Quantitative Trait Loci for Maternal Performance for Offspring Survival in Mice

Andréa C. Peripato,*[,1] Reinaldo A. de Brito,[†] Ty T. Vaughn,[†] L. Susan Pletscher,[†] Sergio R. Matioli* and James M. Cheverud[†]

**Department of Biology/Genetics, IB, Universidade de São Paulo, São Paulo, SP 05508-900 Brazil and [†]Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110*

Manuscript received November 2, 2001
Accepted for publication August 2, 2002

ABSTRACT

Maternal performance refers to the effect that the environment provided by mothers has on their offspring's phenotypes, such as offspring survival and growth. Variations in maternal behavior and physiology are responsible for variations in maternal performance, which in turn affects offspring survival. In our study we found females that failed to nurture their offspring and showed abnormal maternal behaviors. The genetic architecture of maternal performance for offspring survival was investigated in 241 females of an F_2 intercross of the SM/J and LG/J inbred mouse strains. Using interval-mapping methods we found two quantitative trait loci (QTL) affecting maternal performance at *D2Mit17* + 6 cM and *D7Mit21* + 2 cM on chromosomes *2* and *7*, respectively. In a two-way genome-wide epistasis scan we found 15 epistatic interactions involving 23 QTL distributed across all chromosomes except *12*, *16*, and *17*. These loci form several small sets of interacting QTL, suggesting a complex set of mechanisms operating to determine maternal performance for offspring survival. Taken all together and correcting for the large number of significant factors, QTL and their interactions explain almost 35% of the phenotypic variation for maternal performance for offspring survival in this cross. This study allowed the identification of many possible candidate genes, as well as the relative size of gene effects and patterns of gene action affecting maternal performance in mice. Detailed behavior observation of mothers from later generations suggests that offspring survival in the first week is related to maternal success in building nests, grooming their pups, providing milk, and/or manifesting aggressive behavior against intruders.

MATERNAL performance is one of the major components of fitness (Falconer and Mackay 1996) because reproductive success is a consequence not only of the fecundity of the parents, but also of the survival of the offspring. Indeed, environmental effects determined by the mother are more important than any other single factor in determining variation in early offspring size, growth, and survival (Lee *et al.* 1991). Variations in the environment provided by individual mothers for their offspring are due, in part, to genetic differences among mothers. The effect that a mother has on her offspring's phenotypes, independent of the genes she has transmitted, is referred to as her maternal performance and is measured in terms of the effect she has on her offspring's characteristics (Cheverud and Moore 1994). Variations in maternal performance are likely to be due to variations in maternal physiology and behaviors, such as nest building, grooming, and milk production.

Despite recent interest in maternal effects (*e.g.*, see Mousseau and Fox 1998; Wolf *et al.* 1998), maternal performance often has been overlooked in the evolutionary genetic literature. This has occurred because of the difficulty of collecting critical data and because many researchers considered this trait as environmental with regard to the offspring phenotypes. While this is true, variation among mothers in their performance can be due to both genetic and environmental effects operating on the mother. Even so, as a fitness component, maternal performance is expected to have a relatively low additive genetic variance and low heritability (Falconer and Mackay 1996). Despite this expectation, some maternal performance constituents, such as nest-building behavior ($h^2 = 0.27$) in mice (Bult and Lynch 2000) and egg volume ($h^2 = 0.55$) in passerine birds (Potti 1999), have moderate heritability, indicating a substantial genetic basis. A survey of the agricultural literature indicates that maternal performance for offspring growth in mammals has a moderate heritability (Cheverud 1984). Considering the importance of maternal performance, if only one or a few genes were responsible for these traits, most of the genetic variation would have been eliminated by selection and drift (Bult and Lynch 2000), which suggests that maternal performance is a complex quantitative trait.

Observation of certain maternal behaviors allows us to investigate the causes of variation in maternal performance for offspring survival. Many different factors af-

[1]*Corresponding author:* Departamento de Biologia/Genética, Instituto de Biociências, Universidade de São Paulo, Rua do Matão 277 sala 300, São Paulo, SP 05508-900 Brazil. E-mail: peripato@ib.usp.br

fecting maternal behavior have already been identified. During pregnancy, females have extensive hormonal alterations that enhance neural activity and contribute to changes in maternal behavior (Kinsley *et al.* 1999). In rodents, hormonal changes during pregnancy and parturition are an additional factor that contributes to the rapid appearance of maternal behavior (Rosenblatt 1967). Oxytocin has different roles in rats and mice, but it is essential for maternal performance in both, as is estradiol (Pedersen *et al.* 1982; Nishimori *et al.* 1996; Yamamuro and Sensui 1998). Prolactin is required for normal reproduction and mammary gland development in mice (Horseman *et al.* 1997; Alston-Mills *et al.* 1999), and the prolactin receptor (*PRLR*) is a regulator of maternal behavior (Lucas *et al.* 1998). The neurochemical mechanisms involved in maternal behavior have been described as involving interactions among critical areas in the brain such as the hippocampus, the media preoptical area, and several areas of the hypothalamus (Flannelly *et al.* 1986; Bernardis and Bellinger 1996; Lonstein and Stern 1997; Olazabal and Ferreira 1997; Numan *et al.* 1998).

Individual gene effects on behaviors affecting maternal performance have been studied through the knockout of various genes. Several genes involved in maternal care have been identified in this way, all of which are associated with the central nervous system, and particularly with the hypothalamus (Brown *et al.* 1996; Thomas and Palmiter 1997; Lefebvre *et al.* 1998; Lucas *et al.* 1998; Li *et al.* 1999). Although all knockout females (*FosB*, *Dbh*, *Mest*, *PRLR*, and *Peg3* deficient) presented normal olfactory capacity, a prerequisite for maternal behavior in mice (Gandelman *et al.* 1971), they showed various abnormal behaviors associated with these genes. Lack of pup retrieval and crouching over the nest were the only behaviors in common among these knockouts. *Mest*- and *Peg3*-deficient mice performed poorly in nest building (Lefebvre *et al.* 1998; Li *et al.* 1999), a problem that *Dbh*-deficient females did not exhibit (Thomas and Palmiter 1997). *FosB*- and *Dbh*-deficient females had lactation problems (Brown *et al.* 1996; Thomas and Palmiter 1997), and the lack of placentophagia was reported in *Mest*- and *Dbh*-deficient females (Thomas and Palmiter 1997; Lefebvre *et al.* 1998).

Inbred mouse strains are often difficult to maintain because of reduced litter size and maternal failure to nurture offspring (Silver 1995; Falconer and Mackay 1996). This failure can be due to environmental disturbance (especially diseases) or, in some cases, to a deleterious mutation becoming fixed in the strains during the inbreeding process (Festing 1979). During the early stages of inbreeding, many of the animals may be infertile or in poor condition due to the occurrence of deleterious recessive alleles in homozygous states (Silver 1995). This reduction in fitness is usually overcome in F_1 hybrids between different inbred lines. The hybrids often show heterosis and better maternal performance than that of their inbred parents. The recovery of fertility and effective maternal care in hybrids indicates a genetic basis for variations in maternal performance among strains.

While we were establishing recombinant inbred and random bred mouse strains from an intercross of the Large (LG/J) and Small (SM/J) inbred mouse strains (Cheverud *et al.* 1999) for genetic mapping studies, we found females that failed to nurture their offspring and showed abnormal maternal performance. This aberrant behavior often led to loss of the entire litter. Although these behaviors occur in both parental strains, it is much less frequent in F_1 hybrids, indicating a genetic basis for the behavioral complex.

We investigate the genetic basis of maternal performance by studying the association between individual quantitative trait loci (QTL) and abnormal maternal performance for offspring survival in the cross of two inbred strains of mice. Maternal performance was determined on the basis of success or failure of the mother in maintaining at least one offspring alive in the first week after birth. F_2 females from an intercross between the LG/J and SM/J mouse strains were randomly mated to F_2 males and the success or failure of their litters was scored. This study allowed a QTL search on all 19 murine autosomes and the identification of the number and relative size of gene effects and patterns of gene action affecting maternal performance in this cross. Additionally, we examined maternal behavioral components in later generations of the same cross to characterize which maternal behaviors modulated maternal performance for offspring survival in the intercross population.

