Detecting Maternal-Effect Loci by Statistical Cross-Fostering

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ABSTRACT Great progress has been made in understanding the genetic architecture of phenotypic variation, but it is almost entirely focused on how the genotype of an individual affects the phenotype of that same individual. However, in many species the genotype of the mother is a major determinant of the phenotype of her offspring. Therefore, a complete picture of genetic architecture must include these maternal genetic effects, but they can be difficult to identify because maternal and offspring genotypes are correlated and therefore, partially confounded. We present a conceptual framework that overcomes this challenge to separate direct and maternal effects in intact families through an analysis that we call "statistical cross-fostering." Our approach combines genotype data from mothers and their offspring to remove the confounding effects of the offspring's own genotype on measures of maternal genetic effects. We formalize our approach in an orthogonal model and apply this model to an experimental population of mice. We identify a set of six maternal genetic effect loci that explain a substantial portion of variation in body size at all ages. This variation would be missed in an approach focused solely on direct genetic effects, but is clearly a major component of genetic architecture. Our approach can easily be adapted to examine maternal effects in different systems, and because it does not require experimental manipulation, it provides a framework that can be used to understand the contribution of maternal genetic effects in both natural and experimental populations.

MATERNAL effects occur when mothers have an indirect causal influence on the expression of traits in their offspring independent of genes passed from mothers to offspring (see Cheverud and Wolf 2009; Wolf and Wade 2009). These maternal effects, which arise from a diversity of factors such as maternally derived mRNA (Berleth *et al.* 1988), maternal provisioning (Bowen 2009), and maternally determined dispersal (Donohue 1998), have been shown to have important influences on offspring development across a diversity of taxa (Mousseau and Fox 1998; Maestripieri and Mateo 2009). The importance of maternal effects in evolution and ecology has become more broadly recognized (Mousseau and Fox 1998), where maternal effects have been shown to play a role in the evolutionary response to selection (see Kirkpatrick and Lande 1989), mate choice and sexual selection (Wolf *et al.* 1997, 1999), adaptive evolution (*e.g.*, Badyaev *et al.* 2002), dynamics of population size (Ginzburg 1998), and niche construction (Odling-Smee *et al.* 2003). Genetically based maternal effects ("maternal genetic effects") are of particular importance to many processes because they contribute to the genetic architecture of traits and can, as a result, contribute in nonintuitive ways to evolutionary change (Kirkpatrick and Lande 1989; Cheverud and Wolf 2009). For example, maternal genetic effects can contribute "hidden" variation that can allow for rapid evolution (*e.g.*, Badyaev *et al.* 2002) and can be the cause of evolutionary time lags, constraints, or momentum (Kirkpatrick and Lande 1989). However, genetic studies of maternal effects have been limited because of the difficulty in applying experimental paradigms, such as cross-fostering, in both natural and experimental populations.

Maternal genetic effects are a form of an indirect genetic effect (Moore *et al.* 1997; Wolf *et al.* 1998), where genes in the mother have their phenotypic effect in her offspring. As a result, the phenotypic consequences of maternal genetic effects appear not in mothers, but in their offspring, where the phenotype of offspring is, to some degree, a "property" of the maternal genotype. Therefore, to study maternal genetic effects, one must be able to attribute phenotypic

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variation in offspring to genetic variation in their mothers, but because mothers and their offspring share half of their genes, the two effects are partially confounded. This confounding can make it difficult or sometimes impossible to distinguish the influence of the maternal genome from the influence of the offspring genome on the expression of offspring traits (but see Wolf *et al.* 2011).

Many approaches have been developed to overcome the confounding of direct and maternal effects, but existing approaches still suffer from limitations (see Roff 1997 for a review of empirical approaches to separating direct and maternal effects). For example, approaches that rely on asymmetrical patterns of resemblance of relatives in crossing schemes (*e.g.*, Kever and Rotman 1987; Jarvis *et al.* 2005) cannot directly differentiate maternal effects from other potential causes of asymmetry, such as genomic imprinting (Hager *et al.* 2008) or uniparental inheritance such as cytoplasmic or sex chromosome inheritance (Wolf and Wade 2009). Other approaches, such as experimental cross-fostering (Bateman 1954; Cox *et al.* 1959; White *et al.* 1968), can detect maternal effects but cannot differentiate between maternal genetic and environmental sources unless a more complex design using related mothers is followed (Wilson *et al.* 2005; Wilson and Festa-Bianchet 2009).

