affecting a trait and one locus driven towards fixation by selection or genetic drift, epistatic variance will be eroded as there will be no more variation among alleles at the fixed locus. Nevertheless, the variation present at the other segregating locus will be liberated by the interaction and expressed as additive and/or dominance variance.

Here we investigate the genetic basis of litter size in mice by studying the association between microsatellite genotypes and litter size in the cross of two inbred strains, LG/J and SM/J. This population has been well studied for the genetics of growth (Cheverud *et al.*, 1996; Vaughn *et al.*, 1999), body size and composition (Cheverud *et al.*, 2001), and aspects of morphology (Cheverud *et al.*, 1997; Leamy *et al.*, 1999). In each of these studies, many quantitative trait loci (QTL) of small effect were found segregating in the intercross population. Many small epistatic effects have also been mapped for these traits (Cheverud, 2000; Cheverud *et al.*, 2001). These traits display an additive architecture in the cross of LG/J and SM/J, with the F₁ hybrids being intermediate between the parental strains (Kramer *et al.*, 1998). The present study analyses genetic effects for litter size, a fitness-related trait. Given the expected observation of low heritability, we expect the genetic architecture of litter size to differ from the architectures previously observed for body size, growth and morphology.

Material and methods

Mouse strains and breeding

The mouse strains used in this study are the Large (LG/J) and the Small (SM/J) inbred lines acquired from Jackson Laboratories (for details of animal husbandry, see Cheverud *et al.*, 1996; Kramer *et al.*, 1998; Vaughn *et al.*, 1999). This cross has been the subject of many QTL studies mapping loci affecting body size growth and composition and skeletal morphology (Cheverud *et al.*, 1996, 1997, 2001; Leamy *et al.*, 1999; Vaughn *et al.*,

1999). As part of these studies data were collected on litter size. First, 10 SM/J males were mated with 10 LG/J females producing 41 heterozygous isogenic offspring. The F₁ hybrids were randomly mated producing 510 F₂ progeny. Litter size information was scored at the date of birth and survival was monitored daily for the first week in which all animals alive were individually identified by toe clipping. Three weeks after birth the litter was weaned and placed in single-sex cages with at most five animals per cage. Animals were fed *ad libitum* with Purina PicoLab Rodent Chow 20 (5353) (St Louis, MO, USA). At 10 weeks, 241 F₂ females were randomly mated with F₂ males to produce 1632 F₃ progeny. This F₃ generation was the source for an Advanced Intercross Line (AIL) maintained by pseudo-random mating (close inbreeding was avoided) of more than 60 mating pairs per generation with one male and one female breeding animal chosen for the breeding pool from each fertile mating of the previous generation. This line has been continued beyond the F₁₅ generation of random mating. Males were removed from the breeding cage when their mate was pregnant. In addition, the Large (LG/J) and Small (SM/J) inbred lines have been maintained in our mouse facility.

Data analyses

Litter size was scored as the number of offspring born per female in the F₁, F₂ and F₃ generations. Information for parental generation animals (SM/J and LG/J strains) was based on litter size for these lines maintained in our laboratory. As we crossed two distinct inbred lines (SM/J and LG/J), we checked for heterosis for litter size (measured as the deviation from the mid-parent value) in the F₁ generation using a *t*-test (Sokal & Rohlf, 1995). With detection of heterosis, we performed an additional *t*-test to further investigate whether changes in average litter size over generations fit with expected values based on a dominance model of heterosis. If heterosis is purely because of dominance we expect it to decrease by half from the F₁ to the F₂ generation (Falconer & Mackay, 1996).

Differences in litter size between the parental lines were tested using ANOVA in SAS (SAS Institute Inc., 1998). We calculated the narrow-sense heritability of litter size using the regression of F₃ females' litter size on the litter size produced by their F₂ mothers. As F₂ and F₃ females were mated only once, repeatability for litter size was determined from 133 females that had more than one litter in generations F₉ and F₁₅ of the AIL derived from this cross.

We also estimated the number of segregating factors responsible for litter size variation (effective number of factors, n_e) in our cross by using the Castle-Wright estimator (Lynch & Walsh, 1998). This estimator uses statistical properties of phenotype distributions of the two parental lines and their line-cross derivatives to infer

indirectly the number of independent segregating factors that are likely to be responsible for them. It assumes additive gene action, unlinked loci, equality of allelic effects, and that all genes with a positive influence on the trait are sorted into one line and all those with negative influences into the other.