MATERIALS AND METHODS

Mouse strains and breeding: The mouse strains used in this study are the LG/J and the SM/J inbred lines. The history and details of animal husbandry are available in Cheverud *et al.* (1996), Kramer *et al.* (1998), and Vaughn *et al.* (1999). We utilize these strains because their genomes have been characterized extensively and because they show heterosis in the cross for the traits we are investigating. Ten SM/J males were mated with 10 LG/J females, producing 41 hybrids autosomally identical to one another and heterozygous at each locus that differs between the parental strains. The F_1 hybrids were randomly mated, producing 510 F_2 progeny. Animals born were individually identified within the first week. Three weeks after birth they were 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). At 10 weeks 241 F_2 females were randomly mated with F_2 males to form F_3 progeny. Males were removed from the breeding cage when their mate was determined to be pregnant. Each female's litter size and litter survival through the first week was recorded. These intercrosses have continued over generations to produce recombinant inbred (RI) and advanced intercross (AI) lines. To produce these lines, two different systems of mating were used: brother-sister and random mating to produce, respectively, RIs and AIs. Furthermore, we continue to breed the parental strains LG/J and SM/J in our mouse facility.

Scoring maternal performance for offspring survival: The first week of life is an important period for offspring survival. The major factor determining the life or death of the litter in this phase is maternal performance (Lee *et al.* 1991). In our cross we examined data from these females and scored whether or not they lost the whole litter in the first week after birth. Usually, in our cross, primiparous females with loss of the entire litter maintained this pattern in consecutive parities, so this abnormal maternal performance was not just random failure due to stress or inexperience. Maternal performance for offspring survival was scored as the ability of the female to maintain the litter alive through the first week of life. If only part of the litter was lost, the mother still was considered successful, whereas mothers that lost the entire litter were considered unsuccessful. We scored litter size and maternal performance on the basis of offspring survival in mothers from LG/J and SM/J inbred lines, 21 F_1 and 241 F_2 generation females. Offspring survival for these 241 F_2 females was scored as an all-or-none trait and used in a QTL study of maternal performance for offspring survival in this cross.

Molecular genotyping: Total cellular DNA was extracted from liver using DNA QIAamp tissue kit (QIAGEN, Chatsworth, CA). PCR amplification of microsatellite loci was performed according to the protocol described by Dietrich *et al.* (1992) and modified by Routman and Cheverud (1994). PCR product was visualized using 5–6% agarose gels and ethidium bromide staining. Ninety-six polymorphic loci were scored in our study to cover all 19 autosomes as completely as possible (Vaughn *et al.* 1999; Cheverud *et al.* 2001). The *X* chromosome was excluded from this study because of a relative lack of microsatellite marker variability (Routman and Cheverud 1995; Cheverud *et al.* 1996). The relative positions of these markers are given in the Mouse Genome Database (2001). However, map distances are known to vary between crosses so for this study map distances were calculated using MAPMAKER 3.0b (Lander *et al.* 1987; Lincoln *et al.* 1992) as described in Vaughn *et al.* (1999) and Cheverud *et al.* (2001).

Statistical procedures: *Interval mapping:* The presence of potential QTL and their relative positions were determined by interval mapping (Lander and Bolstein 1989) using multiple regression analysis (Haley and Knott 1992). This analysis consists of regressing the phenotype (maternal performance, or MP) on genotype scores every 2 cM along each chromosome. Genotype scores are calculated using the probability that an individual is homozygous for either parent allele or heterozygous at the specified location. These probabilities are multiplied by −1, 0, and 1 for homozygous SM/J or heterozygous or homozygous LG/J, respectively, and summed to obtain an additive genotype score (X_a). Likewise, the dominance genotypic score (X_d) is calculated as the probability of heterozygosity at the arbitrary intermediate location. Genotypic scores are imputed using the genotypes measured at the flanking markers and the rates of recombination between the location of interest and the flanking markers (Haley and Knott 1992). We used the model,

$$\mathrm{MP} = \mu + aX_a + dX_d + e,$$

where μ is a constant, a is the additive genotypic value, X_a is the additive genotype score, d is the dominance genotypic value, X_d is the dominance genotype score, and e is the residual. These regression coefficients are estimates of the additive (a) and dominance (d) genotypic values if a QTL occurs at the tested position (Falconer and Mackay 1996). The probability of a gene affecting the character at that specific location was obtained using the SETCOR procedure in SYSTAT 8.0 (Cohen and Cohen 1983; Cohen and Wilkinson 1997).

We use parametric models here even though the dependent and independent variables—maternal performance for offspring survival and genotype score—are not in interval scale. Hilton (1976) argues that the significance tests obtained from this model are accurate as established by Kort (1973). Other studies have confirmed the feasibility of using interval mapping for QTL studies of binary traits in intercrosses and backcrosses (Visscher *et al.* 1996; Rebai 1997; McIntyre *et al.* 2001) or even in multifamily half-sib designs (Kardamideen *et al.* 2000).

Significance levels: Statistical significance of one-QTL models was evaluated using LOD scores. Because of multiple comparison problems (Lander and Kruglyak 1995), we adjusted pointwise probabilities 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). The effective number of independent tests was calculated using the variance of the eigenvalues of the intermarker correlation matrix calculated separately for each chromosome. A Bonferroni-corrected significance threshold was then obtained by dividing the pointwise threshold (0.05) by the number of independent tests. We calculated two threshold levels to distinguish between chromosome-wide and genome-wide significance levels. The chromosome-wide significance threshold was obtained by dividing 0.05 by the number of independent tests calculated for each chromosome, while the genome-wide threshold was obtained by dividing 0.05 by the number of independent tests summed over all the chromosomes. This approach has been validated using simulation results (see Cheverud 2001 for details). A chromosome-wide significance value is very informative in that it strikes a balance between eliminating false-positive results and avoiding false negatives (Rao 1998; Weller *et al.* 1998). The genome-wide threshold is an important value because LOD scores above the genome-wide threshold indicate highly significant evidence of linkage, while those exceeding only the appropriate chromosome-wide threshold suggest linkage, requiring confirmation from additional data (Lander and Kruglyak 1995).

We also evaluated statistical significance of the single-locus QTL genome scan by running a permutation test (Churchill and Doerge 1994; Doerge and Churchill 1996) in QTL Cartographer (Basten *et al.* 1994, 2002). Experiment-wise statistical significance is obtained by rearranging phenotypes to genotypes 1000 times. The single-locus QTL detected in these analyses were the same as we obtained using the Haley and Knott (1992) model, and their likelihood estimates were also very similar. Thus the use of the Haley and Knott (1992) model with a binary dependent variable does not compromise the analysis.

Epistasis: The interaction among single-locus QTL detected by interval mapping was tested using the "physiological" epistasis model (Cheverud and Routman 1995; Routman and Cheverud 1997; Cheverud 2000) in SAS (SAS Institute 1998). In this model, epistatic genotypic values are defined and their contributions to additive, dominance, and interaction variances are all included in significance testing for epistasis. For this study, each pair of maternal performance QTL was investigated for epistatic interactions, where 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 ($X_{a1} \times X_{a2}$), additive by dominance ($X_{a1} \times X_{d2}$), dominance by additive ($X_{d1} \times X_{a2}$), and dominance by dominance ($X_{d1} \times X_{d2}$), were considered as independent variables and maternal performance as the dependent variable. Statistical significance was tested as described in Routman and Cheverud (1997) and Cheverud (2000).

We used an interchromosomal two-way genome-wide scan

performed at every 2 cM along the mouse chromosomes to test for epistasis across the whole genome (Cheverud 2000). At each pair of locations, a test for genic epistasis was performed. We used a generalization of multiple regression procedures that allows us to regress the dependent variable(s) on the four interaction components ($X_{a1} \times X_{a2}$, $X_{a1} \times X_{d2}$, $X_{d1} \times X_{a2}$, $X_{d1} \times X_{d2}$) holding the main effects ("a" and "d" for each of the two loci) constant (Cohen and Cohen 1983). This was done to obtain a single, joint significance test for the interaction terms in the model independent of their direct effects. The model is

$$\mathrm{MP} = \mu + aaX_{a1}X_{a1} + adX_{a1}X_{d2} + daX_{d1}X_{a2} + ddX_{d1}X_{d2}|X_{a1}, Xd_1, X_{a2}, X_{d2},$$

where MP is the 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}, and X_{d2} at the specified location. The *aa*, *ad*, *da*, and *dd* regression coefficients measure additive-by-additive, additive-by-dominance, dominance-by-additive, and dominance-by-dominance genotypic values for epistasis, respectively. The "|" indicates that the independent variables listed to the right are partialed out of the independent variables to the left. This provides a joint test for the interaction terms independent of tests for the single-locus scores. The probability obtained and parameters estimated are the same as in a standard multiple regression (Cohen and Cohen 1983). We tested the two-locus interactions separately from the single-locus effects because we had already done a single-locus scan, in this case finding only two single-locus QTL.

