

## Genetic Influences on Maternal Care

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**ABSTRACT:** The basis of social evolution in mammals is the mother-offspring relationship. It is also the primary and most important instance of indirect genetic effects, where genetic variation in one individual affects phenotypic variation among others. This relationship is so important in mammals that often the major factor determining the life or death of newborns is the environment provided by their mother. Variations in these environments can be due to variations in maternal genotypes. In our work with the intercross of two mouse inbred strains, LG/J and SM/J, we uncovered a very severe variation in maternal performance. These females failed to nurture their offspring and showed abnormal maternal behaviors leading to loss of their litter. Rather than this being due to a single gene variant as in knockout mice, we uncovered a complex genetic basis for this trait. The effects of genes on maternal performance are entirely context dependent in our cross. They depend on the alleles present at the same or other epistatically interacting loci. Genomic locations identified in this study include locations of candidate genes whose knockouts displayed similar aberrant maternal behavior. Behaviors significantly associated with maternal performance in this study include suckling, nest building, placentophagia, pup grooming, and retrieval of pups after disturbance.

*Keywords:* maternal care, QTL, mammals, genetic architecture, epistasis.

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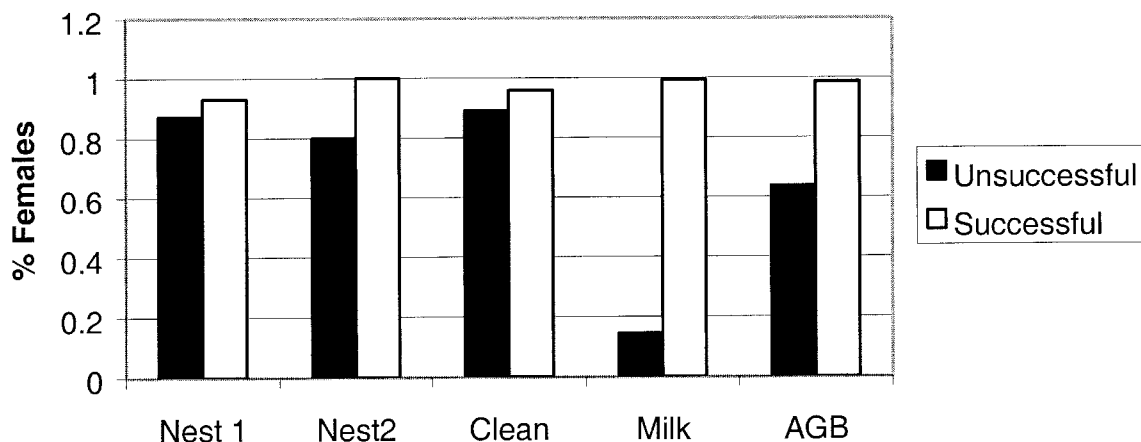
During gestation and after birth, the environment provided by mothers to their offspring is the primary and most significant instance of indirect genetic effects affecting offspring's phenotype, and it is referred to as maternal performance for specific offspring traits (Cheverud and Moore 1994). In fact, environmental effects due to 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 these environments are, in part, due to genetic differences among mothers. Therefore, maternal performance as an indicator of maternal care is a result of variations in maternal physiology and behaviors and may determine the life or death of newborns. In this article, we review factors influencing maternal care in mammals and their genetic basis. We put special emphasis on our experiments with maternal care in a mouse population (Peripato et al. 2002), showing the feasibility of a strategy to investigate the genetics of a complex behavioral trait.

In mammals we find different forms of care of the young that may be performed either by females (in most cases), by males (approximately 5% of the species), or by both, although cooperative care may also be found among a small number of mammals (Clutton-Brock 1991; Woodroffe and Vincent 1994). Maternal care varies among species, depending on gestation length, litter size, age at weaning, body size, maternal experience, and so forth. Usually, eutherian mammals have a long and inflexible gestation length that produces large offspring (Clutton-Brock 1991), while, in contrast, marsupials do not, and their young continue to develop outside the uterus, often for weeks or months, depending on the species (Low 1978). After birth, lactation represents the most energetically demanding component of reproduction in mammals, forcing lactating mothers to acquire most of their energy from diet (requiring more energy intake) than from maternal stores (van Raaij et al. 1991; Rogowitz 1998). Because of the cost of lactation, early age at weaning and small litter sizes may be advantageous in mammals with respect to maternal survival and future reproduction. Generally, maternal care is related to stage of lactation, with care declining as weaning approaches, and to offspring development. However, the intensity of maternal care varies among mammals, decreasing between parturition and weaning in most mammals (McLean and Speakman 1997) but extending after weaning in others such as Kloss's gibbons (Tilson 1981). Kloss's gibbons are attended by their parents until they find a mate. Maternal experience can also be an additional component affecting maternal care. The frequency of offspring rejections declines with maternal experience in ewes, though the maternal behavior of a primiparous

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**Figure 1:** Behaviors significantly associated with maternal performance. *Nest 1*, nest building before birth; *Nest 2*, nest building after birth; *Clean*, placentophagia and pup grooming; *Milk*, milk provision; *AGB*, aggressive behavior with retrieval of pups after disturbance. Successful and unsuccessful females differed significantly for these traits (nest 1,  $P = .033$ ; nest 2,  $P = 1.5 \times 10^{-7}$ ; clean,  $P = .0739$ ; milk,  $P = 7.57 \times 10^{-36}$ ; AGB,  $P = 1.0 \times 10^{-9}$ ).

ewe is reasonably predictive of her behavior in subsequent pregnancies (Dwyer and Lawrence 2000). Maternal experience may also influence the behavior of offspring (Beattie et al. 1996).

