INTRODUCTION

Maternal care is critical to offspring growth and survival, which is greatly improved by building an effective nest. Some suggest that genetic variation and underlying genetic effects differ between fitness-related traits and other phenotypes. We investigated the genetic architecture of a fitness-related trait, nest building, in F2 female mice intercrossed from inbred strains SM/J and LG/J using a QTL analysis for six related nest phenotypes (Presence and Structure pre- and postpartum, prepartum Material Used and postpartum Temperature). We found 15 direct-effect QTLs explaining from 4 to 13% of the phenotypic variation in nest building, mostly with non-additive effect. Epistatic analyses revealed 71 significant epistatic interactions which together explain from 28.4 to 75.5% of the variation, indicating an important role for epistasis in the adaptive process of nest building behavior in mice. Our results suggest a genetic architecture with small direct effects and a larger number of epistatic interactions as expected for fitness-related phenotypes.

Keywords: maternal behavior, fitness, QTL, epistasis, mice

Maternal care is a complex and flexible phenotype with critical importance to fitness (Mousseau and Fox, 1998). The environment created by the mother modifies several phenotypes such as survival, growth, body size, and the emotional development of their offspring (Francis and Meaney, 1999; Caspi and Moffitt, 2006; Räsänen and Kruuk, 2007), and is more important than any other single factor during the first days of life (Lee et al., 1991). The performance of a mother is related to specific maternal care characteristics such as milk production and ejection (Horsemann et al., 1997), aggression against intruders (Bateson, 1994), and nest building (Lynch, 1981, 1994; Bult and Lynch, 1997).

Nest building has an essential role in offspring viability (Wallace, 1981), being important for protection against predators (Boake, 1994), for temperature maintenance (Lynch, 1994; Gaskill et al., 2011), for fostering pup’s development by an increase of growth hormone (Marple et al., 1972; Zimmerberg and Shartrand, 2004), for reduction of water loss (Friedman and Bruno, 1976), and for keeping pups in closer contact with their mother (Fleming et al., 1999). Nest building is also common in adult mice (males and non-pregnant females) for thermoregulation (Weber and Olsson, 2008), and the behavior for maternal and non-maternal nests probably shares some genetic basis (Lynch, 1981). Mice selected for adaptations to cold temperatures build better adult nests (Barnett and Dickson, 2008) which lead to more successful offspring (Bult and Lynch, 1997). General nest building behavior also has, in male and female mice, a high to moderate broad-sense heritability (H2 from 0.4 to 0.6) and low narrow-sense heritability (h2 around 0.2; Lee, 1973; Lynch, 1994), suggesting that natural selection is removing disadvantageous additive genetic variation.

Although there is abundant information on nest building, little is known about the evolution of this behavior and its genetic architecture (Boake, 1994; Weber and Olsson, 2008). By genetic architecture of a phenotype, we refer to the total number of genetic regions involved, their direct effects (additivity), inter-allelic interactions at the same locus (dominance), interactions between different loci (epistasis), and the extension of effects across other phenotypes (pleiotropy) (Falconer and Mackay, 1996; Erickson, 2005). Taking into account multiple genetic components simultaneously allows the understanding of phenotypes with complex genetic architectures that would be unpredictable if we consider only one component at a time (Rockman, 2008). Knowledge of the underlying genetic architecture leads to inferences about the causal physiological networks involved and aids in the discovery of genes responsible for phenotypic differences. Also, genes’ interactions and effects contain important information about past and future phenotypic evolution (Templeton, 2006; Levy and Siegal, 2008; Parter et al., 2008).

The genetic architecture for phenotypes critical to survival and reproduction (fitness-related traits) may be dramatically different from that of phenotypes not directly related to fitness (Peripato et al., 2004). Fitness-related traits usually have a larger number of underlying genes, and, consequently, higher levels of additive and interaction variance because there are more targets for mutational events (Houle et al., 1996). However, due to natural selection acting on the direct effects through generations, fitness-related traits are expected to have less relative (per gene) additive genetic variation and, because of the high number of possible interactions, more dominance, and epistatic genetic variation than other complex traits (Lynch and Walsh, 1998; Merilä and Sheldon, 1999). Moreover, traits related to fitness may have proportionally more genes with intermediate effects in comparison with other phenotypes due to the balance between natural selection and genetic drift (Ohta, 1973; Merilä and Sheldon, 1999).
We analyzed all mothers during the first week after delivery for a fitness-related trait. As soon as pregnancy was detected, females were each maintained in a single cage and provided with pieces of cotton of similar sizes (average 3.67 ± 0.48 g) at the cage's food/water level to be used for nest building. To compare possible differences in material usage described in some studies (Sherwin, 1997; van de Weerd et al., 1998), we also added paper (average 1.08 ± 0.03 g) under the cotton for most animals. The final phenotype of each female was the sum of those weighed daily after birth, 5/7 in the third day after birth, and progressively. For the postpartum period, the highest number of days was 7, so we multiplied each value by: 7/7 in the first day after birth, 6/7 in the second day after birth, 5/7 in the third day after birth, and progressively. The final phenotype of each female was the sum of those weighed values divided by the sum of days phenotypes were measured for that female. In order to bring this back to the correct scale, we then multiply each value by the number of days each phenotype was measured (multiplied by the maximum number of days measured, in our case, seven) divided by the sum of indices considering all days in which measurements were taken. By doing this, we also reduced biases due to different number of days being scored for different animals, like the ones caused by differences in pregnancy detection during the prepartum period.

**NEST BUILDING**

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Presence, Nest Closure, and Nest Height in a PCA for each period. We performed a separate PCA for Cotton Used and Paper Used and maintained the original values of Temperature Difference. The analysis, then, allowed us to reduce the number of meaningful phenotypes for the subsequent QTL analysis by using, for each female, factor scores (values of the best planes explaining all variance) instead of the original values. With this, we increased the phenotypes’ repeatability, since the new variables aggregate similar measures with minimal error, while also increasing the degrees of freedom for the epistatic QTL analysis. We used six phenotypic variables for the QTL analysis: Prepartum Nest Structure (1) and Nest Presence (2) resulting from standardized vectors of the PCA between original prepartum phenotypes Nest Presence, Nest Closure, and Nest Height; Material Used (3) from the resulting standardized vector of the PCA between original phenotypes of Cotton Used and Paper Used; Postpartum Nest Presence (4) and Nest Structure (5) from the standardized vectors of the PCA between original postpartum phenotypes Nest Presence, Nest Closure, and Nest Height; and finally Temperature Difference (6), unchanged from original.

**GENOTYPING**
We genotyped 234 F2 females for 101 microsatellites markers previously shown to be polymorphic for SM/J and LG/J strains that uniformly cover all 19 autosomal chromosomes and the sex chromosome X (Figure 1).