Litter size indices

Litter size typically has a low heritability (Falconer, 1960; Avalos & Smith, 1987; Falconer & Mackay, 1996; Johnson *et al.*, 1999). Traits with low heritability present difficulties in gene mapping studies. However, additional useful information can be gained if we use information from relatives to obtain a more accurate measurement of an individual's breeding value (Falconer & Mackay, 1996). As we have information on litter size from both F_2 females and their F_3 daughters, we combined all the information available into a litter size index. This index represents the best linear prediction of an individual's breeding value (value of an individual measured by the average value of its offspring) using a multiple regression of breeding values on all sources of information (Falconer & Mackay, 1996). The index was calculated using the equation:

$$I = \frac{h^2(1-k)L_2}{1-K} + \frac{h^2(k-K)L_3}{K(1-K)},$$

where $K=[1+(n-1)t]/n$, $k=[1+(n-1)r]/n$ and h^2 is heritability of litter size, L_2 is the F_2 's litter size, L_3 is the F_2 's daughters average litter size, t is the phenotypic correlation between F_3 full sibs and r the coefficient of relationship between the relative and the individual whose index is calculated, 0.5 for mothers and their offspring. This index of litter size is rescaled relative to the original scale in terms of numbers of pups per litter but the rescaling does not affect the statistical findings (Falconer & Mackay, 1996).

We calculated indices for 166 females including all F_2 females that succeeded in having litters that also produced offspring. These litter size indices were used to perform interval mapping and epistasis analysis. We also calculate the accuracy of this index, which is the correlation between index values and breeding values (Falconer & Mackay, 1996) to determine how much additional information is gleaned from the index in comparison with the F_2 phenotypes themselves.

Statistical procedures

We used 96 polymorphic loci covering the 19 mouse autosomes for this analysis. These markers specify 77 intervals averaging 21.5 cM in length. Molecular genotyping methods and the marker map are described in Vaughn *et al.* (1999) and Cheverud *et al.* (2001). The relative positions of these markers are given in the Mouse Genome Database (2001). However, map distances are

known to vary between crosses. For this study, map distances were calculated from F_2 animals of this intercross (Vaughn *et al.*, 1999; Cheverud *et al.*, 2001) using MAP-MAKER 3.0b (Lander *et al.*, 1987; Lincoln *et al.*, 1992).

Interval mapping of QTL (Lander & Bolstein, 1989) was undertaken by regressing litter size index (LS) on genotype scores every 2 cM along each chromosome as described by Haley & Knott (1992). Genotype scores are calculated using the probability that an individual is homozygous for either parental allele or heterozygous at the specified location as determined from the recombination rates between the position of interest and the flanking markers and the genotypes observed at those markers (Haley & Knott, 1992). These probabilities are multiplied by -1 , 0 and 1 , for homozygous SM/J, heterozygous, and homozygous LG/J genotypes, respectively, and then summed to obtain an additive genotype score (X_a). Likewise, the dominance genotype score (X_d) is calculated as the probability of heterozygosity at the arbitrary intermediate location.

We used the following model at each location for the interval mapping analysis:

$$LS = \mu + aX_a + dX_d + e,$$

where μ is a constant, a is the additive genotypic value, X_a is additive genotype score, d is dominance genotypic value, X_d is dominance genotype score and e is the residual. These regression coefficients are unbiased estimates of the additive (a) and dominance (d) genotypic values in an F_2 intercross population when a QTL occurs at the tested position (Lander & Botstein, 1989).

The statistical significance of one-QTL models was evaluated using LOD scores [$LOD = \log_{10}(1/\text{probability})$]. Point-wise probabilities were adjusted for multiple comparisons (Lander & Kruglyak, 1995) using the Bonferroni correction by calculating the number of statistically independent regressions performed on each chromosome and dividing the appropriate probability level by the effective number of independent tests performed (Cheverud, 2001). Two threshold levels were computed to discriminate between chromosome-wide and genome-wide significance levels. Chromosome-wide thresholds were calculated using the effective number of independent markers on each chromosome in a Bonferroni correction while the genome-wide threshold was determined by summing the effective number of independent markers across the 19 autosomes and then performing a Bonferroni correction (for details, see Cheverud, 2001). LOD scores above the genome-wide threshold ($LOD > 3.18$) indicate highly significant evidence of linkage; while values only exceeding the appropriate chromosome-wide threshold level indicate suggestive linkage, requiring confirmation from additional data (Lander & Kruglyak, 1995).

We used the 'physiological epistasis model' (Cheverud & Routman, 1995; Routman & Cheverud, 1997; Cheverud, 2000) implemented in SAS (SAS Institute Inc.,

1998) to test for interactions among single QTL. In this model, epistatic genotypic values are defined and epistatic contributions to additive, dominance and interaction variances are all included in significance testing for epistasis. For this study, each pair of litter size QTL was analysed for epistatic interactions. Multiple regressions were performed at these loci using the additive and dominance genotype scores and their products as the independent variables. The forms of interaction, additive-by-additive ($X_{a1}X_{a2}$), additive-by-dominance ($X_{a1}X_{d2}$)/dominance-by-additive ($X_{d1}X_{a2}$) and dominance-by-dominance ($X_{d1}X_{d2}$) were considered as independent variables and litter size index the dependent variable. Statistical significance was tested as described in Routman & Cheverud (1997) and Cheverud (2000).