#### Maternal Behaviors

Maternal care is observed in most mammals. Behaviors include nest building, placentophagia, grooming, and aggression against intruders. In captivity some mammals display abnormal maternal care due to developmental, ecological, and social factors (Baker 1994; Bahr et al. 1998) in which management strategies are required to promote adequate conditions to insure the reproductive success of animals (Zhang et al. 2000). The preparation of a nest before birth and its maintenance after delivery is a common behavior in nonhuman mammalian females. The grouping of offspring in a nest is important not only to maintain their body temperature (Lynch 1994) and minimize evaporation of body water (Friedman and Bruno 1976) but also to allow touching among infants and mother and facilitate a gamut of maternal care that assures offspring survival (Fleming et al. 1999). Body-heat transfer from mother to offspring is suggested as an important factor in increased serum growth-hormone levels in neonatal rats (Kacsoh et al. 1990). Nest-building behavior has a genetic component, as shown by significant heritabilities in a variety of laboratory mouse populations and response to artificial selection (Lynch 1994; Bult and Lynch 2000).

Interactions between mother and offspring are not spontaneous but an outcome of hormone alterations dur-

ing pregnancy and offspring stimuli, among other factors (Fleming et al. 1999; Kinsley et al. 1999; Nowak et al. 2000). Vocalization and odor signals by offspring and mothers may help in their mutual recognition. In species such as the northern fur seal, mothers returning from foraging at sea that must find their offspring among hundreds of conspecifics use a distinct sound as an important indicator for reciprocal identification (Insley 2001). In addition to vocal signals, odor recognition is used, and distinctive body odor may be due to genetic differences at the major histocompatibility complex in mice, rats, and humans (Singh et al. 1987; Wedekind et al. 1995; Beauchamp et al. 2000). In rodents, females usually groom their offspring after birth by consuming the placenta and licking the entire body surface, with special care given to the anogenital region. This latter behavior stimulates micturition in offspring that generally do not urinate spontaneously until the first week of life. On the other hand, by the consumption of offspring urine, mothers can recover part of the water lost in milk production and also eliminate clues to predators (Friedman and Bruno 1976; Friedman et al. 1981). It is worth mentioning that maternal grooming is associated with the development of individual behavioral and endocrine responses to stress in rats (Francis et al. 1999; Cadji et al. 2000) and with variation in spatial learning and memory (Liu et al. 2000) and may protect newborn mammals against opportunist pathogens (Hart and Powell 1990; McLean and Speakman 1997). Likewise, ingestion of maternal feces by young rats may stimulate their immunological system (Kilpatrick et al. 1983). The aggressive behavior displayed by mothers against intruders