We used a Guanidine Thiocyanate protocol (Nelson and Krawetz, 1992) to extract total DNA from the liver of 234 F2 females previously sacrificed and necropsied. We then used a PCR amplification protocol for microsatellites (Dietrich et al., 1992) modified by Routman and Cheverud (1994) to efficiently genotype a high number of samples. PCR products were visualized and genotyped using 5% agarose gels and ethidium bromide staining.

**STATISTICAL ANALYSIS**

**Nest building**
We used the parametric Multivariate analysis of variance (MANOVA), with the Wilks lambda ($\lambda$) test in SYSTAT 13 (Systat Software Inc., USA) to investigate the differences between nest building phenotypes during pre- and postpartum periods of the F2 females. A significance threshold of 0.05 was used in all phenotypic tests.

**Quantitative trait loci analysis**
We performed QTL analysis of the F2 females in maternal phase to correlate the segregating phenotypes and polymorphic genetic markers covering a genome (Liu, 1997). First, the linkage map of the 101 markers for the F2 females was created using the Kosambi function, with the program Map Manager QTX (Manly et al., 2001). We, then, used interval mapping (2 cM intervals) and multiple regression analysis (Haley and Knott, 1992) to estimate additive (a) and dominance (d) genotypic values for each interval (Falconer and Mackay, 1996). We calculated these values using the MIXED procedure in SAS 9 (SAS Institute Inc., USA).

The statistical significance of the tests was measured by LOD scores in each analysis against two different significance thresholds, chromosome-wide, and genome-wide, based on the multilocus model of Li and Ji (2005). The 5% genome-wide specific threshold value for the F2 mice is 3.27, while the 5% chromosome-wide threshold values ranged from 1.77 to 2.13, depending on the chromosome, with a mean of 1.97. LOD scores higher than the chromosome-wide significance threshold only suggest an association of the genomic region with the phenotype (Weller et al., 1998). LOD scores higher than the genome-wide significance threshold are a strong evidence of such association (Lander and Kruglyak, 1995). Confidence intervals for each QTL were specified by the one-LOD-drop-rule (Lynch and Walsh, 1998).

The epistatic QTL analysis was performed by an interchromosomal two-way genome-wide scan performed at every two cM using the F2 Model (Cockerham, 1954) extended to two loci by Cockerham and Zeng (1996) in SAS 9 (SAS Institute Inc., USA). We did not evaluate epistasis among genotypes within chromosomes because of difficulties in interpretation caused by linkage disequilibrium. For each pair of chromosomes, we plotted all F values generated from these analyses which had probabilities below 0.05. We considered as potential epistatic QTLs only those positions exhibiting the highest F values in a plot. Distant F peaks separated by low F values were taken as distinct epistatic signals (Leamy et al., 2005) and epistatic QTLs found within 10 cM were treated as the same locus. Due to the problem of false positives arising through multiple comparisons, we estimated a significance threshold based in the Bonferroni criteria for gene–gene interactions using the Li and Ji (2005). The total number of independent epistasis tests per phenotype, calculated by the cross products’ sum of markers for all pairs of chromosomes, was 3,137. This suggests that we might expect about 157 tests to be significant at the 5% level, 31 at the 1% level, and 3 at the 0.1% level due of chance alone. In our study, the 0.05 significance threshold for interactions was 0.05/3137 = 1.63 × 10⁻⁵ which corresponds to a LOD score of 4.79. We considered epistatic tests with probability below this P value as significant evidence for epistasis (Peripato et al., 2002; Leamy et al., 2009). We also considered epistasis significant if one of the four types of epistasis (additive by additive, additive by dominant, dominant by additive, and dominant by dominant) was below 4.09 × 10⁻⁶ (1.63 × 10⁻⁵/4) which corresponds to a LOD score of 5.33, even if the overall epistasis model was not significant.

We used a GLM model in SYSTAT 13 (Systat Software Inc., USA) to estimate the percentage of variation in each nest building phenotype explained by direct-effect QTLs. We also estimated the contribution of all direct-effect and epistatic QTLs together in models with and without interactions to evaluate the effect of all regions and the importance of epistasis to nest building.

**RESULTS**

**NEST BUILDING**
Pre- and postpartum nest building data for the F2 females are listed in Table 1. We found significant differences between the pre- and postpartum sets of phenotypes Nest Presence, Nest Closure, and Nest Height (Wilks $\lambda_{3,36} = 0.87, P = 10^{-10}$). Nest Presence is greater in the postpartum period ($F_1 = 13.66, P = 10^{-3}$), but prepartum values are greater for Nest Closure ($F_1 = 29.18, P = 10^{-6}$) and Nest Height ($F_1 = 29.18, P = 10^{-6}$).
**Table 1 | Nest building for pre- and postpartum periods, in F2 females in maternal phase.**

| Phenotype                          | n  | Mean          |
|------------------------------------|----|---------------|
| Prepartum                          |    |               |
| Nest presence (0–1)                | 231| 0.873 ± 0.015a|
| Nest closure (0–1)                 | 224| 0.145 ± 0.015a|
| Nest height (cm)                   | 224| 3.752 ± 0.055c|
| Cotton used (g)                    | 116| 1.862 ± 0.081 |
| Paper used (g)                     | 106| 0.761 ± 0.042 |
| Postpartum                         |    |               |
| Nest presence (0–1)                | 241| 0.958 ± 0.010a|
| Nest closure (0–1)                 | 238| 0.072 ± 0.010b|
| Nest height (cm)                   | 139| 3.217 ± 0.089c|
| Temperature difference (°C)        | 232| 2.613 ± 0.075 |

Means are expressed as mean ± SEM. Upper script letters indicate that there is significant difference ($P < 10^{-6}$) between pre- and postpartum equivalent phenotypes.

**PRINCIPAL COMPONENT ANALYSIS IN NEST BUILDING DATA**

Using the PCA, we reduced the number of nest building phenotypes for the QTL analyses. Prepartum variables Nest Closure, Nest Height, and Nest Presence were represented by the resulting first two vectors, Nest Structure and Nest Presence, which explain 54.6 and 28.7% of the original variation, respectively. The vector Material Used, resulting from the prepartum variables Cotton Used and Paper Used explains 94.6% of their variation. Likewise, postpartum variables Nest Closure, Nest Height, and Nest Presence were represented by the resulting two vectors, Nest Structure and Nest Presence, explaining 51 and 32.9% of the original variation, respectively.