Epistasis across all pairs of chromosomes was tested for using a two-way genome-wide scan performed at every 2 cM along the pairs of mouse autosomes. At each pair of locations, a test for genic epistasis was performed using the following model in SAS (SAS Institute Inc., 1998):

$$LS = \mu + aaX_{a1}X_{a2} + adX_{a1}X_{d2} + daX_{d1}X_{a2} + ddX_{d1}X_{d2} | X_{a1}, X_{d1}, X_{a2}, X_{d2}$$

where LS is the litter size index dependent variable and μ is the constant.

The independent variables are the interaction terms $X_{a1}X_{a2}$, $X_{a1}X_{d2}$, $X_{d1}X_{a2}$ and $X_{d1}X_{d2}$, while the independent partial variables are the genotype scores (X_{a1} , X_{d1} , X_{a2} , X_{d2}) at the specified locations (Cheverud, 2000). The *aa*, *ad*, *da* and *dd* regression coefficients measure the additive-by-additive, additive-by-dominance, dominance-by-additive and dominance-by-dominance genotypic values for epistasis, respectively, in an F_2 intercross population. The symbol '|' indicates that the variables listed to the right are partialled out of the independent variables to the left. This provides a joint statistical test for the interaction terms independent of tests for the single locus scores.

There were 2736 independent tests in the two-way genome-wide scan and we expect 136, 27 and 2.7 'false-positive' significant results at the 0.05, 0.01 and 0.001 point-wise levels, respectively, by chance alone even when there is no epistasis present. In order to eliminate 'false-positive' results without ignoring several true instances of epistasis, we used a significance threshold correction based on the Bonferroni test (Cheverud, 2001). This Bonferroni threshold for epistasis at a pair of locations was obtained by considering the full number of independent epistasis tests in the genome scan and limiting the ratio of false positives to one in 10, which corresponds to a pointwise probability value of 3.7×10^{-5} in our study. While this level may seem less conservative than the 0.05 level usually chosen, we chose the 0.1 significance level to balance between the risk of potentially reporting a false-positive result and the risk of ignoring large numbers of true instances of epistasis. We also considered epistasis significant if one

of the four kinds of epistasis (additive-by-additive, additive-by-dominance, dominance-by-additive and dominance-by-dominance) was significant at the 9.25×10^{-6} level ($3.7 \times 10^{-5}/4$), even if the overall epistasis model was not significant.

Results

The average numbers of offspring born per female in the parental, F_1 , F_2 and F_3 generations are shown in Table 1. The LG/J strain has slightly larger litters, 0.88 pups larger on average, than the SM/J strain ($t_{97} = 2.8608$, $P < 0.01$). The average litter size for F_1 females is significantly larger than the litter size in the parental strains ($t_{68.5} = 10.70$, $P = 1.58 \times 10^{-16}$) indicating strong heterosis for this trait in this cross. The F_1 hybrids have, on average, five more pups per litter than the parental strains. If we assume that all heterosis observed here is due to dominance, we expect heterosis in the F_2 generation to be half as large as that observed in the F_1 generation to be half as large as that observed in the F_1 (Falconer & Mackay, 1996). Given that the average litter size of the parentals is 6.04 and the average litter size of the F_1 is 11.11, we expect an average litter size in the F_2 of 8.57 under a purely dominance model of heterosis. The observed average litter size of 9.43 in the F_2 generation deviates significantly from the expected value ($t_{199.25} = 20.38$, $P = 1.35 \times 10^{-50}$). Therefore, we plotted the observed average litter size across generations against the expected litter size under a dominance model of heterosis (Fig. 1).

The proportion of variance between parental strains for litter size in our cross was estimated as 13%. The narrow-sense heritability, estimated from the offspring on mother regression, is 0.123 ± 0.09 , which is not significantly different from zero. As estimates of litter size, in our cross, had a repeatability of 0.335, the narrow-sense heritability corrected for lack of repeatability would be 37%. The Castle-Wright estimator of the number of additive segregating loci likely to be responsible for litter size variation in this cross was estimated as being close to zero ($n_e = 0.04$).

The accuracy of the litter size index as a measure of breeding value is 0.70, which is twice the accuracy of litter size for F_2 females considered alone (0.35). Therefore, using the index greatly enhances our ability to map litter size QTL in this population. Interval mapping

Table 1 Litter size across generations in the LG/J by SM/J intercross.