More recently, genomic mapping approaches have been implemented to identify maternal genetic effects by mapping from the maternal genotype to offspring phenotypes. For example, Wolf *et al.* (2002) used means of cross-fostered litters to identify maternal effects and Casellas *et al.* (2009) mapped maternal effects in small chromosomal blocks in subcongenic mice by mapping maternal genotype to offspring phenotype using an unspecified linear model. Thus, despite a growing body of information accumulating on maternal effects in general, the technical challenges in design and analysis mean that we still have limited reliable data on maternal genetic effects, especially from populations where experimental manipulation is not possible or long-term pedigree data are not available (but exceptions exist, *e.g.*, Wilson *et al.* 2005). To overcome these challenges Wolf *et al.* (2011) used a linear model approach in a population with cross-fostering to separate the independent effects of the maternal and offspring genotypes on the offspring phenotype. Although that approach was fruitful and identified a number of loci affecting prenatal and postnatal growth, it was limited by the fact that no formal statistical framework was available to explore the properties of the analysis or the nature of the results produced.

Here we develop a simple conceptual approach for the identification and characterization of maternal effects that uses genetic information from parents and offspring to achieve what we call "statistical cross-fostering." This approach uses marker information from parents and their offspring to develop an analysis that accounts for the genomic autocorrelation of mothers and their offspring caused by Mendelian inheritance. These sorts of marker data are becoming widely available and therefore, implementing such an approach could be achieved in most systems. Because this approach does not require any experimental manipulation, it can be applied to natural populations, assuming that appropriate marker information is available. We illustrate this approach using data from an experimental population of mice. We develop a formal statistical model, but stress the conceptual basis for the approach, rather than the specific quantitative techniques used (which can be adapted to the specific nature of other data sets).

A Conceptual Framework for Studying Maternal Genetic Effects

We first present a conceptual model where we illustrate the confounding effects of the offspring and maternal genomes and then use this model to illustrate why cross-fostering allows for the separation and identification of maternal effects. We then illustrate how one can break the correlation between the maternal and offspring genomes without cross-fostering by using subsets of genotypes to achieve what we call statistical cross-fostering. This approach is then formalized into an orthogonal linear model and applied to an experimental population of mice.

We consider a hypothetical population where there are two alleles at a locus, A_1 and A_2, with frequencies p_1 and p_2, respectively. For simplicity, we assume that there is random mating and that these genotypes occur in Hardy–Weinberg proportions. Deviations from these assumptions will alter the specific results, but not the general conclusions that we emphasize. We assume that the locus can have a direct effect and a maternal effect. These effects can be characterized using the traditional additive and dominance genotypic values or effects (Falconer and Mackay 1996). Importantly, however, we define the direct and maternal effects in an idealized model where each is defined in the absence of the other effect. This is done because we wish to define a maternal effect as being attributable to the maternal genotype and a direct effect as being attributable the property of the offspring genotype. Because the two are confounded by relatedness, the presence of both effects makes it problematic to clearly separate direct effects from indirect maternal effects in the whole population when both are present. This is not a limitation of the model *per se*, but rather is a limitation imposed by the biology of maternal effects, and therefore we use the idealized model to illustrate this confounding and then examine how the two can be successfully separated.

For direct effects (in the absence of maternal effects), the additive effect (a_o) is defined as half the difference between the mean phenotypes (the "genotypic values") of the two homozygotes (A_1A_1 mean minus A_2A_2 mean), and the dominance effect (d_o) is defined as the deviation of the heterozygote mean from the midpoint between the two homozygotes (*i.e.*, unweighted mean of the two classes) (see Falconer and Mackay 1996).