**DIRECT-EFFECT AND EPISTATIC QTL ANALYSIS**

We searched for direct-effect QTLs in F2 females in maternal phase using regressions of genotypic values imputed for every 2 cM with the nest building phenotypes Nest Presence pre- and postpartum, Nest Structure pre- and postpartum, Material Used prepartum, and Temperature Difference postpartum. In total, we obtained 15 regions that, individually, are significantly associated with the variation in the six pre- and postpartum phenotypes analyzed (Figure 2), after correcting for multiple tests. Of these, five QTLs are above the genome-wide threshold and the other 10 regions are above the chromosome-wide threshold.

Out of the 10 prepartum direct-effect QTLs (Table 2), four regions have mainly direct (additive) effects: preNP8, preNS7,
preNS17, and preNSX, whereas the other six QTLs showed mostly dominance effects, with four showing overdominance (preNS15, preMU1, preMU10, and preMU16) and two under-dominance (preNP6 and preMU12). Considering the additive and dominance effects, QTLs explain, together, 7% of the variation for Nest Presence, with QTL effects of 3.4 and 4.3%. QTLs explain, together, 16.1% of variation for Nest Structure, with QTL effects ranging from 4.4 to 6.4%, whereas QTLs explain 26.6% of the variation for Material Used, with QTL effects varying from 9.1 to 11.2%.

The five postpartum direct-effect QTLs we found (Table 2) have only significant dominance effects. Heterozygous females have a higher score structured nest than parental ones based on overdominance effect for postNS15. Nonetheless, poor nest performance in heterozygous females (under-dominance) is found at the QTLs postNP1, postNS7, postTD2, and postTD14. The postNP1 QTL explains 9.8% of the variation of this phenotype. QTLs for Nest Structure explain, together, 20.3% of its variation, with QTL effects of 8.7 and 12.9%, whereas QTLs explain 7% of the variation for Temperature Difference, with QTL effects of 3.1 and 4.6%.

We found several interactions in the analysis of epistatic QTL for nest building phenotypes in the F2 females in maternal phase. We detected close to 1,900 significant tests for epistasis at the 5% level considering all the phenotypes here investigated, which is about twice as many as we would have expected by chance. Furthermore, 500 were significant at the 0.1% level though we would expect 19 by chance and 71 that were significant at the corrected 5% level ($P < 1.63 \times 10^{-5}$), though we expected less than one by chance. The analyses on prepartum variables revealed 10 significant epistatic interactions at the corrected 5% level affecting Nest Presence involving 17 regions (Figure 3A; Table A1 in Appendix), seven interactions for Nest Structure involving seven regions (Figure 3C; Table A2 in Appendix) and 10 for Material Used involving 15 regions (Figure 3E; Table A3 in Appendix). The comparison of epistatic QTLs for prepartum nest building phenotypes revealed a region at medial chromosome 7 which is common for Nest Presence and Nest Structure and another at proximal chromosome 11 which is common for Nest Presence and Material Used. We found direct-effect
Table 2 | Direct-effect QTLs of pre- and postpartum nest building phenotypes in F2 females in maternal phase.

| Nest building                  | QTL name | Chr | Location (CI) | Additive effect | Dominance effect | Variance explained (%) | LOD score |
|-------------------------------|----------|-----|---------------|-----------------|------------------|------------------------|-----------|
| Prepartum nest presence       | preNP6   | 6   | 10 (0–20)     | −0.531*         | −0.423*          | 4.3                    | 2.10      |
| Prepartum nest structure      | preNS7   | 7   | 82 (74–90)    | −0.747*         | −0.292           | 6.4                    | 3.58      |
| Prepartum material used       | preMU1   | 1   | 10 (0–24)     | 0.787*          | 0.562*           | 11.2                   | 2.77      |
| Postpartum nest presence      | postNP1  | 1   | 36 (22–48)    | 0.281           | −0.762*          | 3.6                    | 9.80      |
| Postpartum nest structure     | postNS7  | 7   | 74 (60–82)    | −0.793*         | −0.917*          | 3.9                    | 12.90     |
| Postpartum temperature difference| postTD2 | 2   | 80 (66–98)    | −0.409*         | −0.442*          | 2.5                    | 4.60      |

Direct-effect QTLs are listed by phenotype affected, name, chromosome, location from centromere in centiMorgans and Confidence Interval of 1 LOD (CI), Standardized additive effect, $2a/\sigma_p$, and Standardized dominant effect, $d/\sigma_p$ (with asterisks indicating significance), and percentage of variation explained. Genome-wide threshold is 3.27.

FIGURE 3 | Location of epistatic QTLs for nest presence and nest structure pre- and postpartum, material used prepartum, and temperature difference postpartum across the total 20 chromosomes.

(A) Nest presence prepartum; (B) Nest Presence postpartum; (C) Nest Structure prepartum; (D) Nest Structure postpartum; (E) Material Used prepartum; (F) Temperature Difference postpartum.
QTLs located inside the confidence interval of some of the epistatic QTLs detected, at chromosome 6 for prepartum Nest Presence (0–20 cM) and at chromosomes 1, 12, and 16 for Material Used.

In the postpartum period, we found 26 epistatic interactions for Nest Presence involving 29 regions (Figure 3B; Table A4 in Appendix), 13 interactions for Nest Structure involving 22 regions (Figure 3D; Table A5 in Appendix), and five for Temperature Difference involving 10 regions (Figure 3F; Table A6 in Appendix). Among epistatic QTLs of postpartum nest building phenotypes, we found regions in common in proximal chromosome 3, distal chromosomes 5, 7, and 11, and medial chromosomes 13 and 16 for Nest Presence and Nest Structure. There were direct-effect and epistatic QTLs in common at chromosome 1 for postpartum Nest Presence, at chromosome 7 for postpartum Nest Structure and at chromosome 2 for Temperature Difference.

When we contrasted pre- and postpartum phenotypes, we found several regions in common for Nest Presence, at chromosomes 4, 10, 13, 15, and X, whereas for Nest Structure we found common regions at chromosomes 4, 9, and 19.

All direct-effect and epistatic QTLs and their interactions together explain 28.4% of Nest Presence prepartum, 45.1% of Nest Structure prepartum, 62.3% of Material Used, 75.7% of Nest Presence postpartum, 34.9% and of Nest Structure postpartum, 40% for Temperature Difference.