Generations	N	Mean	Variance	SE
SM/J	64	5.60	2.01	0.177
LG/J	35	6.48	2.54	0.269
F1	21	11.11	1.67	0.282
F2	166	9.44	3.76	0.150
F3	166	9.62	4.86	0.171

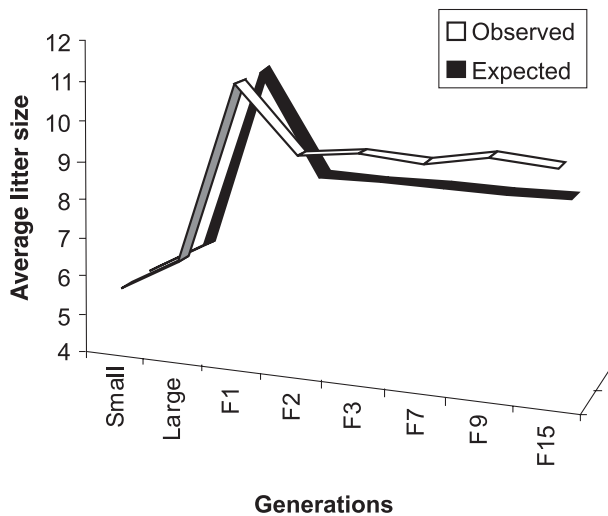


Fig. 1 Average litter size across generations. Clear band refers to the observed litter size in our cross and the dark band is the expected litter size assuming the heterosis by dominance model.

analyses located two QTL affecting litter size index, one on chromosome 7 and another on 12 (Table 2). Both LOD scores exceed the chromosome-wide 5% level (2.10 and 1.87 respectively), but they do not reach the genome-wide threshold (3.18). In a full genome scan we expect one false-positive result at the chromosome-wide threshold even when there is no gene effect. The QTL that lies at *D7Mit21* + 0 cM is additive while an overdominant QTL lies at *D12Mit6* + 8 cM. They explain, respectively, 7.41 and 6.52% of the variation for this phenotype. Epistatic interactions between these two markers were not significant ($F_4 = 0.598$, $P = \text{n.s.}$).

The two-way genome-wide scan detected 271 significant epistatic interactions at 0.05 point-wise level, twice the value expected by chance (135). Of the 271, 108 were significant at the 0.01 level and 27 at the 0.001 level, the first one four times (27) and the latter 10 times (2.7) the value expected by chance under the null model. These results show substantial evidence for epistasis. Regardless of the variety of interactions detected, we only further consider epistasis tests that significantly exceeded the Bonferroni-corrected significance criterion. We do this although only three of the 27 results significant at the even less restrictive 0.001 pointwise level are likely to

be false positives. This restricts our report to those few results that are most likely not one of the three predicted false-positive results. Only four interactions among QTL affecting litter size satisfied the strict Bonferroni criterion, two at the 0.05 level (1.9×10^{-5} , 4.75×10^{-6}) and another two at the 0.1 level (3.7×10^{-5} , 9.25×10^{-6}) (see Table 3). Eight chromosomal regions on seven different chromosomes (chromosomes 2, 4, 5, 11, 14, 15 and 18) were involved in epistatic interactions. We did not find significant interactions among the single litter size QTL identified on chromosome 7 and 12 and other regions across the genome. All forms of epistasis are represented in the results. In the additive-by-additive epistatic interaction between chromosomes 11 and 14 (Fig. 2) the genotypic values indicates that the highest genotypic values are for the parental double homozygotes. Additive-by-dominance/dominance-by-additive epistasis (Fig. 3) shows, in this case, that the double heterozygote has a larger litter size than the midpoint of the parental double homozygotes, although the SS double homozygotes have the largest litters. Dominance-by-dominance interactions are represented in Figs 4 and 5 indicating that all single heterozygotes have larger litters than the double homozygotes and/or the double heterozygote.

The two single locus QTL accounted for 12.6% (10.4% multiple r^2 adjusted for number of parameters) of the overall variation in litter size. When we add the main effects of the epistatic QTL to the single locus model, main effect QTL account for 25.6% of the variance (14.5% adjusted multiple r^2) but the model does not fit significantly better than the original model including only the two main effect QTL ($\chi^2_{22} = 23.34$, $P = \text{n.s.}$). When the significant epistatic effects are added to the model there is a significant increase ($\chi^2_6 = 28.13$, $P < 0.0001$) in variance explained to 48.3% (39.5% adjusted) of the litter size variation in our cross. Thus, the epistatic effects make a large, significant contribution to the genetic variance in this cross by increasing the variance explained by genetic factors by 36 percentage points (29 percentage points when adjusted for number of parameters).