Maternal genetic effects (in the absence of direct effects) are defined by the average phenotype of the offspring of

Table 1 Expected offspring phenotype as a function of their own genotype and the genotype of their mother

	Offspring genotype			
	A_1A_1	A_1A_2	A_2A_2	Maternal litter average
Maternal genotype				
A_1A_1	$+a_m +a_o$ (p_1^3)	$+a_m +d_o$ $(p_1^2p_2)$	—	$+a_m + p_2d_o + p_1a_o$
A_1A_2	$+d_m +a_o$ $(p_1^2p_2)$	$+d_m +d_o$ $(p_1^2p_2 + p_1p_2^2)$	$+d_m -a_o$ $(p_1p_2^2)$	$+d_m + \frac{1}{2}d_o + \frac{1}{2}a_o(p_1 - p_2)$
A_2A_2	—	$-a_m +d_o$ $(p_1p_2^2)$	$-a_m -a_o$ (p_2^3)	$-a_m + p_1d_o - p_2a_o$
Offspring average	$+a_o + p_2d_m + p_1a_m$	$+d_o + \frac{1}{2}d_m + \frac{1}{2}a_m(p_1 - p_2)$	$-a_o + p_1d_m - p_2a_m$	

Each cell gives the direct (subscripted "o") and maternal (subscripted "m") effects that contribute to the phenotype of an offspring as a function of the maternal–offspring genotype combination. The cells with dashes exist under Mendelian inheritance. The expected phenotypes of the offspring associated with each maternal genotype are given as row means and the average phenotype of each offspring genotype is given by the column means. The frequencies of each maternal–offspring genotype combination are shown in parentheses (Wade 1998).

mothers of a particular genotype. The additive maternal effect (a_m) is defined as half the difference in the mean phenotypes of the offspring of the two types of homozygous mothers (again, the A_1A_1 mean minus A_2A_2 mean), while the dominance maternal effect (d_m) is defined as the deviation of the mean phenotype of offspring that have heterozygous mothers from the midpoint between the mean phenotypes of the offspring that have homozygous mothers.

Maternal genetic effects in intact families

The idealized direct and maternal effects we have defined above can be used to characterize the phenotypes of offspring as a function of their own genotype and the genotype of their mother (see Table 1), where the offspring phenotype is the sum of the direct and maternal effects. In doing so, it is important to note that, because mothers homozygous for one allele cannot have offspring that are homozygous for the alternate allele, there are two maternal–offspring genotype combinations that cannot occur (Table 1). These missing combinations are wholly responsible for the confounding of maternal and offspring effects (Wade 1998) and hence are the main obstacle to studying maternal genetic effects.

Using the genotypic values of offspring defined as a function of the direct effect of their genotype and the maternal effect of their mother's genotype, we can examine the mean phenotypes of offspring of the different genotypes or the means of offspring produced by the different types of mothers (these are the row and column means in Table 1). These mean phenotypes clearly demonstrate that the presence of a maternal effect is manifested in the average phenotypes associated with the offspring genotypes and, likewise, the direct effect is manifested in the average phenotypes of the offspring associated with the different maternal genotypes. For example, offspring with the A_1A_1 genotype have an average phenotype of $+a_o + p_2d_m + p_1a_m$, illustrating that the additive and dominance maternal effects contribute to the average phenotype of the A_1A_1 individuals, despite the fact that these effects do not arise from their own genotype, but their contribution is frequency dependent. This frequency dependence occurs because the expected phenotype of A_1A_1 individuals depends on the probability that they have A_1A_1 mothers (and hence experience the $+a_m$ effect) *vs.* A_1A_2 mothers (and experience the $+d_m$ maternal effect) (see Cheverud and Wolf 2009). Likewise, the average phenotype of the offspring of the A_1A_1 mothers is $+a_m + p_2d_o + p_1a_o$, showing the symmetrical confounding of direct effects with maternal effects.