The number of independent tests in the two-way genome-wide scan was estimated by summing the products of the numbers of independent tests for each pair of chromosomes over all chromosome pairs (Cheverud 2000). This is likely to be an overestimate of the true number of independent tests because there will be complex correlations between tests involving different chromosome pairs that share a chromosome. This makes our Bonferroni correction for epistasis conservative. We calculated 2736 independent tests in the two-way genome-wide scan. Given this number of tests, we expect 136 false-positive significant results at the 0.05 pointwise level even in the absence of epistasis. To control for "false-positive" results, we used a significance threshold correction based on the Bonferroni test (Cheverud 2001). A significant epistatic interaction in this study was considered when epistasis at the pairs of positions reached the Bonferroni threshold level of 0.1, which in our case corresponds to the probability value of 3.7×10^{-5} ($= 0.10/2736$). We also considered epistasis significant if one of the four modes of epistasis (additive by additive, additive by dominance, dominance by additive, and dominance by dominance) was significant at the 9.3×10^{-6} level ($3.7 \times 10^{-5}/4$), even if the overall epistasis model was not significant.

A randomization procedure was performed to corroborate the significance values obtained for epistatic interactions. This randomization consisted of creating 1000 data sets by sampling without replacement from the original maternal performance data and keeping the genotypes unchanged. Because of the extremely large number of interactions in a two-way genome-wide scan, the randomization test among all chromosomes was prohibitive; therefore we analyzed the interactions on a subsample of the two-way tests, those involving chromosomes *6* and *10*. These chromosomes were chosen because they are of average size and had one significant epistatic interaction detected using the procedures described above. Every possible interaction between locations on these two chromosomes was investigated for each of the 1000 randomly created data sets using the model previously described. When a significant interaction was detected, a full regression model was prepared and the probability of significant epistasis estimated. We expected 50 of the 1000 randomizations to reach significance by chance at a probability level corresponding to the 0.05 Bonferroni-corrected level. However, only 31 tests displayed interaction that surpassed this level (0.00276 for these two chromosomes) and none achieved the probability level obtained in the analysis of the original data. The observation of 31 false-positive results rather than the expected 50 indicates that our methods are conservative when evaluating a two-way genome-wide scan with a binary dependent variable. The probability of obtaining ≤ 31 false positives rather than the expected 50 is <0.006.

Behavior observation: An ancillary behavioral observation study was undertaken to provide a better understanding of the specific behaviors related to maternal performance for offspring survival in the intercross population. We could not perform these observations in the F_2 generation since these females were no longer alive; hence we studied later generations of the same intercross that were being maintained in our mouse facility. The information obtained from these later generations suggests which maternal features would have been associated with offspring survival in the F_2 generation. Thus, we observed 199 pregnant females from matings performed to produce RI lines and an advanced intercross line (random matings) in the F_9–F_{14} generations. We calculated inbreeding levels using pedigree data and the PEDSYS (Pedigree Data Management System) program (Dyke 1996) using the Quaas-Henderson algorithm (Boyce 1983). The maternal features we investigated included nest building before and after delivery, placentophagia and pup grooming, presence of milk in the stomach of the pup (indicating presence of milk in the mother), aggressive behavior against intruders, and pup retrieval. Maternal behavioral monitoring began when pregnancy was detected and was completed 7 days after delivery. Observation was performed daily for 10–15 min per female.

The analyses of behavioral data were undertaken as follows: association among nominal variables was tested by cross-tabulation using the Pearson chi-square test and the phi coefficient, while association among scalar variables was examined using the general linear model procedures in SYSTAT 8.0. The phi coefficient is a standard measure of association for two-way tables corresponding to the Pearson product moment correlation between binary variables. Because reproductive success has been negatively associated with inbreeding, we first tested the association of inbreeding with behavioral variables using the following model:

$$F = \mu + \mathrm{N1} + \mathrm{N2} + G + M + \mathrm{AB} + e. \quad (1)$$

A second model was used to test the association among variables and female maternal performance for offspring survival (Success):

$$\mathrm{Success} = \mu + \mathrm{N1} + \mathrm{N2} + G + M + \mathrm{AB} + F + e, \quad (2)$$

where μ is the constant, N1 is the prepartum nest, N2 is the postpartum nest, G is placentophagia and grooming pups after birth, M is pup stomachs with milk, AB is aggressive behavior to intruders, F is the inbreeding coefficient, and e is the residual.

RESULTS

Maternal performance for offspring survival: Data from litter size at birth and litter survival in the first week after birth are represented in Table 1. Data were collected from mothers across generations F_1 and F_2 and also from females from LG/J and SM/J inbred lines. To increase sample size of the inbred parental strains, these

TABLE 1

Maternal performance for offspring survival

Generations	*n*	Litter size average	Unsuccessful females	Successful females
SM/J	64	5.60	17	47
LG/J	35	6.48	21	14
F_1	21	11.11	0	21
F_2	241	9.15	31	210

values were estimated from females currently maintained in our laboratory. The LG/J strain shows significantly lower rates of maternal success than those of the SM/J strain (40 *vs.* 74%, respectively). However, each parental strain has a lower success rate than that of the F_1 (100%) or F_2 (85%) hybrids.

Interval mapping: The regression of maternal performance for offspring survival on their genotype scores in the 241 F_2 females at 96 microsatellite loci allowed a genome scan for QTL for this trait. We found highly significant linkage at the genome-wide level on chromosome *7* at *D7Mit21* + 2 cM (Figure 1) using both Bonferroni-correction and randomization-based significance thresholds. This locus is underdominant and explains 6.2% of the phenotypic variance in maternal performance (Table 2). A suggestive linkage at the chromosome-wide level was found on chromosome *2* at *D2Mit17* + 6 cM (Figure 2) using the Bonferroni-corrected significance threshold. This result exceeded the genome-wide 5% significance threshold as determined by randomization. As can be seen in Table 2, this locus is overdominant and accounts for 4.4% of the variance in maternal performance.

Epistasis between markers on chromosome *2* and *7* was not significant for the overall model ($P = 0.123$), although there is borderline significance ($P = 0.025$) at the Bonferroni-corrected level for dominance-by-dominance epistasis (Figure 3).

Epistasis: In the two-way genome-wide epistasis scan, a total of 346 epistasis tests are significant at the 0.05 pointwise level, 2.5 times the number expected by chance (136). Furthermore, 15 of these 346 exceed the Bonferroni-corrected significance criterion, 5 at 0.05 (1.8×10^{-5}) and an additional 10 at the 0.1 (3.7×10^{-5}) levels. Therefore, maternal performance differs among genotypes at one locus, depending on which genotypes are present at another locus. These pairs of loci are summarized in Table 3. Twenty-three chromosomal regions were involved in these 15 interactions (markers with overlapping confidence regions were conservatively considered to be a single locus). Of the two QTL identified in the single-locus analysis, *D2Mit17* + 6 cM interacts significantly with other regions across the genome, but *D7Mit21* + 2 cM does not, although a separate region on chromosome *7* (*D7Nds1* + 0 cM) is involved in epistatic interactions. Chromosomes *12*, *16*, and *17* were the only ones that did not present significant interactions for epistatic QTL for maternal performance. Most loci participate in only one interaction although 7 of 23 loci participated in two or more interactions (Figure 4). These loci are not involved in a single unified network, but form several small separate sets of interacting loci.

All four forms of epistasis are represented in the results. Since we found 25 significant epistasis coefficients among the 15 significant interactions and since we have four forms of epistasis, we would expect to find each form approximately 6 times. Our results did not deviate from this expectation. Additive-by-additive epistasis (*e.g.*, Figure 5) occurred 6 times, additive-by-dominance and dominance-by-additive epistasis (*e.g.*, Figure 6) appeared 11 times, and dominance-by-dominance epistasis (*e.g.*, Fig-

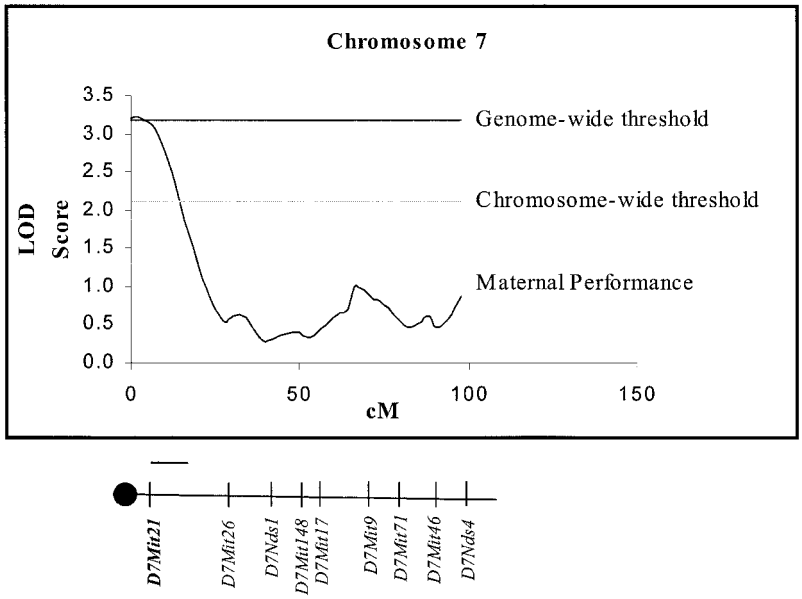

FIGURE 1.—LOD plot of chromosome 7. Significant LOD score (3.21) at genome-wide level (3.18) at 2 cM downstream of the marker *D7Mit21* indicates highly significant evidence of QTL in this position. The chromosome-wide significance threshold for this chromosome is 2.12.