Discussion

The highly significant difference in litter size among parental strains and the F_1 generation indicates heterosis

Table 2 Position 1 is the QTL's distance from the nearest proximal marker on the chromosome while position 2 is the telomeric distance from the most proximal marker on the chromosome, in Haldane's cM. C.R. is the ± 1 LOD confidence region. Also included are the raw and standardized additive (a , $2a/\sigma_p$) and dominance (d , d/σ_p) genotypic values for litter size at each QTL. % VAR represents the percentage of phenotypic variation accounted for QTL with associated LOD score.

Locus	Position 1 (cM)	Position 2 (cM)	C.R. (cM)	a	d	$2a/\sigma_p$	d/σ_p	% VAR	LOD score
<i>D7Mit21</i>	0	0	0–12	0.18	–0.06	0.76	–0.13	7.41	2.70
<i>D12Mit6</i>	8	62	38–72	0.04	0.30	0.17	0.63	6.52	2.35

Table 3 Loci 1 and 2 are QTL involved in significant epistatic interactions affecting litter size. Position 1 is the QTL's distance from the nearest proximal marker on the chromosome while position 2 is the telomeric distance from the most proximal marker on the chromosome, in Haldane's cM. Epistasis types: AA, additive-by-additive; DD, dominance-by-dominance; AD, additive-by-dominance; DA, dominance-by-additive.

Locus 1	Position 1 (cM)	Position 2 (cM)	Locus 2	Position 1 (cM)	Position 2 (cM)	Prob. epistasis	Epistasis type	Genotypic value	Prob. genotypic value
<i>D2Mit17</i>	20	108	<i>D5Mit47</i>	12	12	6.69×10^{-5}	DD	-0.40	9.84×10^{-7}
<i>D2Mit22</i>	14	130	<i>D15Mit2</i>	26	72	0.000152	DD	-0.34	5.53×10^{-6}
<i>D4Mit163</i>	6	22	<i>D18Mit51</i>	6	32	2.05×10^{-5}	AD	0.28	0.000654
							DA	0.29	0.000383
<i>D11Mit333</i>	4	104	<i>D14Mit5</i>	20	66	2.36×10^{-6}	AA	0.27	0.000176
							DD	0.23	8.31×10^{-5}

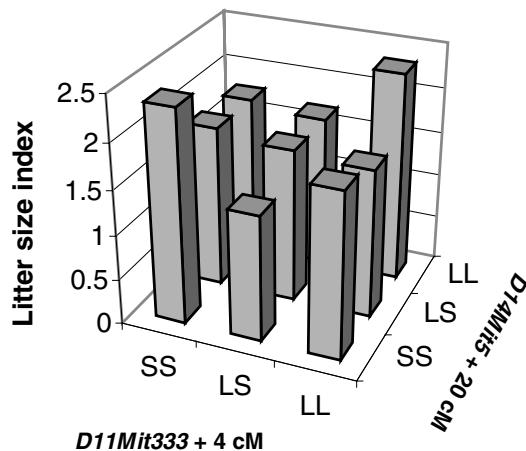


Fig. 2 Additive-by-additive and dominance-by-dominance epistatic interactions between loci on chromosomes 11 and 14 for litter size index (linear prediction of an individual breeding value for litter size). Each column indicates average genotypic value of litter size index for individual with genotypes SS, LS or LL at 4 cM downstream from genetic marker *D11Mit333* and 20 cM downstream from loci *D14Mit5*.

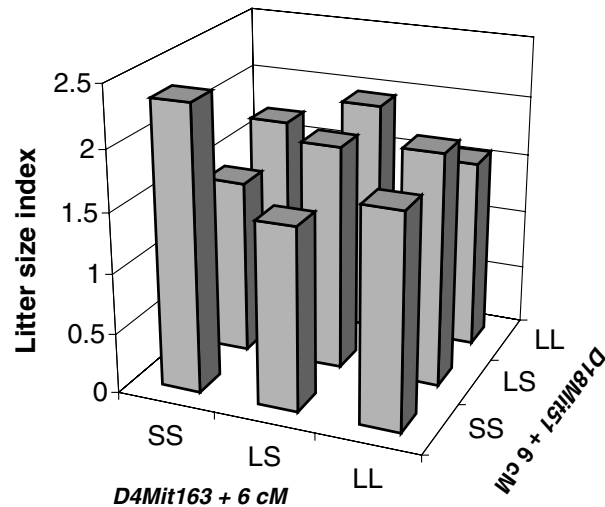


Fig. 3 Litter size index genotypic values for two-locus genotype at 6 cM downstream from marker *D4Mit163* and 6 cM downstream from marker *D18Mit51* illustrating additive-by-dominance/dominance-by-additive interaction.