**DISCUSSION**

Nest building is a common behavior for thermoregulation in adult rodents (Weber and Olsson, 2008) which is typical during the maternal phase for mother/offspring aspects beyond thermoregulation (Marple et al., 1972; Boake, 1994; Lynch, 1994; Zimmerberg and Shartrand, 2004; Gaskill et al., 2011). LG/J and SM/J dams exhibit similar maternal performance of prepartum nest building (Chiavegatto et al., 2012). Additionally, when we contrast the Nest Presence in F2 females in prepartum stage and in adult F2 males and females that were not in maternal phase we found similar values (data not shown), suggesting that they may share some genetic basis (Lynch, 1981). However, F2 females in maternal phase have significantly higher values for Nest Height (on average, 1.28 times higher than F2 males) and Nest Closure (on average, 7.17 times more frequently closed than females that were not in maternal phase; data not shown). These results are similar to studies in rodents showing that maternal nests are more structured than non-maternal nests, two times higher and usually closed (Denenberg et al., 1969; Weber and Olsson, 2008). Maternal nests may also be structurally different depending on maternal stage. Generally, mothers show some programmed hormonal stimulus response during pregnancy and sensorial stimuli after birth coming from pups (Rosenblatt, 1975). Consequently, we would expect differences in nest building behavior in these two phases. Indeed, here we observed significant differences between pre- and postpartum phenotypes of nest building in F2 females. Even though LG/J and SM/J females build a pre- and postpartum nest, the postpartum nest differ in quality between the strains (Chiavegatto et al., 2012) with SM/J females building higher score nests than LG/J females. The LG/J × SM/J genetic background for females from this intercross generates a poor quality postpartum nest, as seen in F2 females. This may suggest interactions among genes modulating maternal performance of nest building. Nonetheless, even if mothers built similar nests before and after giving birth, the pups alone could alter the postpartum phenotypes measured. The decrease of postpartum Nest Closure and Nest Height quality in F2 could come from difficulties faced by mothers to keep nests structured with pups interfering. LG/I and SM/J females have smaller litter size (6.59 and 4.05, respectively) when compared to F2 females (8.73) (Goes, C. P., Sauce, B., and Peripato, A. C., unpublished data) and the interference of pups in the nest may be less pronounced in the parental strains. Since litter size has a positive correlation with Nest Presence (r = 0.21) and negative correlations with Nest Closure (r = -0.25) and Nest Height (r = -0.04) in the F2 females (Sauce, 2010), the number of pups may be impacting the quality of postpartum nest building.

The direct-effect QTL analysis of prepartum nest building detected 10 significant regions that explain, individually, between 3.4 and 11.2% of the variation, which indicates effects of moderate intensity compared to other genetic regions and phenotypes (Ramos et al., 1999; Turri et al., 2001). Six of the 10 direct-effect QTLs detected showed significant non-additive effects of over-and-under-dominance. We also found direct-effect QTLs overlapping in pre- (preNS7 and preNS15) and postpartum (postNS7 and postNS15) nest building phenotypes. Considering that pre- and postpartum nest building may be distinct phenotypes, as indicated by our results on nest quality (closure and height) and considering that quality is also the difference between maternal and non-maternal nests in mice, the presence of common QTLs for pre- and postpartum Nest Structure suggests common genetic components in mothers shared with thermoregulatory nest building behavior in non-pregnant adults (Lynch, 1981; Weber and Olsson, 2008).

The analysis of epistasis revealed a vast number of interactions modulating prepartum nest building variation (Figures 3A,C) that ratify the participation of non-additive effects for this phenotype. We detected half as many direct-effect QTLs for postpartum nest building when compared to the prepartum period. This may not be surprising considering the smaller sample size of the former compared to the later. Interestingly, though, all postpartum QTLs showed over or under-dominance instead of only additive effects, which is not expected if you consider the higher degrees of freedom (sample size) required to detect interactions. It is possible that several of the loci with additive effects have small direct effects that would not show up as significant in our analysis, perhaps because alleles with larger direct effects might have been eliminated by natural selection. Hence, additive effects in the F2 females could be hidden from selection by epistatic interactions or be dependent on changes in environment and genetic architecture (Templeton, 2006). It is expected that, due to the Beavis effect (Beavis, 1998), the number of QTLs detected in analyses performed with less than 1000 individuals may be biased downward whereas their average effects will be biased upward. These effects are more pronounced with dominance, because there is higher variance associated with its detection, since in general there is more power to detect additive than dominance effects. We only detected dominance effects...
for postpartum nest building and a combination of additive and dominance for prepartum nest building. Because pre- and postpartum data have roughly the same sample size, this difference might not be due to the Beavis effect, but, instead, might suggest the existence of more interaction effects in the postpartum, possibly due to increased levels of complexity brought by the offspring. The mother-offspring interaction may be a source of epistasis because genes expressed in the offspring may interact with genes expressed in the mother (Wolf et al., 2002). Furthermore, the care of a mother affects many behaviors in the offspring (Varki et al., 2008), which may create a network of more complex behavioral interactions. This is exactly what we found, with even more complexity for the interactions in postpartum nest phenotypes (Figures 3B,D) that may reflect that pups foster more interactions among hormones than in pregnancy in which pups show only indirect effects. Moreover, because the postpartum period may have greater importance for pup survival (Wallace, 1981; Weber and Olsson, 2008), it is possible that these differences support the hypothesis that phenotypes more closely related to fitness have proportionally less additive genetic variance (Merila and Sheldon, 1999).

Even though this study was performed in a cross between LG/J and SM/J, two strains which have been selected for size and have been maintained in the lab for more than 50 years, we may still consider broad evolutionary considerations to explain our results. Because the phenotypes here studied may be important to fitness, it is possible that the strains were exposed to direct selection favoring their survival in the lab during their formation. Nonetheless, the phenotypes here investigated failed to show significant correlations with size (data not shown), indicating that the alleles here studied were not subject to the same selection pressure as relations with size (data not shown), indicating that the alleles investigated their survival in the lab during their formation. Nonetheless, it is possible that these differences support the hypothesis that phenotypes more closely related to fitness have proportionally less additive genetic variance (Merila and Sheldon, 1999).

The results of the direct-effect and epistatic QTL analyses in the pre- and postpartum periods highlight the complexity in nest building’s genetic architecture. We expect that this phenotype, being a complex maternal behavior and a fitness-related trait, may have intermediate genetic effects and several regions with dominance and epistatic interactions. Using the same intercross, we found the same pattern in other maternal aspects related to fitness such as maternal performance for offspring survival (Peripato et al., 2002), litter size (Peripato et al., 2004), and milk provision (Goes, C. P., Sauce, B., and Peripato, A. C., unpublished data). On the other hand, studying non-fitness-related traits, such as morphology and body composition, in the same intercross, we found more direct-effect locus QTL of small additive effect and fewer epistatic QTLs than compared to those fitness-related traits (Norgard et al., 2008; Leamy et al., 2009). Considering the higher degrees of freedom in direct-effect QTLs tests, we should expect more direct effects than epistatic effects, particularly considering the reduced sample sizes here investigated. Therefore, the greater number of epistatic QTLs when compared to direct-effect QTLs in our study suggests that genetic interactions are modulating maternal behavior of nest building.