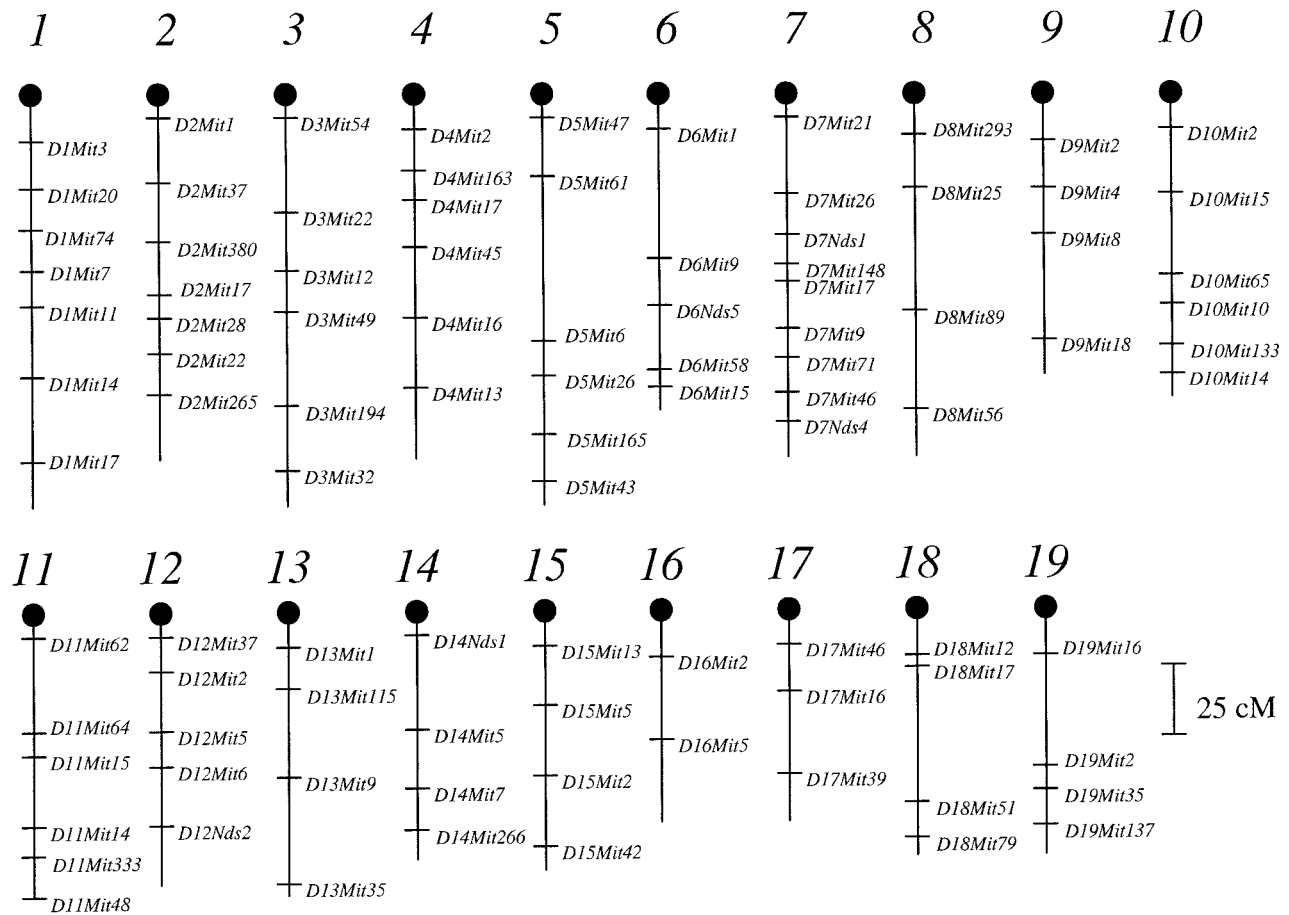


Figure 2: Relative positions of microsatellite markers scored in the intercross of LG/J and SM/J mice

and their protective reaction in response to environmental changes are females' attempts to assure their reproductive success. In the same way, it also serves as a learning experience for offspring to increase their chances of surviving to breed (Bateson 1994).

Maternal behaviors are especially interesting as models for the evolution of social behaviors and interactions because they are the archetypical social relationship in which the attributes of one partner directly affect the phenotypes of the second partner, independent of any shared genes. This is usually conceived of as social partners providing environments that directly affect the phenotypes of the interacting individuals. Variability in these social "environments" can be due to both genetic and environmental sources of variation and can, therefore, evolve. Wolf et al. (1998) describe how these concepts, originating in consideration of maternal-offspring relationships, can be generalized to any interindividual interactions. Experimental

studies in mice have been particularly informative with respect to the genetics of maternal performance for offspring phenotypes because they display many of the typical maternal behaviors and pathologies of development.

We have discovered profound genetic effects on maternal performance in our studies of the intercross of LG/J and SM/J mice (Peripato et al. 2002). Behaviors significantly associated with maternal performance included suckling, nest building, placentophagia, pup grooming, and retrieval of pups after disturbance (fig. 1). In that study, females whose pups survived the first week had built a good nest before and kept it after delivery. Such females usually performed placentophagia, cleaned, provided milk, and protected their offspring against intruders. Significant differences found between successful and unsuccessful females for these variables point out the absence of these maternal behaviors in the latter. A few genes have been found that are associated with similar abnormal maternal

**Table 1:** Quantitative trait loci (QTL) affecting maternal performance

Locus	Position marker (cM)	Position centromere (cM)	CR	<i>a</i>	<i>d</i>	$2a/\sigma_p$	$d/\sigma_p$	Percent VAR	LOD score
<i>D2Mit17</i>	6	94	72–108	.05	.16	.29	.49	4.61	2.43
<i>D7Mit21</i>	2	2	0–14	-.03	-.19	-.17	-.57	6.18	3.21

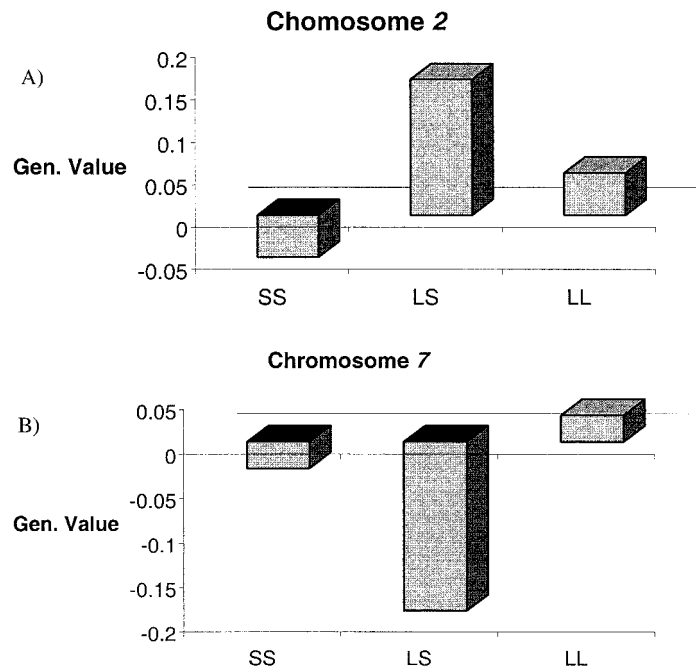
Note: "Position marker" is the QTL's distance from the nearest proximal marker on the chromosome, while "Position centromere" is the telomeric distance from the most proximal marker on the chromosome, in Haldane's centiMorgan. "CR" is the  $\pm 1$  LOD confidence region. Also included are the raw and standardized additive (*a*,  $2a/\sigma_p$ ) and dominance (*d*,  $d/\sigma_p$ ) genotypic values for maternal performance at each QTL. "Percent VAR" represents the percentage of phenotypic variation accounted for QTL with associated LOD score (Peripato et al. 2002).