for this trait. There are two possible mechanisms that cause heterosis, dominance and epistasis (Falconer & Mackay, 1996). The average litter size observed for F_2 females (9.43) differed significantly from the expected value (8.57) based on a pure dominance model indicating that epistatic interactions among loci are affecting litter size heterosis in our cross. Under epistasis, heterosis is expected to decline to an equilibrium value in subsequent generations at a rate that depends on the closeness of the linkage between interacting loci (Falconer & Mackay, 1996). In our experiment, the average litter size did not decrease in later generations (no significant difference among litter size in generations F_3 , F_7 , F_9 and F_{15} , of the AI line, Kruskal-Wallis₂ = 0.252, $P = n.s.$), and the observed litter size remained higher than expected under an epistasis model of heterosis. There

are several alternative explanations that may have contributed to the observed lack of decline in litter size with continued random mating. It is possible that 14 generations of random mating have not yet disassociated loci on the same chromosome involved in heterosis. Another potential explanation for maintenance of heterosis is that unintended selection for litter size may have occurred during the production of the AIL because only animals that produced litters were used in successive mating. However, fertility selection was limited by having each litter contributing equally to the next generation. Even so, some segregating genes involved in epistatic interactions may have favoured facilitating their reunion each generation. A third possibility is that epistatically interacting loci on different chromosomes are in linkage equilibrium in the F_2 generation because of the nonrandom mating of previous generations. In this

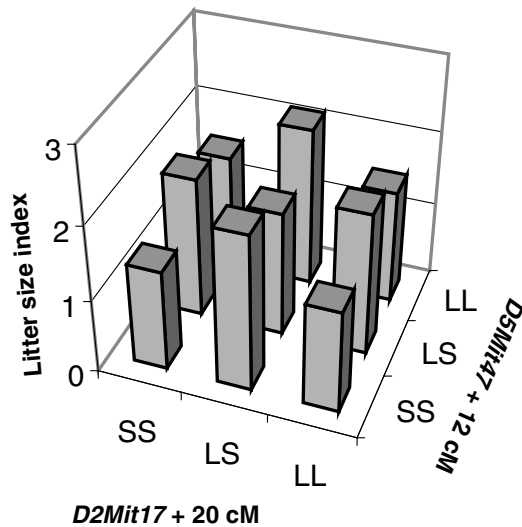


Fig. 4 Litter size index genotypic values indicating dominance-by-dominance interaction between chromosomal regions *D2Mit17* + 20 cM and *D5Mit47* + 12 cM.

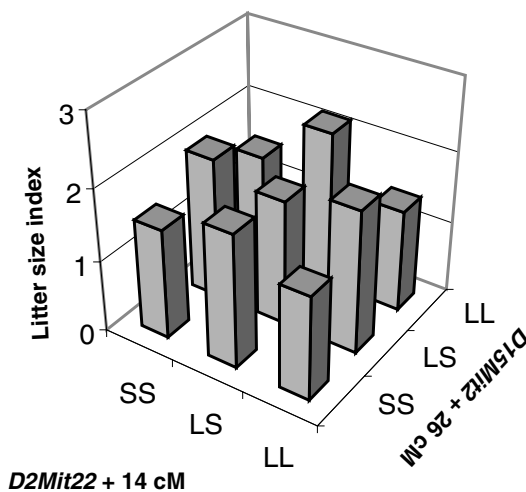


Fig. 5 Litter size index genotypic values indicating dominance-by-dominance interaction between chromosomal regions 14 cM downstream from *D2Mit22* and 26 cM from marker *D15Mit2*.

situation, we do not expect average litter size to decrease over generations because the dissociation of most pairs of epistatic loci involved in heterosis has already occurred in generation F_2 . All the epistasis reported here is between loci on different chromosomes.

The estimate of the effective number of factors involved in our cross was very low ($n_e = 0.04$). This is reflected in our finding only one QTL that fits the model assumed in making this calculation, i.e. additive. The number of segregating loci in the F_2 generation may be masked by 'nullification effect' on both parental lines

(Lynch & Walsh, 1998). This effect may occur because some F_2 individuals exhibit more extreme litter size values than are seen in either parental line (transgressive segregation).

We used an index as an estimator of litter size breeding value in the QTL study. If necessary, this index can be rescaled to the number of pups, although such rescaling would not affect the statistical results (Falconer & Mackay, 1996). The accuracy of the index as a measure of the breeding value was twice that of individual phenotypes, thereby increasing power for QTL detection. As this index is a linear inference of F_2 phenotypes based on F_3 information, 1/4 of the dominance variation and a varying amount of the epistatic genetic variation, depending on the types of epistasis present, are lost in these inferences.