These average phenotypes can be used to examine the apparent additive or dominance direct and maternal effects, where these are defined from the marginal means and include the confounding influence of the maternal and offspring genotypes. It is important to keep in mind that the direct (a_o and d_o) and maternal (a_m and d_m) effects are defined in the absence of the other effect, but here we ask, "What would the apparent direct or maternal effect be if both occur?" These are the values we would observe in a population if we measured individual phenotypes or the phenotypes of the offspring of a set of mothers and used them to infer direct and maternal effects, but did not account for the confounding. The apparent additive direct effect ($a_{o(apparent)}$), calculated as half the difference between the A_1A_1 homozygote and the A_2A_2 homozygote (Table 1), would be

$$a_{o(apparent)} = a_o + \frac{1}{2}d_m(p_2 - p_1) + \frac{1}{2}a_m \qquad (1)$$

(Cheverud and Wolf 2009), which illustrates that the presence of maternal effects contributes to the apparent additive direct effect of the locus. Importantly, maternal effects do not contribute to the apparent dominance effect of the locus (calculated from the marginal means as the deviation of the A_1A_2 phenotypic mean from the unweighted mean phenotype of the A_1A_1 and A_2A_2 genotypes), and so the apparent dominance effect would simply be d_o, *the true effect value*.

Table 2 Expected offspring phenotype under random cross-fostering

	Offspring genotype			
	A_1A_1	A_1A_2	A_2A_2	Maternal litter average
Foster mother genotype				
A_1A_1	$+a_m + a_o$ (p_1^4)	$+a_m + d_o$ $(2p_1^3p_2)$	$+a_m - a_o$ $(p_1^2p_2^2)$	$+a_m + a_o(p_1 - p_2) + 2p_1p_2d_o$
A_1A_2	$+d_m + a_o$ $(2p_1^3p_2)$	$+d_m + d_o$ $(4p_1^2p_2^2)$	$+d_m - a_o$ $(2p_1p_2^3)$	$+d_m + a_o(p_1 - p_2) + 2p_1p_2d_o$
A_2A_2	$-a_m + a_o$ $(p_1^2p_2^2)$	$-a_m + d_o$ $(2p_1p_2^3)$	$-a_m - a_o$ (p_2^4)	$-a_m + a_o(p_1 - p_2) + 2p_1p_2d_o$
Offspring average	$+a_o + a_m(p_1 - p_2) + 2p_1p_2d_m$	$+d_o + a_m(p_1 - p_2) + 2p_1p_2d_m$	$-a_o + a_m(p_1 - p_2) + 2p_1p_2d_m$	

Each cell gives the direct (subscripted "o") and maternal (subscripted "m") effects that contribute to the phenotype of an offspring as a function of the genotype of the offspring and their foster mother. The expected phenotypes of the offspring of each foster mother genotype are given as row means and the average phenotype of each offspring genotype is given by the column means. The frequencies of each foster mother–offspring genotype combination under random cross-fostering are shown in parentheses below the effects.

The apparent additive maternal effect $a_{m(apparent)}$), of the locus, when both maternal and direct effects occur, is

$$a_{m(apparent)} = a_m + d_o(p_2 - p_1) + \frac{1}{2}a_o. \quad (2)$$

Like direct dominance effects, the apparent dominance maternal effect is not confounded with direct effects. Because of the confounding of direct and maternal additive genetic effects, an analysis focused on either direct or maternal effects would detect an apparent effect whenever either is present if the causal effects cannot be identified or controlled for, meaning that empirical analysis of either type of effect in the absence of consideration of the other may produce spurious results (Hager *et al.* 2008).

Analysis of maternal genetic effects with cross-fostering

The confounding of direct and maternal effects can be removed by cross-fostering offspring genotypes across maternal genotypes randomly, such that offspring experience the different maternal genotypes in proportion to their frequency in the population. This scheme is shown in Table 2, which illustrates the expected phenotypes of offspring with different direct and maternal effects, but where the maternal effect arises from the "foster" mother. Note that there are no empty cells in the cross-fostering design since the constraint of Mendelian inheritance has been removed. From the marginal means (mean phenotypes as a function of the direct or maternal genotype) it is clear that, although maternal effects contribute to the mean phenotype of the different offspring genotypes, they contribute the same value to all genotypes (*i.e.*, they contribute $a_m(p_1 - p_2) + 2p_1p_2d_m$ to all offspring genotypes) and therefore do not appear to contribute a direct effect. Likewise, direct effects do not contribute to apparent maternal effects. As a result, the apparent additive direct effect is a_o, and the apparent additive maternal effect is a_m.