TABLE 2

Quantitative trait loci affecting maternal performance

Locus	Position marker (cM)	Position centromere (cM)	C.R. (cM)	a	d	$2a/\sigma p$	$d/\sigma p$	% VAR	LOD score
D7Mit21	2	2	0–14	−0.03	−0.19	−0.17	−0.57	6.18	3.21
D2Mit17	6	94	72–108	0.05	0.16	0.29	0.49	4.61	2.43

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

ure 7) occurred 8 times (7 of them as negative dominance-by-dominance epistasis). A multiple regression model involving only the direct-effect loci on chromosomes *2* and *7* as independent variables has an adjusted multiple r^2 of 10%. A second multiple regression model containing all significant direct effects, the direct effects of loci involved in epistatic interactions, and significant epistatic effects accounts for 34.5% of the phenotypic variation after adjusting for the number of independent variables. Thus a fairly significant proportion of phenotypic variation in maternal performance is due to epistasis.

Behavior observation: The frequency of maternal performance features observed in this study is presented in Figure 8. Observations from later generations indicate that pregnant female mice usually start to build a nest before delivery and maintain it postpartum. This behavior was found in most of the 144 successful females in our behavioral study. Although unsuccessful females (55) sometimes showed pre- and postpartum nest building, the nests built generally were of poor quality (data not shown). Successful and unsuccessful females differed significantly for these traits ($\phi = 0.152$ and $P = 0.033$ for prepartum and $\phi = 0.343$ and $P = 1.5 \times 10^{-7}$ for postpartum nest building). Placentophagia and cleaning pups was undertaken more frequently by successful rather than unsuccessful females, but the difference is of only borderline significance ($\phi = 0.127$, $P = 0.0739$). Presence of milk in the stomach of the pup is a major distinction between the two kinds of females ($\phi = 0.886$, $P = 7.57 \times 10^{-36}$). Unsuccessful females fail to provide milk for their offspring. This failure could also be due to absence of suckling behavior in pups; however, unsuccessful females in most cases do not handle the pups. Consequently, this absence of milk is most likely due to the mothers' failure rather than to failed suckling behavior in their offspring. Aggressive behavior against intruders and pup retrieval was measured together because all females that performed aggressive behavior also retrieved their pups. Unsuccessful females, generally, did not respond to external stimulus and did not rescue pups removed from the nest ($\phi = 0.439$, $P = 1.0 \times 10^{-9}$), in contrast to successful females that would usually attack our hands and, after carrying all pups back to the nest, immediately crouch over them.

Inbreeding values for females used in the behavioral

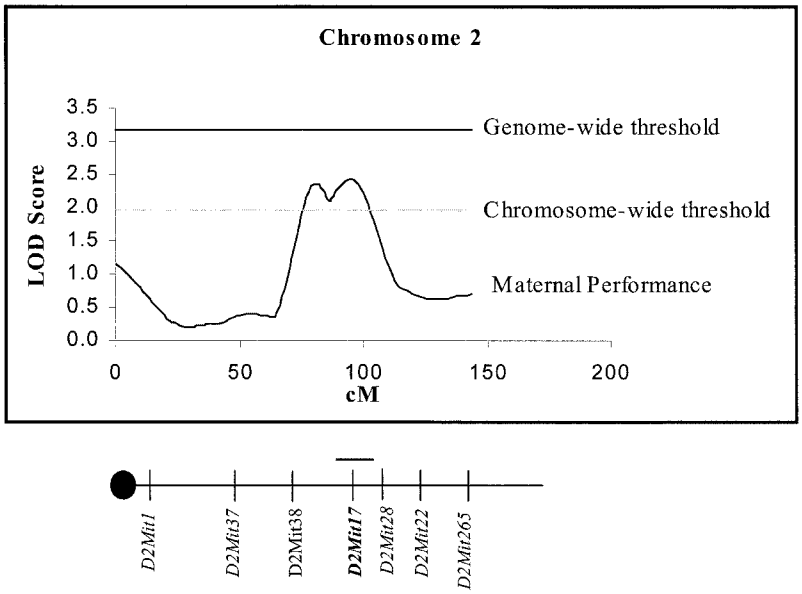

FIGURE 2.—LOD plot of chromosome *2*. The chromosome-wide significance threshold is 2.02. LOD score at significant peak is 2.43, showing suggestive linkage at a chromosome-wide level at *D2Mit17* + 6 cM.

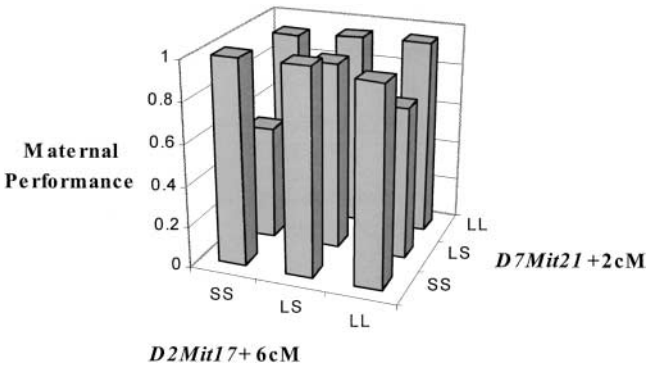

FIGURE 3.—Maternal performance genotypic values for *D2Mit17* + 6 cM and *D7Mit21* + 2 cM.

study are on average 0.561 ± 0.026. Association between inbreeding and success raising a litter was significant (F = 9.1728, $P = 0.0028$). When we examined the relationship between inbreeding and all the observed maternal behavior variables, we found a significant association among them ($P = 0.048$). Nevertheless, this significant result was due primarily to association between inbreeding and pup cleaning and milk provision ($P = 0.034$ and $P = 0.040$, respectively), but not to pre- and postpartum nest building and aggressive behavior against intruders.

When we tested for the association among different behavior variables and female maternal performance for offspring survival, a highly significant value was found ($P = 5.85 \times 10^{-65}$). This analysis indicates that variables primarily responsible for this association are milk provision and aggressive behavior/pup retrieval ($P = 1.0 \times 10^{-17}$ and $P = 0.0004$, respectively). Interestingly, the level of inbreeding is not significantly associated with success independent of the behavioral factors ($P = 0.716$).

DISCUSSION

Interval mapping revealed two QTL affecting maternal performance. The genetic architecture of these QTL indicates a contrasting pattern. Underdominance at the chromosome *7* locus indicates that heterozygous females are, on average, less successful than either homozygote at this locus, while overdominance at the chromosome *2* QTL indicates that the heterozygotes display more successful maternal performance for offspring survival than do the parental genotypes. Although of only borderline significance after Bonferroni correction, ma-

TABLE 3

Epistatic interactions between QTL affecting maternal performance

Locus 1	Position marker (cM)	Position centromere (cM)	Locus 2	Position marker (cM)	Position centromere (cM)	Prob. epistasis	Epistasis type	Genotypic value	Prob. genotypic value
D1Mit3	8	8	*D3Mit194*	14	128	1.22×10^{-5}	AD	−0.14	0.000219
							DA	−0.12	0.002579
D1Mit3	8	8	*D5Mit61*	38	58	3.23×10^{-5}	AA	−0.24	2.79×10^{-5}
D1Mit14	0	78	*D18Mit51*	2	28	2.9×10^{-5}	AA	0.14	0.006313
							DD	−0.15	1.65×10^{-5}
D2Mit380	8	72	*D9Mit4*	12	28	3.06×10^{-5}	AD	0.23	1.14×10^{-5}
D2Mit17	4	92	*D14Nds1*	12	12	3.34×10^{-5}	AA	−0.17	0.010101
							AD	0.23	0.000495
							DD	−0.16	0.009477
D2Mit1	38	38	*D3Mit54*	0	0	0.000245	AD	0.20	5.67×10^{-6}
D3Mit54	26	26	*D19Mit16*	16	16	9.18×10^{-7}	DD	−0.52	1.98×10^{-8}
D3Mit194	12	126	*D4Mit45*	18	66	2.49×10^{-5}	AA	0.12	0.007736
							AD	0.11	0.009347
							DA	−0.12	0.005267
D5Mit26	28	114	*D11Mit333*	8	108	3.47×10^{-6}	DD	−0.20	1.53×10^{-7}
D5Mit61	16	36	*D7Nds1*	0	40	5.07×10^{-5}	DD	0.24	7.74×10^{-6}
D6Mit1	20	20	*D10Mit2*	12	12	1.81×10^{-7}	AA	−0.22	0.000803
							AD	−0.20	0.005546
							DD	−0.31	0.000167
D6Mit58	4	94	*D15Mit5*	0	22	2.3×10^{-5}	AA	0.15	0.004661
							AD	0.14	0.00072
D6Nds5	16	80	*D11Mit15*	16	76	7.31×10^{-5}	DD	−0.23	5.1×10^{-6}
D11Mit15	6	66	*D13Mit115*	28	38	1.22×10^{-5}	DA	0.20	0.00686
							DD	−0.37	1.89×10^{-5}
D8Mit25	22	44	*D15Mit2*	20	66	0.00019	AD	0.29	5.07×10^{-6}

Epistasis types: AA, additive by additive; DD, dominance by dominance; AD, additive by dominance; DA, dominance by additive.