behaviors in mice using gene knockout strategies (Brown et al. 1996; Thomas and Palmiter 1997; Lefebvre et al. 1998; Lucas et al. 1998; Li et al. 1999).

### Hormones and Genes

Observation of particular maternal behaviors has allowed the investigation of the causes of variation in maternal care. Many different factors affecting 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). Estradiol is related to maternal behavior in red-bellied tamarin monkeys, a species in which females showing high prepartum estradiol levels in urine have normal maternal care, while low levels correlate with rejection of offspring (Pryce et al. 1988). 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

**Figure 3:** Additive and dominance genotypic value for QTLs on chromosome 2 (A) and chromosome 7 (B)

**Table 2:** Epistatic interactions between quantitative trait loci (QTL) affecting maternal performance

Locus 1	Position marker (cM)	Position centromere (cM)	Locus 2	Position marker (cM)	Position centromere (cM)	Probability epistasis	Epistasis type	Genotypic value	Probability genotypic value
<i>D1Mit3</i>	8	8	<i>D3Mit194</i>	14	128	$1.22 \times 10^{-5}$	AD	-.14	.000219
							DA	-.12	.002579
<i>D1Mit3</i>	8	8	<i>D5Mit61</i>	38	58	$3.23 \times 10^{-5}$	AA	-.24	$2.79 \times 10^{-5}$
<i>D1Mit14</i>	0	78	<i>D18Mit51</i>	2	28	$2.9 \times 10^{-5}$	AA	.14	.006313
							DD	-.15	$1.65 \times 10^{-5}$
<i>D2Mit38</i>	8	72	<i>D9Mit4<sup>A</sup></i>	12	28	$3.06 \times 10^{-5}$	AD	.23	$1.14 \times 10^{-5}$
<i>D2Mit17</i>	4	92	<i>D14Nds1</i>	12	12	$3.34 \times 10^{-5}$	AA	-.17	.010101
							AD	.23	.000495
							DD	-.16	.009477
<i>D2Mit1<sup>A</sup></i>	38	38	<i>D3Mit54</i>	0	0	.000245	AD	.20	$5.67 \times 10^{-6}$
<i>D3Mit54</i>	26	26	<i>D19Mit16</i>	16	16	$9.18 \times 10^{-7}$	DD	-.52	$1.98 \times 10^{-8}$
<i>D3Mit194</i>	12	126	<i>D4Mit45<sup>A</sup></i>	18	66	$2.49 \times 10^{-5}$	AA	.12	.007736
							AD	.11	.009347
							DA	-.12	.005267
<i>D5Mit26</i>	28	114	<i>D11Mit333</i>	8	108	$3.47 \times 10^{-6}$	DD	-.20	$1.53 \times 10^{-7}$
<i>D5Mit61<sup>A</sup></i>	16	36	<i>D7Nds1<sup>A</sup></i>	0	40	$5.07 \times 10^{-5}$	DD	.24	$7.74 \times 10^{-6}$
<i>D6Mit1</i>	20	20	<i>D10Mit2</i>	12	12	$1.81 \times 10^{-7}$	AA	-.22	.000803
							AD	-.20	.005546
							DD	-.31	.000167
<i>D6Mit58</i>	4	94	<i>D15Mit5</i>	0	22	$2.3 \times 10^{-5}$	AA	.15	.004661
							AD	.14	.00072
<i>D6Nds5</i>	16	80	<i>D11Mit1 5</i>	16	76	$7.31 \times 10^{-5}$	DD	-.23	$5.1 \times 10^{-6}$
<i>D11Mit15</i>	6	66	<i>D13Mit115</i>	28	38	$1.22 \times 10^{-5}$	DA	.20	.00686
							DD	-.37	$1.89 \times 10^{-5}$
<i>D8Mit25</i>	22	44	<i>D15Mit2</i>	20	66	.00019	AD	.29	$5.07 \times 10^{-6}$

Note: Superscripted letters show QTLs in the same confidence interval as QTLs for maternal effect for early growth in mice (Wolf et al. 2002).

have been described as involving interactions among critical areas in the brain such as the hippocampus, the media preoptical (MPO) 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), which then regulate hormone production that modulates maternal behaviors.

Most of the research involving the genetics of maternal behavior has been done in mice, due to facility in breeding, short interval of generations, synteny to other mammals, and the large number of specialized and genetically characterized strains for experimental work (Lyon et al. 1996). Knockout gene technology has been used for identification of individual genes affecting maternal performance. This technology assumes the selective inactivation or “knockout” of a single gene in a manner that leaves all other genes unaffected. Many genes involved in maternal care in mice have been identified this way, all of which are associated with the central nervous system, particularly 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). As described above, maternal care involves many behavioral and physiological alterations that insure offspring survival and growth. Therefore, it is likely that multiple genes influence maternal care and that these genes may interact with one another.