The regression of litter size index on genotype scores detected two QTL with distinct patterns of gene effect. As both QTL were only significant at the chromosome-wide level and one false-positive QTL is expected at this level under the null model, these results require confirmation by further work. Additivity at *D7Mit21* + 0 cM indicates that heterozygous females have litter sizes close to the midpoint of the homozygotes with the LG allele conferring a larger litter. LG/J homozygotes, on average, have 0.36 more pups per litter than SM/J homozygotes at this locus. Overdominance at the locus at *D12Mit6* + 8 cM indicates that heterozygous mothers have the largest litter sizes, consistent with the finding of heterosis in the original cross. Litter size was not affected by interactions between these loci. The relative lack of single locus additive genotypic effects found here is consistent with the low, nonsignificant level of heritability reported for the mother-offspring regression.

The two-way genome-wide scan found many more regions involved in epistatic interactions for litter size than expected under the null model indicating the ubiquity of epistasis for litter size in this cross. Ten times more epistatic pairs were observed at the 0.001 level than would be expected if there were no epistasis. These results are expected for this cross because of the strong heterosis observed in the F_1 hybrids and the observed lack of fit of a pure dominance model. Four interactions were significant at the Bonferroni-corrected level. Regions involved in these epistatic interactions lie on chromosomes 2, 4, 5, 11, 14, 15 and 18. Chromosome 2 has two regions with epistatic interactions whose confidence intervals overlap. No other region participates in more than one interaction.

All four forms of epistasis were found among our four most significant epistatic pairs. In two cases, more than one form of epistasis was involved, generating a more complex pattern of interactions among genotypes. The interaction between chromosomes 11 and 14 involves both additive-by-additive and dominance-by-dominance interaction. Inspection of the pattern of genotypic values indicates that this two-locus epistasis would not produce

heterosis in this cross because the highest genotypic values are for the parental double homozygotes. While there is no additive genetic variance for these two loci in the F_2 population, additive variance would increase as allele frequencies become more extreme. For example, if either locus drifted to fixation while the other locus remained variable, the variable locus would have a high additive genetic variance (Cheverud & Routman, 1996). In this instance the allele producing larger litters at the variable locus would differ depending on which allele happened to be fixed at the alternate locus.

The interaction between chromosomes 4 and 18 involves both additive-by-dominance and dominance-by-additive epistasis. This pair of loci can contribute to heterosis in the cross because the double heterozygote has a larger litter size than the midpoint of the parental double homozygotes, although the double SS homozygotes have the largest litters. The pattern of genotypic values does not lead to generally higher additive variance as allele frequencies become more extreme, as in the first example, but the evolutionary outcome of selection would differ as populations randomly drift. For example, if the LG allele became fixed on chromosome 18, selection would favour the heterozygote on chromosome 4 with its alleles taking on intermediate frequencies at a stable equilibrium, while if the chromosome 18 S allele became fixed, an unstable equilibrium would be produced at chromosome 4, the eventual outcome being determined by random deviations from the equilibrium. A similar scenario of stable vs. unstable equilibria occurs for the chromosome 18 alleles depending on which allele on chromosome 4 happens to be fixed.

In the other two interactions, between *D2Mit17* + 20 cM and *D5Mit47* + 12 cM and between *D2Mit22* + 14 cM and *D15Mit2* + 26 cM, a negative dominance-by-dominance epistasis was found, indicating that all single heterozygotes have larger litters than the double homozygotes and/or the double heterozygote. Patterns such as these contribute to heterosis in the cross because the double heterozygote has larger litters than either parental homozygote, although litter sizes are largest for the single heterozygotes. Dominance-by-dominance epistasis leads to enhanced additive genetic variance as populations drift away from intermediate allele frequencies (Cheverud & Routman, 1996), although this effect is not as extreme as seen for additive-by-additive epistasis like that found between the chromosome 11 and 14 loci. Furthermore, whenever one of the loci involved in dominance-by-dominance interactions happens to become fixed, selection will maintain a balanced polymorphism at the other locus.

Our results indicate two individual gene effects that account for 12.6 % of the variation in litter size, and some strong epistatic interactions. Although litter size has low heritability and low repeatability, taken together, all QTL and interactions explain nearly 49% (39.5% adjusted multiple r^2) of the total phenotypic variation for this

trait in our cross. This increase is a significant improvement to the single locus model and highlights the significance of epistasis in litter size in our cross. The importance of epistasis for litter size is consistent with the original finding of strong heterosis in the cross.