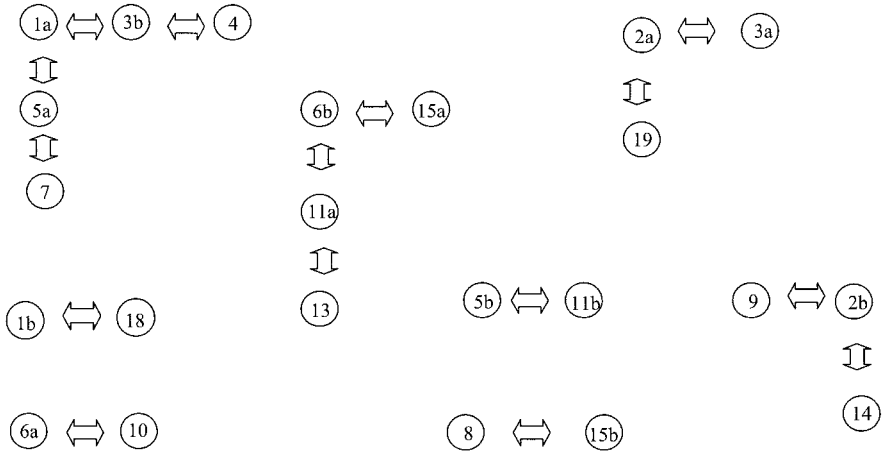

Figure 4.—Epistatic interaction patterns for maternal performance. QTL are represented by respective chromosome number or by chromosome number and a letter when more than one QTL occurred in the same chromosome. QTL 1a refers to *D1Mit3*, 1b to *D1Mit14*, 2a to *D2Mit1*, 2b to both *D2Mit38* and *D2Mit17*, 3a to *D3Mit54*, 3b to *D3Mit194*, 5a to *D5Mit61*, 5b to *D5Mit26*, 6a to *D6Mit1*, 6b to both *D6Mit58* and *D6Mit5*, 11a to *D11Mit15*, 11b to *D11Mit333*, 15a to *D15Mit5*, and 15b to *D15Mit2*. For other chromosomes, check Table 2.

ternal performance differences seem to be affected by interactions between these two loci (Figure 3). If the QTL at *D7Mit21* + 2 cM is homozygous, the QTL at *D2Mit17* + 6 cM is slightly additive; only when the former is heterozygous does the chromosome *2* locus present overdominance. Likewise, underdominance at the chromosome *7* locus is minimal in chromosome *2* heterozygotes but strong in the two homozygous genotypes.

The presence of significant QTL suggests a gene or genes associated with maternal performance for offspring survival at those chromosomal positions. It may be instructive to consider whether genes known to map to those regions include those likely, *a priori*, to have an effect on maternal performance. We searched the Mouse Genome Database (2001) for candidate genes that affect maternal performance located close to these QTL. Although this identification is preliminary, it may suggest potential genes affecting the trait. The FBJ osteosarcoma oncogene B (*FosB*) and the paternally expressed gene 3 (*Peg3*) are appropriate candidate genes because they are in the proximal region of chromosome *7*, 3.0–4.5 cM away from the position of the QTL at *D7M21* + 2 cM. These candidate genes are associated with the lack of nurturing maternal behavior in knockout mice (Brown *et al.* 1996; Li *et al.* 1999). Both *FosB*- and *Peg3*-deficient females fail to retrieve and crouch over their pups; the former also show poor nest-building performance and the latter have lactation problems. These phenotypes are similar to the abnormal maternal behaviors observed in our study. Naturally, it is possible that this chromosome *7* QTL could represent the combined effects of multiple, closely linked loci. Oxytocin (*Oxt*) is a candidate gene for the QTL at *D2Mit17* + 6 cM since it is located in the confidence interval of the QTL at *D2Mit17* (the intermediary region of chromosome *2*) and has an essential role in stimulating maternal behavior. The release of oxytocin during delivery triggers maternal behavior in rats (Pedersen *et al.* 1982); oxytocin-deficient female mice failed to provide milk to the offspring (Nishimori *et al.* 1996), although they had normal maternal behaviors.

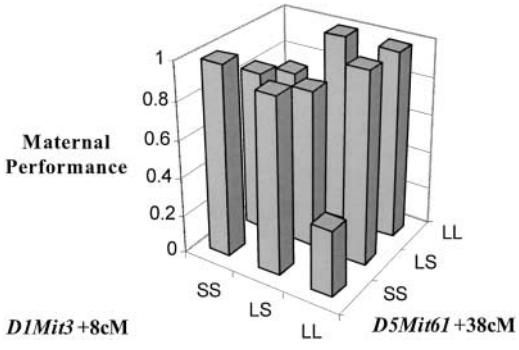

Figure 5.—Genotypic values for maternal performance indicating additive-by-additive interaction between *D1Mit3* + 8 cM and *D5Mit61* + 38 cM.

Many regions across the genome were found to participate in epistatic interactions for maternal performance for offspring survival. Only one of the QTL identified in the single gene mapping (*D2Mit17* + 6 cM) participated in significant epistatic interactions. Although some loci participated in more than one interaction, the 23 chromosomal regions did not show a unified network of interactions. Rather, many separate interaction sets were identified (Figure 4). It is noteworthy to contrast these results with a study investigating QTL for adiposity in the same population, which found a single network connecting all epistatic QTL (Cheverud *et al.* 2001). Maternal performance is a complex trait and distinct mechanisms may be operating separately, affecting maternal behaviors, physiology, or both. These mechanisms combine in a chain of events that lead to the distinct maternal behaviors conducive to offspring survival. Some candidate genes for QTL showing epistatic interactions are shown in Table 4. All five gene knockouts linked to maternal behavior (Bridges 1998; Li *et al.* 1999) are potential candidate genes in our study. Two of them are candidates for single QTL and three of them are found near regions involved in epistatic interactions (Table 4). Other potential candidate genes for QTL are associated with abnormal maternal behav-

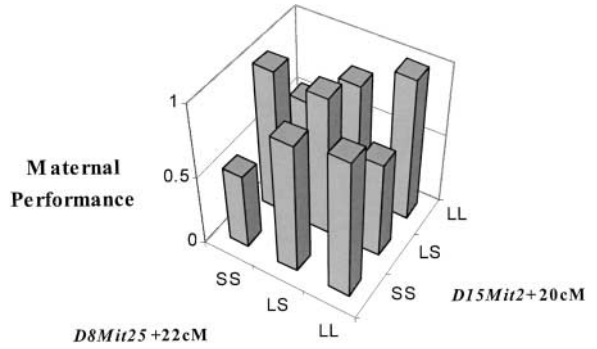

FIGURE 6.—Maternal performance genotypic values for the two-locus genotype at *D8Mit25* + 22 cM and *D15Mit2* + 20 cM illustrates additive-by-dominance interaction.