Nest building in mammals is set by an important array of maternal behaviors that ensures pups survival and it is directly associated with fitness, as well as milk provision (Falconer and Mackay, 1996), suggesting that genetic interactions could play a major role in the adaptive process as stocks of new additive variation (Phillips, 2008). The QTL effects found in pre- and postpartum nest building behaviors show low to moderate values. This is expected in a genetic architecture of fitness-related traits because, according to some models, mutations with intermediate effects are more favored during evolution of traits closely related to fitness (Merila and Sheldon, 1999). In conclusion, our results provide important information about the genetic architecture of the maternal behavior of nest building in mice. We found 15 individual QTLs with effects ranging from 4 to 13% of the phenotypic variation in nest building, but when all significant epistatic interactions (71 overall) are considered, they explain on average 47.7% of the variation per nest building phenotype (ranging from 28.4 to 75.5%), indicative of the important role played by epistasis in the adaptive process of nest building behavior in mice.

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## APPENDIX

### Table A1 | Epistatic QTLs of prepartum nest presence in F$_2$ females.

| Locus 1  | Locus distance to marker (cm) | Locus distance to marker (cm) | Locus 2  | Locus distance to marker (cm) | Locus distance to marker (cm) | Prob. epistasis | Type | Genotypic value | Prob. genotypic value |
|----------|-------------------------------|-------------------------------|----------|-------------------------------|-------------------------------|------------------|------|----------------|-----------------------|
| D4Mit16  | 8                             | 68                            | D7Mit227 | 10                            | 32                            | 4.48 $\times 10^{-8}$ | AD   | 0.645          | 1.53 $\times 10^{-3}$  |
| D5Mit6   | 4                             | 46                            | D6Mit1   | 16                            | 16                            | 1.6 $\times 10^{-5}$    | AD   | -0.952         | 9.51 $\times 10^{-8}$  |
| D6Mit9   | 0                             | 22                            | DXMit172 | 16                            | 58                            | 7.26 $\times 10^{-6}$    | AD   | 0.615          | 5.12 $\times 10^{-5}$  |
| D7Mit227 | 22                            | 44                            | D11Mit62 | 26                            | 26                            | 5.1 $\times 10^{-6}$    | AA   | 0.669          | 6.45 $\times 10^{-4}$  |
| D7Mit71  | 8                             | 92                            | D15Mit143| 2                             | 18                            | 3.52 $\times 10^{-7}$    | AA   | -0.650         | 2.31 $\times 10^{-3}$  |
| D8Mit189 | 8                             | 76                            | D10Mit10 | 14                            | 62                            | 2.53 $\times 10^{-7}$    | AA   | -0.741         | 1.32 $\times 10^{-4}$  |
| D9Mit90  | 22                            | 22                            | D11Mit15 | 0                             | 48                            | 5.13 $\times 10^{-8}$    | DA   | 0.862          | 3.89 $\times 10^{-3}$  |
| D13Mit115| 40                            | 52                            | D15Mit143| 8                             | 24                            | 1.45 $\times 10^{-5}$    | AA   | -0.732         | 2.62 $\times 10^{-4}$  |
| D15Mit143| 10                            | 26                            | DXMit144 | 14                            | 34                            | 6.48 $\times 10^{-7}$    | AA   | -0.534         | 5.23 $\times 10^{-3}$  |
| D17Mit46 | 0                             | 0                             | D18Mit17 | 18                            | 38                            | 1.44 $\times 10^{-6}$    | AA   | -0.308         | 2.36 $\times 10^{-2}$  |

Epistasis type: AA, additive by additive; AD, additive by dominance; DA, dominance by additive; DD, dominance by dominance. Markers in bold were considered the same region in that respective chromosome.

### Table A2 | Epistatic QTLs of prepartum nest structure in F$_2$ females.

| Locus 1  | Locus distance to marker (cm) | Locus distance to marker (cm) | Locus 2  | Locus distance to marker (cm) | Locus distance to marker (cm) | Prob. epistasis | Type | Genotypic value | Prob. genotypic value |
|----------|-------------------------------|-------------------------------|----------|-------------------------------|-------------------------------|------------------|------|----------------|-----------------------|
| D1Mit212 | 8                             | 26                            | D4Mit325 | 6                             | 6                             | 3.79 $\times 10^{-5}$    | DD   | 0.687          | 9.63 $\times 10^{-7}$  |
| D5Mit233 | 6                             | 26                            | D6Mit1   | 0                             | 0                             | 1.38 $\times 10^{-4}$    | DD   | -0.675         | 3.19 $\times 10^{-6}$  |
| D5Mit233 | 4                             | 24                            | DXMit55  | 10                            | 10                            | 5.44 $\times 10^{-5}$    | AD   | 0.805          | 8.12 $\times 10^{-7}$  |
| D6Mit188 | 10                            | 62                            | D9Mit8   | 14                            | 68                            | 2.26 $\times 10^{-7}$    | AD   | 0.681          | 5.82 $\times 10^{-9}$  |
| D7Nds1   | 2                             | 52                            | D14Mit225| 8                             | 48                            | 7.05 $\times 10^{-5}$    | AD   | 0.827          | 1.54 $\times 10^{-6}$  |
| D9Mit4   | 10                            | 46                            | D11Mit14 | 0                             | 66                            | 6.56 $\times 10^{-6}$    | AD   | 0.461          | 5.51 $\times 10^{-4}$  |
| D19Mit35 | 8                             | 66                            | DXMit55  | 18                            | 18                            | 9.28 $\times 10^{-6}$    | AA   | 0.351          | 3.38 $\times 10^{-2}$  |

Epistasis type: AA, additive by additive; AD, additive by dominance; DA, dominance by additive; DD, dominance by dominance. Markers in bold were considered the same region in that respective chromosome.
Table A3 | Epistatic QTLs of prepartum Material Used in F2 females.