One can certainly question the generality of our findings in that they were obtained from a single, synthetic population produced by the intercross of inbred mouse strains. While this is clearly not a natural population, our results still have some general significance for evolutionary studies. First, any single natural or synthetic population is unique in its genetic characteristics (alleles present and their frequencies) making results difficult to generalize to other populations with other unique histories. Thus a lack of generality in this sense affects all population studies whether of natural or synthetic populations. However, our specific synthetic population is highly unusual in that all segregating loci have intermediate allele frequencies, when this situation is unlikely to be true of natural populations. Thus, to generalize from any population study one needs to consider population characteristics under alternate allele frequencies, as in our discussion above of the effects of fixation of one or the other allele on population processes.

The generality of our synthetic population can also be considered in the light of its history. The exact origins of the LG/J and SM/J mouse strains are unclear with regard to their relationships with other inbred strains (Goodale, 1938; MacArthur, 1944; Beck *et al.*, 2000) but studies of genetic markers in these strains indicate that they are independently derived from a base population of mice that gave rise to the recognized modern strains (Silver, 1995; R. Williams & J. Cheverud, unpublished data). In general, the alleles carried by any given strain can be considered as having been randomly drawn from this basal mouse population. Thus, alleles segregating in an intercross are a random sample of alleles present in the base population and can be taken as representing that base in the manner of their phenotypic effects and interactions. The situation with LG/J and SM/J is a bit more complicated because each strain was selected to fix large and small body size alleles at 63 days, so the alleles affecting body size are not randomly taken from the base population but, instead, they are selected for their effects on body size. In our population the correlation between body size and litter size is minimal ($r = 0.05$, n.s.), suggesting that the allelic effects examined here are for a random set of alleles. In this sense, the allelic effects discovered here can still be generalized to populations at large. We are examining the effects on litter size of a random set of alleles segregating at a random set of loci. The fact that we are examining them in an F_2 intercross population rather than a natural population is an advantage because that is the situation in which the alleles produce the largest genetic variance and are most easily detected. Thus our synthetic intercross population

can be considered as a test bed for the effects of variable alleles present in mice and has generality in that sense.

The genetic architecture of litter size in this cross contrasts strongly with that found for a wide range of morphological and body composition traits (Cheverud *et al.*, 1996, 1997, 2001; Leamy *et al.*, 1999) previously analysed. While, like litter size, these traits are often affected by epistatic interactions (Routman & Cheverud, 1997; Cheverud, 2000; Leamy *et al.*, 2002), they do not display heterosis in the LG/J by SM/J cross and are affected by many single locus QTL of small additive effect. These differences in genetic architecture are not due to differences in allele frequencies at loci affecting litter size and morphological traits, as all loci in this segregating F₂ population have intermediate allele frequencies. Instead, architectural differences between morphological and life-history traits are due to differences in patterns of genotypic values for the traits.

The relative lack of single locus effects for litter size is only matched by maternal performance for offspring survivorship (Peripato *et al.*, 2002; Peripato & Cheverud, 2002) and fluctuating asymmetry for mandibular size (Leamy *et al.*, 2002). All three of these traits share a genetic architecture composed of many interacting small effect loci combined with a relative lack of difference between the parental strains. They also tend to lack heritability in segregating populations (Falconer & Mackay, 1996).

As discussed above, the low level of heritability displayed by fitness-related traits has been thought to be due either to a lack of heritable variance because of fixation at loci affecting fitness or to a relatively high level of nonheritable genetic variance at such loci. The genetic architecture displayed by litter size in this experiment is consistent with models where there is a low level of heritable variation combined with a substantial underlying pool of nonheritable genetic variation. In this QTL study most of the genetic variation we detected for litter size had nonheritable dominance and epistatic effects. As the effects of different individual loci change depending on gene frequencies at other loci in an epistatic system (Goodnight, 1995, 2000), single locus additive studies are not the most effective methodology to study gene effects on such traits. An approach that can account for genetic interactions, such as the one presented here, is necessary to reveal the genetic basis of trait variation. We also show that in our population the genetic architecture for fitness-related traits, such as litter size, is dramatically different from that for somatic traits and morphologies. While epistasis is important for all of these traits, heritable additive effects are largely absent for fitness. In a few cases of epistasis chosen for detailed examination, epistasis patterns for litter size often lead to heterozygote superiority at one locus when alleles at another locus are fixed, as with additive-by-dominance, dominance-by-additive and dominance-by-dominance forms of epistasis. After random fixation of one of the

pairs of loci variation at the second locus would be maintained by balancing selection. The importance of nonadditive genetic variation detected here is even more impressive if one considers that the usage of a linear index to estimate litter size is expected to lead to a loss of dominance and epistasis variation but not of additive variation. Furthermore, although heritability is nil in the F₂ generation with its intermediate allele frequencies, we expect new additive variance for litter size to be produced by genetic drift as allele frequencies become more extreme.

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