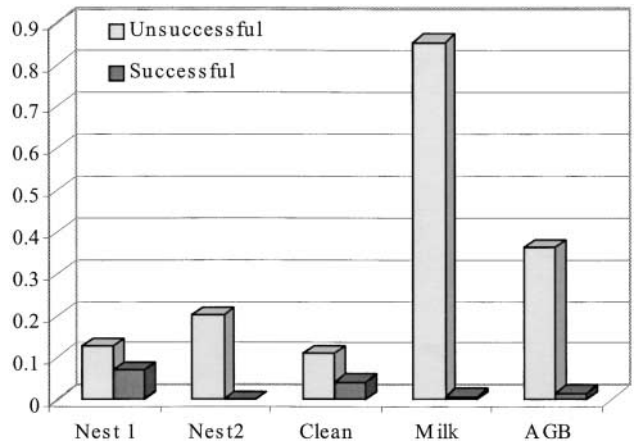

FIGURE 8.—Frequency of maternal features significantly associated with maternal performance for offspring survival. The frequency given is for lack of the feature. "Nest 1" refers to lack of nest building before birth and "Nest 2" to nest building after birth; "Clean" indicates absence of placentophagia and pup grooming; "Milk" means failure in milk provision; and "AGB" refers to lack of aggressive behavior with retrieval of pups after disturbance. Successful and unsuccessful females differed significantly for these traits.

ior and complex behavioral traits. Candidate genes suggested here do not cover all QTL found in our study. Furthermore, it is possible that other genes linked to the candidate genes we identified are actually responsible for the observed variation. At any rate, the use of the QTL approach enables the identification of regions in the genome affecting the trait of interest that may be investigated further.

All forms of epistasis are represented in the interactions found here (see Table 3). Additive-by-additive epistasis (*e.g.*, Figure 5) is a pattern that can interfere with our ability to find single-locus QTL in a genetic mapping study. This "epistatic nullification" occurs because epistasis cancels out the effects of each single locus at the intermediate allele frequencies found in an F_2 population. It causes QTL analyses to underestimate the number of loci involved in complex traits (ROUTMAN and CHEVERUD 1997). In additive-by-additive epistasis, additivity is detected only within classes of genotypes at the interacting locus. In Figure 5, for example, if *D5Mit61* + 38 cM is SS, *D1Mit3* + 8 cM is additive with SS having the highest maternal performance. On the other hand, if *D5Mit61* + 38 cM is LL, the pattern is reversed. *D1Mit3* + 8 cM is still additive, but LL has the best maternal performance. When we average genotypic effects at one locus across all genotypes at the second locus, the effects nullify each other and may have led to our failure to detect single-locus effects on one or both of these chromosomes.

Additive-by-dominance epistasis shows the same pattern as dominance-by-additive epistasis, but with the roles of the loci reversed. Figure 6 represents additive-by-dominance epistasis between chromosome *8* and chromosome *15*. Note that *D8Mit25* + 2 cM has additive effects with the LL homozygote displaying superior maternal performance for both *D15Mit2* + 20 cM homozygotes but that this effect is reversed in *D15Mit2* + 20 cM heterozygotes. Likewise, *D15Mit2* + 20 cM shows overdominance among *D8Mit25* + 2 cM SS animals and underdominance among *D8Mit25* + 2 cM LL animals.

Most observed cases of dominance-by-dominance epistasis are negative (Table 3). An example of this kind of interaction (Figure 7) shows that double heterozygotes have a lower genotypic value than that of single heterozygotes for each locus. Despite this, double heterozygotes still have better maternal performance than that of parental or recombinant homozygotes. This heterosis may explain why no unsuccessful females were observed in the F_1 generation. While investigating body weight in this same population we found a similar pattern in which the majority of the dominance-by-dominance epistasis was negative (CHEVERUD 2000). However, our findings do not predict hybrid dysgenesis because double heterozygotes were better than the parentals, although worse than single heterozygotes at either locus. A separate study on maternal effects on early growth in this same population found five single-locus QTL and 10 interactions for maternal performance for offspring growth

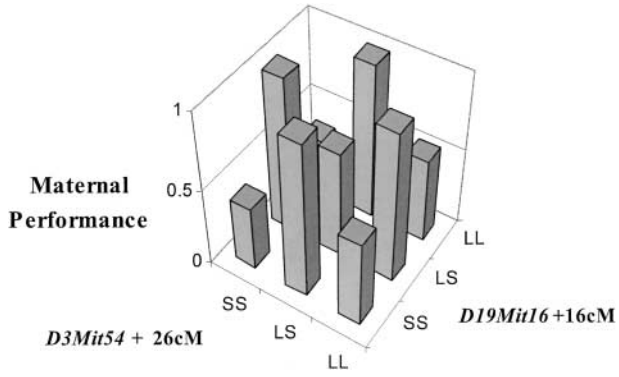

FIGURE 7.—Dominance-by-dominance interaction between *D3Mit54* + 26 cM and *D19Mit16* + 16 cM.

TABLE 4

Potential candidate genes for QTL interacting epistatically

QTL	Position (cM)	Candidate gene	Gene name	Position (cM)	Phenotype	References
D1Mit14	78	*Htr5b*	5-Hydroxytryptamine (serotonin) receptor 5B	63	Anxiety and depression	Clement *et al.* (1996)
D2Mit380	72	*Slc30a4*	Solute carrier family 30 (zinc transporter), member 4	69	Lethal milk	Dickie (1969)
D2Mit1	38	*Dbh*	Dopamine β-hydroxylase	15.5	Lack of placentophagia/ lactation problems	Thomas and Palmiter (1997)
D3Mit54	0	*Crh*	Corticotropin-releasing hormone	8	Abnormal maternal behavior	Pedersen *et al.* (1991)
D6Mit1	20	*Mest*	Mesoderm-specific transcript	7.5	Lack of placentophagia/ poor nest	Lefebvre *et al.* (1998)
		Ghrhr	Growth hormone releasing hormone receptor	26	Failure to nurse first litters	Eicher and Beamer (1976)
D7Nds1	40	*Herc2*	Hect [homologous to the E6-AP (UBE3A) carboxyl terminus] domain and RCC1 (CHC1)-like domain (RLD) 2	27	Abnormal maternal behavior	Lehman *et al.* (1998)
D8Mit25	44	*Slc6a2*	Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	45	Complex behavior traits	Fritz *et al.* (1998)
D9Mit4	28	*Foxb1b (Mf3)*	Forkhead box B1b	41	Lactation problems	Labosky *et al.* (1997)
D11Mit15	66	*Crhr*	Corticotropin-releasing hormone receptor	62	Abnormal maternal behavior	Pedersen *et al.* (1991)
D13Mit115	38	*Prl*	Prolactin	14	Abnormal maternal behavior	Lucas *et al.* (1998)
D15Mit15	22	*Prlr*	Prolactin receptor	4.6	Abnormal maternal behavior	Lucas *et al.* (1998)
D18Mit51	28	*CamK2a*	Calcium/calmodulin-dependent protein kinase IIα	33	Aggressive behavior	Chen *et al.* (1994)

(Wolf *et al.* 2002). Seven of these QTL were found in similar positions to those described here. Maternal performance for offspring survival and offspring growth may be directly related. The same maternal features that affect offspring survival may also affect offspring growth. Therefore we expect that some genes may modulate both traits.

The results of this genome-wide scan for maternal performance contrast strikingly with those obtained for a wide variety of morphological traits, including mandibular morphology (Cheverud *et al.* 1997), cranial morphology (Leamy *et al.* 1999), growth and age-specific body weights (Cheverud *et al.* 1996; Vaughn *et al.* 1999), adiposity (Cheverud *et al.* 2001), and binary skeletal nonmetric traits (Leamy *et al.* 1998). Each of these genome scans found large numbers of main-effect QTL spread throughout the genome. Most QTL discovered had significant additive effects. For maternal performance we found only two direct-effect QTL and neither had a significant additive effect. The only comparable result has been for fluctuating asymmetry for mandibular morphology, which had no direct genetic effects (Leamy *et al.* 1997) but many significant epistatic interactions (Leamy *et al.* 2002).

Maternal performance was inferred from the analysis of litter survival. To corroborate this inference, we must show that females with low maternal performance present specific maternal behaviors that affect their success in rearing litters. Behaviors significantly associated with maternal performance include suckling, nest building, placentophagia and pup grooming, and retrieval of pups after disturbance. In our study, females whose pups survived the first week built a good nest before and kept it after delivery. Such females usually performed placentophagia, groomed pups, provided milk, and protected their offspring against intruders. Significant differences found between successful and unsuccessful females for these variables point out the lack of these maternal behaviors in the latter. Mice with knockout genes involved in maternal care displayed similar abnormal behaviors (Brown *et al.*1996; Thomas and Palmiter 1997; Lefebvre *et al.* 1998; Lucas *et al.* 1998; Li *et al.* 1999).

Inbreeding is also involved with this behavioral alteration, since maternal failure to nurture offspring is one of the causes of inbred mouse strain failure (Silver 1995; Falconer and Mackay 1996). In our study, inbreeding was negatively associated with female success ($P = 0.0028$) through its effects on some of the maternal

behaviors we studied, such as pup cleaning and milk provision. It had no significant effect independent of these behaviors.