Quantitative trait locus (QTL) analysis provides an appropriate framework to elucidate the genetic basis for complex traits (Falconer and Mackay 1996) such as maternal performance. There are two major approaches to QTL studies: the candidate-locus approach and the marker-locus approach (Cheverud and Routman 1993). In the first, genotypes are measured at loci with known physi-

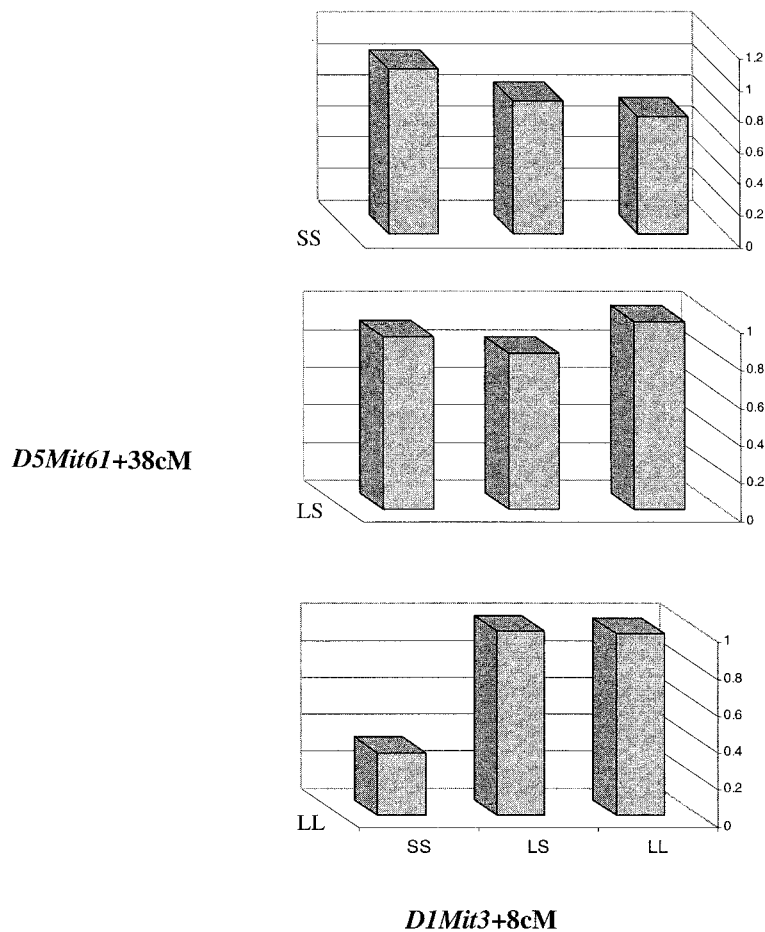
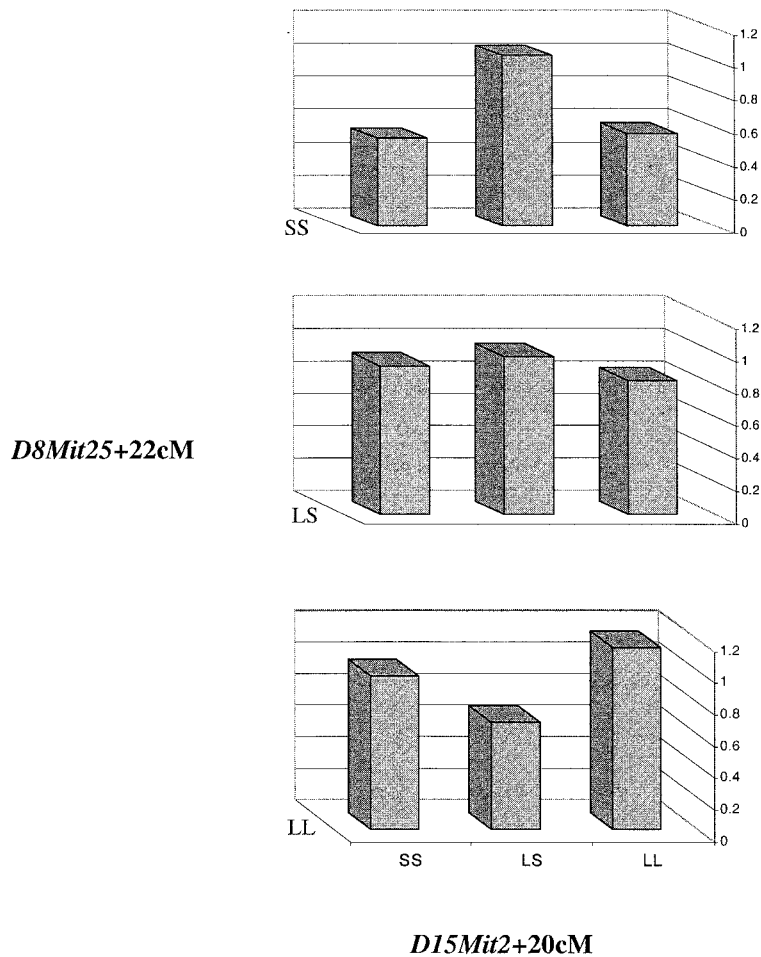