| Locus 1 | Locus distance to marker (cm) | Locus 2 | Locus distance to marker (cm) | Locus 3 | Locus distance to marker (cm) | Locus 4 | Locus distance to marker (cm) | Locus 5 | Locus distance to marker (cm) | Prob. epistasis | Type | Genotypic value | Prob. genotypic value |
|---------|-------------------------------|---------|-------------------------------|---------|-------------------------------|---------|-------------------------------|---------|-------------------------------|-----------------|------|-----------------|----------------------|
| D1Mit14 | 2                             | D3Mit22 | 6                             | 28      | D5Mit59                       | 6       | 70                           | 1.97 x 10^{-5} | DD   | 1.038           | 2.60 x 10^{-6}      |
| D1Mit3  | 6                             | D5Mit59 | 6                             | 70      | D5Mit59                       | 6       | 70                           | 1.72 x 10^{-4} | AA   | -1.071          | 2.80 x 10^{-6}      |
| D1Mit11 | 2                             | D7Mit21 | 0                             | 0       | D5Mit59                       | 6       | 70                           | 5.63 x 10^{-6} | AD   | 0.789           | 1.89 x 10^{-4}      |
|         |                               |         |                               |         |                               |         |                               | 0.781             | DD   | 3.85 x 10^{-5} |                       |
| D1Mit14 | 2                             | D16Mit5 | 8                             | 30      | D5Mit59                       | 6       | 70                           | 9.71 x 10^{-8} | AA   | 0.802           | 7.06 x 10^{-4}      |
|         |                               |         |                               |         |                               |         |                               | 0.916             | DA   | 6.19 x 10^{-6} |                       |
|         |                               |         |                               |         |                               |         |                               | 0.916             | DA   | 6.19 x 10^{-6} |                       |
| D5Mit338 | 10                            | D12Mit2 | 10                            | 28      | D5Mit59                       | 6       | 70                           | 8.17 x 10^{-6} | AD   | 0.869           | 7.51 x 10^{-5}      |
|         |                               |         |                               |         |                               |         |                               | 0.732             | DD   | 3.29 x 10^{-4} |                       |
| D5Mit59 | 2                             | D19Mit43| 10                            | 10      | D5Mit59                       | 10      | 28                           | 1.19 x 10^{-5} | AA   | -0.734          | 3.11 x 10^{-2}      |
|         |                               |         |                               |         |                               |         |                               | -0.774           | DA   | 6.03 x 10^{-3} |                       |
|         |                               |         |                               |         |                               |         |                               | -0.796           | DD   | 6.67 x 10^{-3} |                       |
| D8Mit58 | 6                             | D17Mit10| 18                            | 60      | D5Mit59                       | 6       | 70                           | 6.98 x 10^{-7} | DA   | 2.402           | 2.27 x 10^{-8}      |
| D11Mit349 | 0                            | D17Mit46| 18                            | 18      | D5Mit59                       | 6       | 70                           | 2.74 x 10^{-6} | AD   | -2.401          | 1.49 x 10^{-7}      |
| D11Mit62 | 0                             | D18Mit17| 0                             | 20      | D5Mit59                       | 6       | 70                           | 4.62 x 10^{-5} | DD   | 0.950           | 2.46 x 10^{-6}      |
| D17Mit46 | 22                            | D18Mit10| 14                            | 14      | D5Mit59                       | 6       | 70                           | 5.28 x 10^{-6} | AA   | 0.945           | 4.98 x 10^{-2}      |
|         |                               |         |                               |         |                               |         |                               | 1.529             | DA   | 1.09 x 10^{-2} |                       |
|         |                               |         |                               |         |                               |         |                               | 2.897             | DD   | 1.81 x 10^{-6} |                       |

*Epistasis type: AA, additive by additive; AD, additive by dominance; DA, dominance by additive; DD, dominance by dominance. Markers in bold were considered the same region in that respective chromosome.*
Table A4 | Epistatic QTLs of postpartum nest presence in F2.

| Locus 1 | Locus distance to marker (cm) | Locus distance (cm) | Locus 2 | Locus distance to marker (cm) | Locus distance (cm) | Prob. epistasis | Type | Genotypic value | Prob. genotypic value |
|---------|-------------------------------|--------------------|---------|-------------------------------|--------------------|----------------|------|----------------|------------------------|
| D1Mit3  | 6                             | 10                 | D9Mit355 | 14                             | 88                 | 1.04 x 10^-7 | AA   | 1.395          | 1.23 x 10^-7            |
|         |                               |                    |         |                               |                    |                | DA   | -0.631         | 5.61 x 10^-3            |
| D1Mit3  | 4                             | 8                  | D9Mit4*  | 12                             | 48                 | 1.24 x 10^-6 | AA   | 1.181          | 1.27 x 10^-6            |
| D1Mit7  | 6                             | 48                 | D11Mit325 | 0                             | 58                 | 6.67 x 10^-7 | AA   | 0.944          | 2.88 x 10^-4            |
|         |                               |                    |         |                               |                    |                | AD   | 0.624          | 1.42 x 10^-3            |
|         |                               |                    |         |                               |                    |                | DD   | 0.495          | 1.12 x 10^-2            |
| D1Mit3  | 8                             | 12                 | DXMit172 | 12                             | 54                 | 1.94 x 10^-5 | AA   | 0.609          | 1.17 x 10^-2            |
| D2Mit22 | 20                            | 128                | D4Mit235 | 10                             | 10                 | 1.57 x 10^-5 | AA   | 0.761          | 2.09 x 10^-3            |
|         |                               |                    |         |                               |                    |                | AD   | 0.761          | 2.09 x 10^-3            |
|         |                               |                    |         |                               |                    |                | DD   | 0.618          | 3.23 x 10^-3            |
| D2Mit22 | 12                            | 120                | D9Mit355 | 18                             | 92                 | 9.13 x 10^-7 | AA   | 0.838          | 6.54 x 10^-4            |
|         |                               |                    |         |                               |                    |                | DD   | 1.095          | 2.1 x 10^-6             |
| D2Mit22 | 10                            | 118                | D12Mit5  | 6                              | 44                 | 1.21 x 10^-8 | AA   | -0.943         | 6.92 x 10^-5            |
|         |                               |                    |         |                               |                    |                | DD   | 1.122          | 4.51 x 10^-8            |
| D2Mit22 | 12                            | 120                | DXMit55  | 2                              | 2                  | 3.28 x 10^-6 | AA   | 0.762          | 1.87 x 10^-3            |
|         |                               |                    |         |                               |                    |                | DA   | -0.653         | 1.28 x 10^-3            |
|         |                               |                    |         |                               |                    |                | DD   | -0.719         | 1.67 x 10^-3            |
| D3Mit22 | 4                             | 26                 | D6Nds5  | 0                              | 36                 | 2.64 x 10^-7 | AA   | -1.831         | 5.22 x 10^-5            |
|         |                               |                    |         |                               |                    |                | AD   | 1.052          | 1.15 x 10^-3            |
|         |                               |                    |         |                               |                    |                | DD   | -0.750         | 3.21 x 10^-2            |
|         |                               |                    |         |                               |                    |                | DD   | 0.915          | 2.64 x 10^-3            |
| D4Mit235 | 10                         | 10                 | D5Mit59  | 18                             | 82                 | 1.7 x 10^-10 | AA   | -0.748          | 7.23 x 10^-3            |
|         |                               |                    |         |                               |                    |                | AD   | 1.073          | 9.98 x 10^-7            |
|         |                               |                    |         |                               |                    |                | DD   | -0.817         | 2.16 x 10^-4            |
|         |                               |                    |         |                               |                    |                | DD   | 0.735          | 8.58 x 10^-5            |
| D4Mit16* | 8                           | 68                 | D11Mit14 | 14                             | 80                 | 1.61 x 10^-6 | DA   | -0.745         | 1.74 x 10^-4            |
|         |                               |                    |         |                               |                    |                | DD   | 0.781          | 8.72 x 10^-6            |
| D4Mit235 | 4                           | 4                  | D15Mit143 | 8                             | 24                 | 7.52 x 10^-5 | AD   | -1.372         | 3.74 x 10^-6            |
| D4Mit2  | 4                            | 18                 | D16Mit2  | 2                              | 2                  | 1.39 x 10^-6 | DA   | -1.379         | 5.16 x 10^-7            |
| D4Mit16* | 16                          | 76                 | D17Mit10 | 26                             | 68                 | 2.63 x 10^-6 | AA   | -1.051         | 7.50 x 10^-4            |
|         |                               |                    |         |                               |                    |                | DA   | -1.038         | 1.93 x 10^-5            |
| D7Mit17  | 12                          | 78                 | D13Mit115 | 34                             | 46                 | 3.28 x 10^-6 | DA   | 1.513          | 8.79 x 10^-8            |
| D9Mit355 | 12                          | 86                 | D12Mit5  | 10                             | 48                 | 8.25 x 10^-13| AA   | -1.097         | 2.92 x 10^-6            |
| D9Mit4*  | 2                            | 38                 | D13Mit115 | 40                             | 52                 | 2.54 x 10^-8 | AA   | -1.035         | 3.31 x 10^-4            |
|         |                               |                    |         |                               |                    |                | AD   | 0.654          | 6.66 x 10^-3            |
|         |                               |                    |         |                               |                    |                | DA   | -0.970         | 3.30 x 10^-5            |
|         |                               |                    |         |                               |                    |                | DD   | 0.663          | 3.60 x 10^-3            |
| D10Mit15 | 22                          | 44                 | D15Mit143 | 18                             | 34                 | 2.62 x 10^-11| AA   | -1.518         | 1.76 x 10^-4            |
|         |                               |                    |         |                               |                    |                | AD   | 1.369          | 2.65 x 10^-5            |
|         |                               |                    |         |                               |                    |                | DA   | -1.174         | 2.66 x 10^-4            |
|         |                               |                    |         |                               |                    |                | DD   | 1.327          | 2.91 x 10^-6            |
| D10Mit14 | 2                            | 66                 | D18Mit17 | 0                              | 20                 | 5.76 x 10^-10| AA   | 1.738          | 3.36 x 10^-4            |
|         |                               |                    |         |                               |                    |                | AD   | -1.770         | 3.77 x 10^-6            |
|         |                               |                    |         |                               |                    |                | DA   | -1.320         | 1.93 x 10^-4            |
|         |                               |                    |         |                               |                    |                | DD   | 1.035          | 1.32 x 10^-3            |