Even though the behaviors were observed in later generations, the strong association between these variables and maternal performance suggests that unsuccessful females in the F_2 generation shared the same behaviors found in later generations; *i.e.*, they did not display maternal care after parturition and had problems with lactation.

Maternal performance, as a fitness component, is under stringent selection pressure. Consequently, it is expected to have low additive genetic variation (Falconer and Mackay 1996). However, high levels of total genetic variation have been associated with fitness traits (Houle 1992; Fowler *et al.* 1997; Potti 1999; Bult and Lynch 2000) and there is selection pressure to supply genetic variation for fitness traits, so most of this variation should be due to epistatic and dominance interactions. Thus, epistasis plays an important role in variation for fitness traits (Armbruster *et al.* 1997). Our results indicate a few individual dominant gene effects that account for 10% of the variation in maternal performance, but a large number of epistatic interactions. Taken together, all QTL and interactions explain almost 35% of the phenotypic variation for this trait.

This study revealed QTL affecting maternal performance in mice of the F_2 generation of the SM/J and LG/J cross. To ratify the present results, future QTL studies for maternal performance should include fine-scale mapping and the investigation of RI lines. We expect that if these QTL indeed affect maternal performance, they should show different frequencies in successful and unsuccessful RI strains.

Maternal performance intrigues researchers due to its complexity and its role as an environmental influence on the phenotypes of relatives. Identification of individual genes has been mostly restricted to the use of the knockout gene technology. Nevertheless, multiple genes affect maternal behaviors and QTL analysis provides an appropriate framework not only to determine chromosomal localization of putative candidate genes, with *a posteriori* gene identification, but more importantly, to investigate how these regions interact to produce success or failure in maternal performance.

We thank T. Ehrich, S. Cropp, J. Wolf, A. Moore, and C. Boake for comments. This work is supported by National Science Foundation grant DEB-9726433, National Institutes of Health grant DK52514, and Fundação de Amparo à Pesquisa do Estado de São Paulo grant 98/16139-6 to A.C.P. and S.R.M.

LITERATURE CITED

Alston-Mills, B., A. C. Parker, E. J. Eisen, R. Wilson and S. Fletcher, 1999 Factors influencing maternal behavior in the *hubb/hubb* mutant mouse. Physiol. Behav. **68:** 3–8.

Armbruster, P., W. E. Bradshaw and C. M. Holzapfel, 1997 Evolution of the genetic architecture underlying fitness in the pitcher-plant mosquito, *Wyeomyia smithii*. Evolution **51:** 451–458.

Basten, C. J., B. S. Weir and Z-B. Zeng, 1994 Zmap—a QTL cartographer, pp. 65–66 in *Proceedings of the 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software*, Vol. 22, edited by C. Smith, J. S. Gavora, B. Benkel, J. Chesnais, W. Fairfull *et al.* Organizing Committee, 5th World Congress on Genetics Applied to Livestock Production, Guelph, Ontario, Canada.

Basten, C. J., B. S. Weir and Z-B. Zeng, 2002 QTL Cartographer, Version 1.16. Department of Statistics, North Carolina State University, Raleigh, NC.

Bernardis, L. L., and L. L. Bellinger, 1996 The lateral hypothalamic area revisited: ingestive behavior. Neurosci. Biobehav. Rev. **20:** 189–287.

Boyce, A. J., 1983 Computation of inbreeding and kinship coefficients on extended pedigrees. J. Hered. **74:** 400–404.

Bridges, R. S., 1998 The genetics of motherhood. Nat. Genet. **20:** 108–109.

Brown, J. R., H. Ye, R. T. Bronson, P. Dikkes and M. D. Greenberg, 1996 A defect in nurturing in mice lacking the immediate early gene *fosB*. Cell **86:** 297–309.

Bult, A., and C. B. Lynch, 2000 Breaking through artificial selection limits of an adaptive behavior in mice and the consequences for correlated responses. Behav. Genet. **30:** 193–206.

Chen, C., D. G. Rainnie, R. W. Greene and S. Tonegawa, 1994 Abnormal fear response and aggressive behavior in mutant mice deficient for alpha-calcium-calmodulin kinase II. Science **14:** 291–294.

Cheverud, J. M., 1984 Evolution by kin selection: a quantitative genetic model illustrated by maternal performance in mice. Evolution **38:** 766–777.

Cheverud, J. M., 2000 Detecting epistasis among quantitative trait loci, pp. 58–81 in *Epistasis and the Evolutionary Process*, edited by J. Wolf, E. Brodie, III and M. Wade. Oxford University Press, New York.

Cheverud, J. M., 2001 A simple correction for multiple comparisons in interval mapping genome scans. Heredity **87:** 52–58.

Cheverud, J. M., and A. J. Moore, 1994 Quantitative genetics and the role of the environment provided by relatives in behavioral evolution, pp. 67–100 in *Quantitative Genetic Studies of Behavioral Evolution*, edited by C. R. B. Boake. The University of Chicago Press, Chicago.

Cheverud, J. M., and E. J. Routman, 1995 Epistasis and its contribution to genetic variance components. Genetics **139:** 1455–1461.

Cheverud, J. M., E. J. Routman, F. A. M. Duarte, B. van Swinderen, K. Cothran *et al.*, 1996 Quantitative trait loci for murine growth. Genetics **142:** 1305–1319.

Cheverud, J. M., E. J. Routman and D. K. Irschick, 1997 Pleiotropic effects of individual gene loci on mandibular morphology. Evolution **51:** 2004–2014.

Cheverud, J. M., T. T. Vaughn, L. S. Pletscher, K. J. King-Ellison, C. Erickson *et al.*, 1999 Epistasis and the evolution of additive genetic variance in populations that pass through a bottleneck. Evolution **53:** 1009–1018.

Cheverud, J. M., T. T. Vaughn, L. S. Pletscher, A. C. Peripato, E. S. Adams *et al.*, 2001 Genetic architecture of adiposity in the cross of LG/J and SM/J inbred mice. Mamm. Genome **12:** 3–12.

Churchill, G. A., and R. W. Doerge, 1994 Empirical threshold values for quantitative trait mapping. Genetics **138:** 963–971.

Clement, Y., K. H. Kia, G. Daval and D. Verge, 1996 An autoradiographic study of serotonergic receptors in a murine genetic model of anxiety-related behaviors. Brain Res. **709:** 229–242.

Cohen, J., and P. Cohen, 1983 *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences*, Ed. 2. Lawrence Erlbaum, Hillsdale, NJ.

Cohen, J., and L. Wilkinson, 1997 *Systat 8.0 Statistics*, pp. 817–840. SPSS, Chicago.

Dickie, M. M., 1969 lm. Mouse News Lett. **41:** 30–31.

Dietrich, W., H. Katz, S. Lincoln, H.-S. Shin, J. Friedman *et al.*, 1992 A genetic map of the mouse suitable for typing intraspecific crosses. Genetics **131:** 423–447.

Doerge, R. W., and G. A. Churchill, 1996 Permutation tests for multiple loci affecting a quantitative character. Genetics **142:** 285–294.

Dyke, B., 1996 *PEDSYS: A Pedigree Data Management System*, Version 2.0. Southwest Foundation for Biomedical Research, San Antonio, TX.

Eicher, E. M., and W. G. Beamer, 1976 Inherited ateliotic dwarfism

in mice. Characteristics of the mutation, little, on chromosome 6. J. Hered. **67:** 87–91.

Falconer, D. S., and T. Mackay, 1996 *Introduction to Quantitative Genetics*. Longman Press, New York.

Festing, M. F. W., 1979 *Inbred Strains in Biomedical Research*. Oxford University Press, New York.

Flannelly, K. J., E. D. Kemble, D. C. Blanchard and R. J. Blanchard, 1986 Effects of septal-forebrain lesions on maternal aggression and maternal care. Behav. Neural. Biol. **45:** 17–30.

Fowler, K., C. Semple, N. H. Barton and L. Partridge, 1997 Genetic variation for total fitness in *Drosophila melanogaster*. Proc. R. Soc. Lond. Ser. B **264:** 191–199.

Fritz, J. D., L. D. Jayanthi, M. A. Thoreson and R. D. Blakely, 1998 Cloning and chromosomal mapping of the murine norepinephrine transporter. J. Neurochem. **70:** 2241–2251.

Gandelman, R., M. X. Zarrow, V. H. Denenberg and M. Myers, 1971 Olfactory bulb removal eliminates maternal behavior in the mouse. Science **171:** 210–211.

Haley, C. S., and S. A. Knott, 1992 A simple regression method for mapping quantitative trait loci in line crosses using flanking markers. Heredity **69:** 315–324.

Hilton, G., 1976 *Intermediate Politometrics*. Columbia University Press, New York.