Figure 4: Genotypic values for maternal performance, indicating additive-by-additive interaction between *D1Mit3* + 8 cM and *D5Mit61* + 38 cM

ological and biochemical relationships to the phenotype of interest, and it concentrates on relevant genomic regions for this trait, as in the gene knockout studies. In the marker-locus approach, genotypic values are measured at a large number of marker loci of no particular phenotypic effect. This approach surveys the entire genome, searching for associations between traits and polymorphic markers. A significant association between traits of interest and the markers may be evidence of a QTL near the markers. Quantitative trait locus analyses via the marker-locus approach not only allow chromosomal localization of putative candidate genes, with a posteriori gene identification, but also, more importantly, can be used to investigate how these regions interact to produce the phenotype of interest, such as maternal performance for offspring survival.

#### Quantitative Trait Loci Affecting Maternal Care in Mice

The genetic basis of maternal care in mice may be determined by the association between individual QTLs and traits involved in maternal care. Briefly, this procedure initially searches for regions in each chromosome that are associated with maternal care. This analysis allows the identification of regions that affect the traits of interest, in a context-independent fashion. In the next step, QTL interactions are investigated across the entire genome. Epistatic QTLs are context dependent; that is, the effect of specific genotypes at one locus depends on which genotypes are present at another locus. These interaction effects are often overlooked because the relevance of epistasis has been generally ignored. The ability of QTL analysis to



**Figure 5:** Maternal performance genotypic values for two-locus genotype at *D8Mit25* + 22 cM and *D15Mit2* + 20 cM illustrates additive-by-dominance interaction.

detect interactions shows the utility of QTL studies relative to other technologies used to identify genes affecting traits.

We will use our study to explain how these procedures work (Peripato et al. 2002). An  $F_2$  intercross between the LG/J and SM/J mouse strains (see Cheverud et al. 1996; Kramer et al. 1998; and Vaughn et al. 1999 for details of animal husbandry) was randomly mated to produce an  $F_3$  litter. An intercross involves hybridizing the parental strains (LG/J and SM/J) and then intermating the genetically identical  $F_1$  hybrids. The parental strains exhibit the abnormal maternal performance for offspring survival of 26% for SM/J and 60% for LG/J (Peripato et al. 2002). The  $F_1$  females had normal maternal performance, keeping at least one pup alive through the first week of life. Nevertheless, 31 of 241  $F_2$  females (12%) failed to keep their offspring alive through the first week. We used the  $F_2$  in-

formation to investigate QTLs for maternal performance for offspring survival. In order to do so, we amplified and scored 96 polymorphic loci to cover all 19 autosomes as completely as possible (fig. 2). The presence of potential single QTL and their relative positions were determined by interval mapping (Lander and Bolstein 1989) using multiple regression analysis (Haley and Knott 1992). Statistical significance of one-QTL models was evaluated using likelihood of odds (LOD) scores in which LOD score values above the genome-wide significance level provide highly significant evidence for a QTL, while effects significant at a chromosome-wide level are suggestive of linkage (see Cheverud 2001 for levels of significance when correcting for multiple comparisons).

We found two single QTLs affecting maternal performance for offspring survival (table 1). The additive and

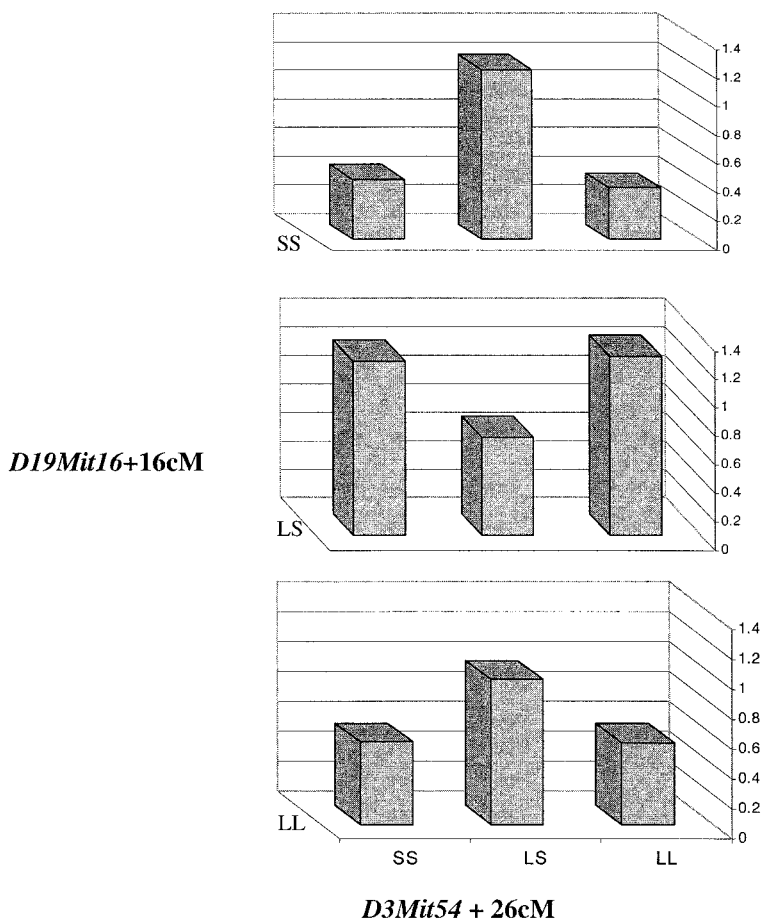


Figure 6: Dominance-by-dominance interaction between *D3Mit54 + 26 cM* and *D19Mit16 + 16 cM*