It is clear that, by randomizing offspring genotypes across maternal genotypes, one can remove the issue of confounding and study maternal or direct effects without a concern for the other. However, this approach can identify only maternal effects that occur after the time of the cross-fostering and, therefore, many maternal effects will be missed because it may be very difficult or impossible to perform cross-fostering early enough in development, especially when there are complex connections between maternally and offspring-derived tissues (*e.g.*, moving embryos prenatally in mammals or prior to hatching in species developing within eggs or disentangling embryos from maternal components of seeds) (but see Brumby 1960; Cowley *et al.* 1989; Atchley *et al.* 1991; Rhees *et al.* 1999 for exceptions). Furthermore, even when cross-fostering is technically possible, it may not be practical or even allowable when data come from natural populations where one cannot manipulate natural families. Nonetheless, this approach can be used to identify maternal genetic effects arising after the timing of the cross-fostering, such as postnatal effects in mammals (*e.g.*, Wolf *et al.* 2002). However, it generally means that most information on maternal effects that is derived from cross-fostering schemes comes from the period of external development in species where offspring can be swapped between nests or litters (Price 1998; Maestripieri and Mateo 2009).

Analysis of maternal genetic effects through statistical cross-fostering

When one cannot implement random cross-fostering across maternal genotypes, it may be possible to use intact families and to achieve the statistical separation of direct and maternal effects, as long as marker data are available for both mothers and their offspring. Importantly, the critical issue with regard to the analysis of maternal effects in intact families is the confounding of maternal and offspring genotypes. This confounding cannot be removed without experimental manipulation, but we can use the mother–offspring classes that exist to separately infer direct and maternal effects. The key to this approach is the fact that heterozygous offspring can be produced by all maternal

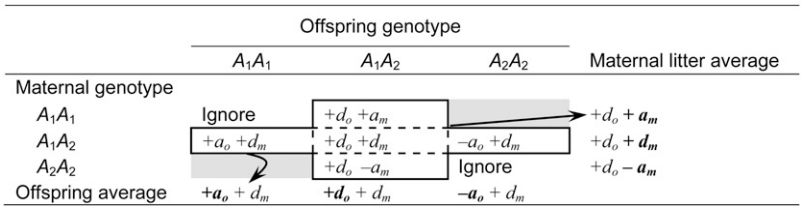

Figure 1 The confounded cells containing homozygous offspring from homozygous mothers are removed. The phenotypes of the offspring of heterozygous mothers are used as the estimates of the phenotype for each offspring genotype. Likewise, the phenotypes of heterozygous offspring are used as the measures of the phenotypes produced by each maternal genotype.

genotypes and heterozygous mothers can produce all types of offspring. Hence, when studying maternal effects one can limit the scope of inference to only heterozygous offspring at a locus and thereby produce a population where genetically variable mothers all have genetically identical offspring (again at that locus, but it is only at that locus that their genomes are confounded). This is illustrated in Figure 1, where the average phenotypes of the different offspring genotypes are simply the phenotypes of the offspring from heterozygous mothers and, likewise, the average phenotypes of the offspring associated with the different genotypic classes of mothers are simply the phenotypic values from the heterozygous offspring. Therefore, the phenotypes are not marginal means (because they are not averaged over different genotype classes), but rather are estimated by directly holding the other effect constant. From the values in Figure 1, it is clear that, as is the case for random cross-fostering across maternal genotypes, maternal effects can contribute to the mean phenotype of all genotypes of offspring, but they contribute the same value to all genotypes. The same is true for the mean phenotype of offspring produced by all genotypes of mothers, where direct effects contribute to the mean, but have the same contribution to all maternal genotypes. Therefore, neither contributes an apparent effect to the other. So the apparent direct effect is, as expected, a_o and the apparent maternal effect is, as expected, a_m (and again, apparent dominance effects are correctly measured as d_o and d_m).

Thus, by removing the confounded genotype combinations from an analysis, one can construct an idealized analysis at a locus by using genetically identical offspring when testing for a maternal effect and genetically identical mothers when testing for a direct effect. When both are present, this analysis also allows one to identify pleiotropic effects, where a locus has both a direct and a maternal effect. Such pleiotropy may be expected on theoretical grounds (Wolf and Brodie 1998; Wolf 2001) and appears significant in many systems where it has been examined (generally appearing as a genetic correlation between direct and maternal effects) (Roff 1997).