Horseman, N. D., W. Zhao, E. Montecino-Rodriguez, M. Tanaka, K. Nakashima *et al.*, 1997 Defective mammopoiesis, but normal hematopoiesis, in mice with a targeted disruption of prolactin gene. EMBO J. **16:** 6926–6935.

Houle, D., 1992 Comparing evolvability and variability of quantitative traits. Genetics **130:** 195–204.

Kadarmideen, H. N., L. G. Janss and J. C. M. Dekkers, 2000 Power of quantitative trait locus mapping for polygenic binary traits using generalized and regression interval mapping in multi-family half-sib designs. Genet. Res. **76:** 305–317.

Kinsley, C. H., L. Madonia, G. W. Gifford, K. Tureski, G. R. Griffin *et al.*, 1999 Motherhood improves learning and memory. Nature **402:** 137–138.

Kort, F., 1973 Regression analysis and discriminant analysis. Am. Polit. Sci. Rev. **67:** 555–559.

Kramer, M., T. T. Vaughn, L. S. Pletscher, K. King-Ellison, E. Adams *et al.*, 1998 Genetic variation for body weight gain and composition in the intercross of Large (LG/J) and Small (SM/J) inbred strains of mice. Genet. Mol. Biol. **21:** 211–218.

Labosky, P. A., G. E. Winnier, T. L. Jetton, L. Hargett, A. K. Ryan *et al.*, 1997 The winged helix gene, Mf3, is required for normal development of the diencephalon and midbrain, postnatal growth and the milk-ejection reflex. Development **124:** 1263–1274.

Lander, E. S., and D. Bolstein, 1989 Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics **121:** 185–199.

Lander, E. S., and L. Kruglyak, 1995 Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat. Genet. **11:** 241–247.

Lander, E. S., P. Green, J. Abrahamson, A. Barlow, M. Daley *et al.*, 1987 MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1:** 174–181.

Leamy, L. J., E. J. Routman and J. M. Cheverud, 1997 A search for quantitative trait loci affecting asymmetry of mandibular characters in mice. Evolution **51:** 957–969.

Leamy, L. J., E. J. Routman and J. M. Cheverud, 1998 Quantitative trait loci for fluctuating asymmetry of quasi-continuous skeletal characters in mice. Heredity **80:** 509–518.

Leamy, L., E. Routman and J. Cheverud, 1999 Quantitative trait loci for early and late developing skull characters in mice: a test of the genetic independence model of morphological integration. Am. Nat. **153:** 201–214.

Leamy, L., E. Routman and J. Cheverud, 2002 An epistatic genetic basis for fluctuating asymmetry of mandible size in mice. Evolution **56:** 642–653.

Lee, P. C., P. Majluf and I. J. Gordon, 1991 Growth, weaning and maternal investment from a comparative perspective. J. Zool. **225:** 99–114.

Lefebvre, L., S. Viville, S. C. Barton, F. Ishino, E. B. Keverne *et al.*, 1998 Abnormal maternal behavior and growth retardation associated with loss of the imprinted gene *Mest*. Nat. Genet. **20:** 163–168.

Lehman, A. L., Y. Nakatsu, A. Ching, R. T. Bronson, R. J. Oakey *et al.*, 1998 A very large protein with diverse functional motifs is deficient in rjs (runty, jerky, sterile) mice. Proc. Natl. Acad. Sci. USA **95:** 9436–9441.

Li, L.-L., E. B. Keverne, S. A. Aparicio, F. Ishino, S. C. Barton *et al.*, 1999 Regulation of maternal behavior and offspring growth by paternally expressed *Peg3*. Science **284:** 330–333.

Lincoln, S., M. Daly and E. Lander, 1992 *Constructing Genetic Maps with MAPMAKER/EXP 3.0*, Ed. 3. Whitehead Institute Technical Report, Whitehead Institute, Cambridge, MA.

Lonstein, J. S., and J. M. Stern, 1997 Role of the midbrains periaqueductal gray in maternal nurturance and aggression: *c-fos* and electrolytic lesion studies in lactating rats. J. Neurosci. **17:** 3364–3378.

Lucas, B. K., C. J. Ormandy, N. Binart, R. S. Bridges and P. A. Kelly, 1998 Null mutation of prolactin receptor gene produces a defect in maternal behavior. Endocrinology **139:** 4102–4107.

McIntyre, L. M., C. J. Coffman and R. W. Doerge, 2001 Detection and location of a single binary trait locus in experimental populations. Genet. Res. **78:** 79–92.

Mouse Genome Database, 2001 Mouse genome informatics (http://www.informatics.jax.org/). The Jackson Laboratory, Bar Harbor, ME.

Mousseau, T. A., and C. W. Fox, 1998 *Maternal Effects as Adaptations*. Oxford University Press, New York.

Nishimori, K., L. J. Young, Q. Guo, W. Zuoxin, T. R. Insel *et al.*, 1996 Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. Proc. Natl. Acad. Sci. USA **93:** 11699–11704.

Numan, M., M. J. Numan, S. R. Marzella and A. Palumbo, 1998 Expression of *c-fos*, *fos B*, and *egr-1* in the medial preoptic area and bed nucleus of the stria terminalis during maternal behavior in rats. Brain Res. **792:** 348–352.

Olazabal, D. E., and A. Ferreira, 1997 Maternal behavior in rats with kainic acid-induced lesions of the hypothalamic paraventricular nucleus. Physiol. Behav. **61:** 779–784.

Pedersen, C. A., J. A. Ascher, Y. L. Monroe and A. J. Prange, Jr., 1982 Oxytocin induces maternal behavior in virgin female rats. Science **216:** 648–650.

Pedersen, C. A., J. D. Cadwell, M. McGuire and D. L. Evans, 1991 Corticoprotein-releasing hormone inhibits maternal behavior and induces pup-killing. Life Sci. **49:** 1537–1546.

Potti, J., 1999 Maternal effects and the pervasive impact of nestling history on egg size in a passerine bird. Evolution **53:** 279–285.

Rao, D. C., 1998 CAT scans, PET scans, and genomic scans. Genet. Epidemiol. **15:** 1–18.

Rebai, A., 1997 Comparison of methods for regression interval mapping in QTL analysis with non-normal traits. Genet. Res. **69:** 69–74.

Rosenblatt, J. S., 1967 Nonhormonal basis of maternal behavior in the rat. Science **156:** 1512–1514.

Routman, E. J., and J. M. Cheverud, 1994 A rapid method of scoring simple sequence repeat polymorphisms with agarose gel electrophoresis. Mamm. Genome **5:** 187–188.

Routman, E. J., and J. M. Cheverud, 1995 Polymorphism for PCR-analyzed microsatellites: data for two additional inbred mouse strains and the utility of agarose gel electrophoresis. Mamm. Genome **6:** 401–404.

Routman, E. J., and J. M. Cheverud, 1997 Gene effects on a quantitative trait: two-locus epistatic effects measured at microsatellite markers and at estimated QTL. Evolution **51:** 1654–1662.

SAS Institute, 1998 *SAS/STAT User's Guide*, Version 6, Vol. 1, Ed. 4. SAS Institute, Cary, NC.

Silver, L. M., 1995 *Mouse Genetics*. Oxford University Press, New York.

Thomas, S. A., and R. D. Palmiter, 1997 Impaired maternal behavior in mice lacking norepinephrine and epinephrine. Cell **91:** 583–592.

Vaughn, T. T., L. S. Pletscher, A. Peripato, K. King-Ellison, E. Adams *et al.*, 1999 Mapping quantitative trait loci for murine growth: a closer look at genetic architecture. Genet. Res. **74:** 313–322.

Visscher, P. M., C. S. Haley and S. A. Knott, 1996 Mapping QTLs

for binary traits in backcross and F2 populations. Genet. Res. **68:** 55–63.

Weller, J. I., J. Z. Song, D. W. Heyen, H. A. Lewin and M. Ron, 1998 A new approach to the problem of multiple comparison in the genetic dissection of complex traits. Genetics **150:** 1699–1706.

Wolf, J. B., E. D. Brodie, III, J. M. Cheverud, A. J. Moore and M. J. Wade, 1998 Evolutionary consequences of indirect genetic effects. Trends Ecol. Evol. **13:** 64–69.

Wolf, J. B., T. T. Vaughn, L. S. Pletscher and J. M. Cheverud, 2002 Contribution of maternal effect QTL to genetic architecture of early growth in mice. Heredity **89:** 300–310.

Yamamuro, Y., and N. Sensui, 1998 Exogenous oxytocin attenuates suckling-induced prolactin release but not maternal or infant behavior in lactating rats. Physiol. Behav. **63:** 939–943.

Communicating editor: G. A. Churchill