dominance genotypic values show overdominance at the QTL on chromosome 2, indicating that the heterozygotes display more successful maternal performance for offspring survival than the parental genotypes (fig. 3A). On the other hand, underdominance at the QTL on chromosome 7 indicates that heterozygous females are, on average, less successful than either homozygote at this locus (fig. 3B).

Because maternal performance for offspring survival is a complex trait, many genes may be influencing it, making it appropriate to determine whether epistasis plays an important role in shaping the variation of this trait. This is especially true given the heterosis displayed by the  $F_1$  hybrids from the LG/J by SM/J intercross, where the  $F_1$  hybrids fail to express the abnormal maternal performance seen in the parental strains. Heterosis in a cross is due to interactions between alleles at the same (dominance) or different (epistasis) loci. Given the limited evidence found

for single-locus effects, it seems that epistatic interactions are likely to be responsible for much of the variation in maternal performance in this cross. Although some studies involving behavioral traits have not considered epistasis (Gershenfeld and Paul 1997; Mayeda and Holstetter 1999; Ramos et al. 1999; Cohen et al. 2001), interest in epistatic interactions explaining variation in such traits has been increasing. Shimomura et al. (2001) reveal that the majority of the loci involved in circadian behavior in mice are involved in interactions stressing the importance of epistatic analyses to uncover genetic variation affecting complex behavioral traits.

We searched for epistasis across the whole genome using an interchromosomal, two-way genome-wide scan performed at every 2 cM (Peripato et al. 2002). In order to correct for multiple comparisons and eliminate “false positive” results, we used a significance threshold correction based on the Bonferroni test (Cheverud 2001). Thus, an

**Table 3:** Potential candidate genes for single-locus quantitative trait loci (QTL) and QTLs interacting epistatically for maternal performance

QTL	Position (cM)	Candidate gene	Gene name	Position (cM)	Phenotype	References
<i>D1Mit14</i>	78	<i>Htr5b</i>	5-hydroxytryptamine (serotonin) receptor 5B	63	Anxiety and depression	Clement et al. 1996
<i>D2Mit17</i>	92	<i>Slc30a4</i>	Solute carrier family 30 (zinc transporter), member 4	69	Lethal milk	Dickie 1969
<i>D2Mit1</i>	94	<i>Oxt</i>	Oxytocin	73.5	Lactation problems	Nishimori et al. 1996
	38	<i>Dbh</i>	Dopamine $\beta$ -hydroxylase	15.5	Lack of placenta-phagia/lactation problems	Thomas and Palmiter 1997
<i>D3Mit54</i>	26	<i>Crh</i>	Corticotropin-releasing hormone	8	Abnormal maternal behavior	Pedersen et al. 1991
<i>D6Mit1</i>	20	<i>Mest</i>	Mesoderm-specific transcript	7.5	Lack of placenta-phagia/poor nest	Lefebvre et al. 1998
		<i>Ghrhr</i>	Growth hormone-releasing hormone receptor	26	Failure to nurse first litters	Eicher and Beamer 1976
<i>D7Mit21</i>	2	<i>FosB</i>	FBJ osteosarcoma oncogene B	5	Abnormal maternal behavior	Brown et al. 1996
		<i>Peg3</i>	Paternally expressed gene 3	6.5	Abnormal maternal behavior	Li et al. 1999
<i>D7Nds1</i>	40	<i>Herc2</i>	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
<i>D8Mit25</i>	44	<i>Slc6a2</i>	Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2	45	Associated with complex behavior traits	Fritz et al. 1998
<i>D9Mit4</i>	28	<i>Foxb1b (Mf3)</i>	Forkhead box B1b	41	Lactation problems	Labosky et al. 1997
<i>D11Mit15</i>	76	<i>Crhr</i>	Corticotropin-releasing hormone receptor	62	Abnormal maternal behavior	Pedersen et al. 1991
<i>D13Mit115</i>	38	<i>Prl</i>	Prolactin	14	Abnormal maternal behavior	Lucas et al. 1998
<i>D15Mit15</i>	22	<i>Prlr</i>	Prolactin receptor	4.6	Abnormal maternal behavior	Lucas et al. 1998
<i>D18Mit51</i>	28	<i>CamK2a</i>	Calcium/calmodulin-dependent protein kinase II $\alpha$	33	Aggressive behavior	Chen et al. 1994