(Continued)
Table A4 | Continued

| Locus 1 | Locus distance to marker (cm) | Locus 2 | Locus distance to marker (cm) | Locus distance (cm) | Prob. epistasis | Type | Genotypic value | Prob. genotypic value |
|---------|--------------------------------|---------|--------------------------------|---------------------|----------------|------|----------------|-----------------------|
| D11Mit14 | 14 | 80 | D13Mit15 | 28 | 40 | 6.07 × 10^{-10} | AA | 1.753 | 3.67 × 10^{-8} |
|         |     |     |        |        |     |     | AD | -1.233 | 1.09 × 10^{-4} |
|         |     |     |        |        |     |     | DA | -0.515 | 3.13 × 10^{-2} |
|         |     |     |        |        |     |     | DD | 0.631 | 3.36 × 10^{-2} |
|         |     |     |        |        |     |     | Prob. epistasis | Type | Genotypic value | Prob. genotypic value |
| D11Mit349 | 6 | 42 | D16Mit2 | 0 | 0 | 5.11 × 10^{-7} | AA | -1.147 | 4.18 × 10^{-5} |
|         |     |     |        |        |     |     | AD | 0.621 | 5.39 × 10^{-3} |
|         |     |     |        |        |     |     | DA | 0.671 | 5.62 × 10^{-4} |
| D13Mit115 | 34 | 46 | D15Mit2* | 20 | 56 | 3.44 × 10^{-7} | AA | -1.097 | 2.56 × 10^{-4} |
|         |     |     |        |        |     |     | AD | -0.952 | 1.06 × 10^{-4} |
|         |     |     |        |        |     |     | DA | 0.871 | 3.01 × 10^{-3} |
| D13Mit115 | 8 | 20 | D17Mit10 | 24 | 66 | 2.02 × 10^{-7} | AA | 4.388 | 2.94 × 10^{-4} |
|         |     |     |        |        |     |     | AD | -3.092 | 8.69 × 10^{-4} |
|         |     |     |        |        |     |     | DA | -3.191 | 5.02 × 10^{-4} |
|         |     |     |        |        |     |     | DD | 1.819 | 2.52 × 10^{-2} |
| D13Mit147 | 26 | 98 | DXMit172 | 10 | 52 | 4.83 × 10^{-6} | AA | -0.939 | 3.10 × 10^{-4} |
|         |     |     |        |        |     |     | DD | 0.822 | 1.78 × 10^{-5} |
| D14Mit5 | 10 | 38 | DXMit55 | 0 | 0 | 3.5 × 10^{-7} | AA | 0.712 | 7.08 × 10^{-4} |
|         |     |     |        |        |     |     | AD | -0.754 | 1.99 × 10^{-5} |
|         |     |     |        |        |     |     | DA | -0.439 | 1.45 × 10^{-2} |
| D15Mit2* | 20 | 56 | D16Mit5 | 14 | 36 | 1.31 × 10^{-6} | AA | -0.953 | 5.11 × 10^{-5} |

Epistasis type: AA, additive by additive; AD, additive by dominance; DA, dominance by additive; DD, dominance by dominance. Markers in bold were considered the same region in that respective chromosome. *After marker means a different set that is considered as same region in the same chromosome.
Table A5 | Epistatic QTLs of postpartum nest structure in F2 females.