Confounding effects of genomic imprinting

Hager *et al.* (2008) demonstrated that the presence of an additive maternal genetic effect could lead to the appearance of parent-of-origin–dependent effect (*i.e.*, the scenario where the effect of an allele depends on which parent it is inherited from) that mimics genomic imprinting and, likewise, that the presence of a parent-of-origin–dependent effect caused by genomic imprinting could lead to the appearance of a pattern consistent with an additive maternal genetic effect when the genomic imprinting effect is not accounted for (Casellas *et al.* 2009; Cheverud and Wolf 2009). Here the parent-of-origin effect is simply defined by a difference in the average phenotypes of reciprocal heterozygotes (*i.e.*, A_1A_2 and A_2A_1, with the first allele coming from the father and the second from the mother) (Wolf *et al.* 2008b). We assume that a true parent-of-origin effect results from genomic imprinting because the reciprocal heterozygotes differ in the parent of origin of the two alleles (it is therefore referred to as the "imprinting effect" hereafter). Again, the confounding of genomic imprinting with maternal effects occurs because the homozygous

Table 3 Expected offspring phenotype as a function of their own genotype and the genotype of their mother when there is a genomic imprinting effect

	Offspring genotype				
	A_1A_1	A_2A_1	A_1A_2	A_2A_2	Maternal litter average
Maternal genotype					
A_1A_1	$+a_m + a_o$ (p_1^3)	$+a_m + d_o - \mathbf{i_o}$ $(p_1^2p_2)$	—	—	$+a_m + a_op_1 + d_op_2 - i_op_2$
A_1A_2, A_2A_1	$d_m + a_o$ $(p_1^2p_2)$	$d_m + d_o - i_o$ $(p_1p_2^2)$	$d_m + d_o + i_o$ $(p_1^2p_2)$	$d_m - a_o$ $(p_1p_2^2)$	$+ d_m + \frac{1}{2}d_o + \frac{1}{2}(a_o + i_o)\ (p_1 - p_2)$
A_2A_2	—	—	$-a_m + d_o + \mathbf{i_o}$ $(p_1p_2^2)$	$-a_m - a_o$ (p_2^3)	$- a_m - a_op_2 + d_op_1 + i_op_1$
Offspring average	$a_mp_1 + d_mp_2 + a_o$	$a_mp_1 + d_mp_2 + d_o - i_o$	$-a_mp_2 + d_mp_1 + d_o + i_o$	$-a_mp_2 + d_mp_1 - a_o$	

The format matches Table 1, except that, for the offspring, the reciprocal heterozygotes are separated into two classes that differ in the parent of origin of their alleles (with the paternally inherited allele listed first). The cells with dashes are the ones that would be used in the identification of additive maternal effects in the statistical cross-fostering design (Figure 1). The imprinting effect in these cells is boxed in boldface type.

mothers produce only one of the two possible reciprocal heterozygotes (*e.g.*, heterozygous offspring of A_1A_1 mothers must have received the A_1 allele from their mother). This scenario is illustrated in Table 3.

The imprinting effect is measured by the parameter i_o, which is defined as half the difference between the reciprocal heterozygote means, with the sign of the effect defined by the paternally inherited allele (Wolf *et al.* 2008b), meaning that i_o is defined as the mean of the A_1A_2 heterozygote (who gets the A_1 allele from its father, which is the "plus" allele as defined by the sign of the additive effect above) minus the mean of the A_2A_1 heterozygote (who gets the A_2 allele from its father). From the values in Table 3 it is clear that the heterozygous offspring produced by the A_1A_1 and A_2A_2 mothers (Table 3) differ by a factor of $-2i_o$. As a result, the additive maternal effect estimated using just these offspring would yield an estimated additive maternal effect of $-i_o$. Because this is the approach we advocate above for identifying maternal effects under the statistical cross-fostering design, the presence of genomic imprinting could result in the appearance of a nonexistent maternal effect. Therefore, when identifying maternal-effect loci using statistical cross-fostering, it is important to recognize that the apparent additive maternal effects (but not dominance) may actually be caused by genomic imprinting.