epistatic interaction was considered significant in this study when epistasis at the pairs of positions reached the Bonferroni threshold level of 0.1, which, in our case, corresponds to a pointwise probability of  $3.7 \times 10^{-5}$ . We also considered epistasis significant if one of the four modes of epistasis was significant at the  $9.3 \times 10^{-6}$  level ( $[3.7 \times 10^{-5}]/4$ ). We chose the 0.1 significance level to balance between the danger of potentially reporting a false positive result and the danger of ignoring large numbers of true instances of epistasis. These pairs of loci are summarized in table 2. We found 23 chromosomal regions interacting epistatically, involving 16 different chromosomes (markers with overlapping confidence regions were conservatively considered to be a single locus), affecting variation in maternal performance. These different pat-

terns of interactions may be represented by a combination of four basic forms of epistasis (additive by additive, additive by dominance, dominance by additive, and dominance by dominance; Cheverud and Routman 1995). In additive-by-additive epistasis (e.g., fig. 4), if *D5Mit61* is SS, *D1Mit3* is additive with SS having the highest maternal performance. In contrast, if *D5Mit61* is LL, the pattern at *D1Mit3* is reversed. *D1Mit3* is still additive, but LL has the best maternal performance. Because a specific genotype reverses its effect on the trait depending on a second locus, we may fail to detect them as single-locus QTLs (Routman and Cheverud 1997). Additive-by-dominance epistasis shows the same pattern as dominance-by-additive epistasis but with the roles of the loci reversed. Figure 5 represents additive-by-dominance epistasis between chromosome 8

and chromosome 15. Note that *D8Mit25* has additive effects with the LL homozygote displaying superior maternal performance for both *D15Mit2* homozygotes, but also note that this effect is reversed in *D15Mit2* heterozygotes. Likewise, *D15Mit2* shows overdominance among *D8Mit25* SS animals and underdominance among *D8Mit25* LL animals. Most cases of dominance-by-dominance epistasis detected are negative (table 2). An example of this sort of interaction (fig. 6) shows that double heterozygotes have a lower genotypic value than single heterozygotes for each locus. Despite this, double heterozygotes still have better maternal performance than parental or recombinant homozygotes.

The results of our experiments indicate how important epistasis can be in explaining variation in maternal performance and how much of this genetic variation would be missing by ignoring it. If we were looking only for single QTLs, we would have found few individual dominance gene effects that account for 10% of the variation in maternal performance, but when epistatic interactions were included, we are able to explain nearly 60% (34.5% adjusted) of the phenotypic variation for this trait.

The existence of significant QTLs indicates the presence of a gene (or genes) associated with maternal performance for offspring survival at those chromosomal positions. It can then be enlightening 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 located close to our single-locus and epistatic QTLs. Although this identification is preliminary, it may suggest potential genes affecting the trait. All five knockout genes that have been linked to maternal behavior (Bridges 1998; Li et al. 1999) are potential candidate genes for QTLs detected in our study. Two of them are candidates for single QTLs and three of them are found near regions involved in epistatic interactions (table 3). Other potential candidate genes for QTLs are associated with abnormal maternal behavior and complex behavioral traits (table 3). A separate study on maternal performance on early offspring growth in this same cross found six single-locus QTLs and 10 interactions for maternal performance for offspring growth (Wolf et al. 2002). Five of these QTLs were found in similar positions to QTLs described here (table 2). Out of these five, two candidate genes for QTLs for maternal performance for offspring survival lie in similar positions to QTLs affecting maternal performance for offspring growth that are involved in lactation, an important factor for growth in mammals. 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 among surviving

pups. Therefore, we expect some genes to be modulating both traits.

Candidate genes suggested here do not cover all QTLs 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 QTL approach enables the identification of regions in the genome affecting the trait of interest that may be investigated further.

### Considerations and Future Directions

Our experiments have revealed QTLs affecting maternal performance for offspring survival in  $F_2$  mice from the SM/J by LG/J intercross. In order to support these results, future QTL studies of maternal performance should include fine-scale mapping and the investigation of recombinant inbred (RI) lines. We expect that if these QTLs are indeed affecting maternal performance, they should show different frequencies in successful and unsuccessful RI strains. We will also study maternal behaviors in detail and search for QTLs affecting specific behaviors, such as grooming, nest building, milk provision, and aggressive behavior. Ultimately, we hope that individual genes affecting maternal performance may be identified and extrapolated to other mammals, due to synteny among them.

The strategy presented here to investigate the genetic factors underlying a complex behavioral trait indicates the existence of many genes interacting in a complex web of epistasis. Therefore, the genetics of maternal performance is context dependent. Not only do alleles at the same loci interact but their expression is altered by alleles present at other loci. This indicates that investigation of specific genes via knockout gene technology should be performed using different genetic backgrounds, such as different strains of mice. The effect of a gene is not an absolute property of that gene but one dependent on the genetic context in which it is expressed.

Maternal care has been of interest to researchers due to its complexity and its role as an environmental influence on the phenotypes of relatives. The context dependence revealed here and by Shimomura et al. (2001) indicates that a program to investigate complex behavioral traits needs to take interaction into account in order to be effective. We have provided an example of how to analyze one of these interactions: that among intrinsic genetic factors of an individual. Extrinsic components should also be considered. Environmental factors, such as the experience obtained during rearing and consecutive parities, influence the behavior mothers exhibit. Likewise, genes expressed in the offspring interact with genes expressed in the mothers (Wolf et al. 2002) modulating behaviors to ensure offspring survival and growth.

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