| Locus 1 | Locus distance to marker (cm) | Locus distance to marker (cm) | Locus 2 | Locus distance to marker (cm) | Locus distance to marker (cm) | Prob. epistasis | Type | Genotypic value | Prob. genotypic value |
|---------|-------------------------------|-------------------------------|---------|-------------------------------|-------------------------------|----------------|------|----------------|-----------------------|
| D1Mit14 | 14                            | 92                            | D9Mit8  | 6                             | 60                            | 7.73 × 10^{-6} | AA   | 0.672          | 5.02 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | DA   | -0.764         | 1.30 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | DD   | 0.505          | 7.24 × 10^{-3}         |
| D1Mit541| 2                             | 96                            | D11Mit14| 14                            | 80                            | 1.95 × 10^{-9} | AA   | 0.844          | 5.04 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | AD   | -0.676         | 4.72 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | DA   | -0.839         | 2.43 × 10^{-5}         |
|         |                               |                               |         |                               |                               |                | DD   | 0.579          | 8.92 × 10^{-4}         |
| D2Mit307| 0                             | 88                            | D4Mit235| 12                            | 12                            | 1.12 × 10^{-5} | AA   | -0.767         | 2.55 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | DA   | 0.620          | 3.63 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | DD   | 1.025          | 1.15 × 10^{-8}         |
| D2Mit380| 18                            | 68                            | D16Mit5 | 4                             | 26                            | 6.36 × 10^{-8} | DA   | 0.412          | 3.22 × 10^{-2}         |
|         |                               |                               |         |                               |                               |                | DD   | -1.025         | 1.15 × 10^{-8}         |
| D2Mit307| 0                             | 88                            | D19Mit39| 14                            | 42                            | 7.67 × 10^{-6} | AD   | -0.606         | 1.63 × 10^{-2}         |
|         |                               |                               |         |                               |                               |                | DD   | 1.019          | 2.82 × 10^{-6}         |
| D3Mit54 | 20                            | 20                            | D7Mit71 | 6                             | 90                            | 8.12 × 10^{-7} | AA   | 1.358          | 3.51 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | AD   | 1.000          | 6.03 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | DA   | 0.903          | 6.52 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | DD   | 1.152          | 1.81 × 10^{-4}         |
| D5Mit59 | 12                            | 76                            | D7Mit71 | 0                             | 84                            | 6.26 × 10^{-9} | AA   | -1.037         | 2.61 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | AD   | -0.774         | 3.67 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | DA   | 0.505          | 3.50 × 10^{-2}         |
|         |                               |                               |         |                               |                               |                | DD   | 0.905          | 1.16 × 10^{-5}         |
| D5Mit338| 8                             | 58                            | D12Mit6 | 14                            | 64                            | 7.34 × 10^{-6} | AA   | 1.074          | 8.66 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | AD   | 0.792          | 1.32 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | DA   | 0.637          | 9.6 × 10^{-3}          |
| D7Mit17 | 12                            | 78                            | D13Mit15| 18                            | 30                            | 1.25 × 10^{-6} | DD   | 1.706          | 5.79 × 10^{-8}         |
|         |                               |                               |         |                               |                               |                | DA   | 0.752          | 3.50 × 10^{-2}         |
|         |                               |                               |         |                               |                               |                | DD   | 1.155          | 8.93 × 10^{-5}         |
| D7Mit46 | 8                             | 116                           | D17Mit46| 20                            | 20                            | 1.26 × 10^{-5} | DD   | -0.928         | 7.99 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | DA   | 1.155          | 8.93 × 10^{-5}         |
| D10Mit10| 8                             | 56                            | D15Mit143| 10                           | 26                            | 3.9 × 10^{-6}  | AA   | 1.220          | 1.66 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | AD   | -1.097         | 2.49 × 10^{-4}         |
| D10Mit213| 10                           | 10                            | D19Mit39| 28                            | 56                            | 8.18 × 10^{-6} | DA   | -0.14         | 4.57 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | DD   | 0.912          | 1.12 × 10^{-4}         |
| D12Mit6 | 14                            | 64                            | D19Mit43| 22                            | 22                            | 5.29 × 10^{-7} | AA   | -0.752         | 2.82 × 10^{-3}         |
|         |                               |                               |         |                               |                               |                | AD   | 0.800          | 3.44 × 10^{-4}         |
|         |                               |                               |         |                               |                               |                | DA   | -0.767         | 1.95 × 10^{-4}         |

Epistasis type: AA, additive by additive; AD, additive by dominance; DA, dominance by additive; DD, dominance by dominance. Markers in bold were considered the same region in that respective chromosome.
Table A6 | Epistatic QTLs of postpartum temperature difference in F2 females.

| Locus 1    | Locus 1 distance to marker (cm) | Locus 2    | Locus 2 distance to marker (cm) | Locus 3 | Locus 3 distance to marker (cm) | Prob. epistasis | Type     | Genotypic value | Prob. genotypic value |
|------------|---------------------------------|------------|---------------------------------|---------|---------------------------------|-----------------|----------|----------------|---------------------|
| D2Mit380   | 18                              | D19Mit35   | 6                               | 64      | 1.95 x 10^{-6}                 | AA -0.569       | DD 0.666 | 5.06 x 10^{-3} | 3.71 x 10^{-4}   |
|            |                                 |            |                                 |         |                                 | DA 0.630       | DD 0.552 | 9.76 x 10^{-4} |                    |
|            |                                 |            |                                 |         |                                 | DA -0.638      | DD -0.496 | 3.21 x 10^{-3} |                    |
|            |                                 |            |                                 |         |                                 | DA -0.496      | DD -0.630 | 1.1 x 10^{-2}  |                    |
|            |                                 |            |                                 |         |                                 | DD 0.882       | DD 1.314 | 9.00 x 10^{-4} |                    |
| D6Nds5     | 14                              | D10Mit15   | 0                               | 22      | 1.19 x 10^{-6}                 | AD -0.352       | DA 0.352 | 2.39 x 10^{-2} |                    |
|            |                                 |            |                                 |         |                                 | DA -0.702      | AA 0.702 | 6.59 x 10^{-6} |                    |
|            |                                 |            |                                 |         |                                 | DA -0.882      | AA 0.927 | 3.87 x 10^{-7} |                    |
| D9Mit385   | 24                              | D14Mit225  | 16                              | 56      | 3.08 x 10^{-10}                | AD -0.882       | DA 0.882 | 8.70 x 10^{-12} |                    |
| D10Mit14   | 6                               | D13Mit1    | 0                               | 0       | 1.54 x 10^{-6}                 | AD 0.702        | AA 0.702 | 6.59 x 10^{-6} |                    |
|            |                                 |            |                                 |         |                                 | DA -0.882      | AA 0.927 | 3.87 x 10^{-7} |                    |
| D13Mit147  | 20                              | D18Mit17   | 18                              | 38      | 3.69 x 10^{-6}                 | AD 0.882        | AA 0.927 | 6.59 x 10^{-6} |                    |

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