This confounding of maternal effects and genomic imprinting effects does not, however, represent an insurmountable problem because, by using genotype data from mothers and offspring, one can infer the phase of the reciprocal heterozygotes, and therefore, whenever statistical cross-fostering can be achieved, it should also be possible to differentiate between the reciprocal heterozygotes and estimate the imprinting effect. The imprinting effect can be analyzed the same way that additive and dominance effects are under the statistical cross-fostering design. The imprinting effect is estimated as the difference between the phenotypes of the reciprocal heterozygote offspring produced by heterozygous mothers. The phase of the alleles in the mothers does not need to be accounted for since genomic imprinting is reset each generation, and therefore both of the reciprocal heterozygote mothers can produce either of the reciprocal heterozygote offspring. This can be clearly seen in Table 3, where the expected phenotypes of the A_2A_1 and A_1A_2 heterozygotes produced by heterozygous mothers (the middle row of Table 3) are $-i_o$ and $+i_o$, respectively. Using the offspring from heterozygous mothers, we would estimate the parent-of-origin effect to be $+i_o$. Thus, if genomic imprinting is leading to the appearance of an apparent maternal effect at a locus, we expect the additive maternal effect to be similar in magnitude and opposite in sign to the parent-of-origin effect at the locus. Because these individuals have genetically identical mothers at this locus, the imprinting effect cannot be attributed to a maternal effect (Hager *et al.* 2008). Consequently, by applying the statistical cross-fostering design to ordered genotypes one should be able to identify the imprinting effects and reconcile the appearance of an additive maternal effect at these loci as being caused by genomic imprinting and, therefore, eliminate these loci as maternal-effect loci.

Thus, when significant additive effects are attributable to true maternal effects, not genomic imprinting, we generally expect to find that the reciprocal heterozygote offspring raised by the different homozygous mothers are phenotypically different, but this difference is not seen among the reciprocal heterozygote offspring raised by heterozygous mothers. In contrast, we expect that genomic imprinting effects should generally make the reciprocal heterozygote offspring phenotypically different, regardless of whether their mothers are homozygotes or heterozygotes. Finally, it is important to keep in mind that it is also possible that a locus could show both imprinting and maternal effects, so that it may not be possible to cleanly distinguish between the two.

An Orthogonal Model of Statistical Cross-Fostering

The statistical cross-fostering scheme can be achieved by simply subdividing the data into separate analyses of direct and maternal effects, as described above (and illustrated in Figure 1), or developed in a single analysis, where these effects are fitted simultaneously as orthogonal components. We present the conceptual framework without providing an explicit statistical model for an analysis because it can be adapted to the specific nature of any given data set. We build on the conceptual framework for the statistical cross-fostering scheme presented above by developing this framework into a linear model where a single analysis is used to simultaneously fit direct and maternal effects as orthogonal components. Because of the potential confounding effects of genomic imprinting effects, we base this model on ordered genotypes in the offspring generation (*i.e.*, matching Table 3). If one were to use unordered genotypes, then the model would be identical, except that the two reciprocal heterozygotes produced by heterozygous mothers would be combined into one cell and the index variable for the parent-of-origin effect would be removed from the design matrix (see below).

To develop a model for direct and maternal effects we assign a set of index values to each of the eight maternal–offspring genotype combinations that can be used in a regression model to provide estimates of the direct and maternal genetic-effect parameters. These index values define the structure of the relationship between the measured phenotypes and measured genotypes in the model and are used, therefore, to estimate the corresponding genetic effects. The index values are chosen to reflect the statistical cross-fostering design described above, but using all of the genotype data from the population in a single analysis. With eight classes of maternal–offspring genotypes we define eight coefficients in the model, with one being the intercept [or reference point, R (Álvarez-Castro and Carlborg 2007)] and the other seven being the three direct-effect parameters (a_o, d_o, and i_o), two maternal-effect parameters (a_m and d_m),

and two new parameters (c_{mo} and dd_{mo}) not present in the framework presented above. The first of these new parameters, c_{mo}, is the confounded effect and the second, dd_{mo}, is the interaction between the dominance effects of the maternal and offspring genomes (see below). These coefficients are shown in Table 4, where each maternal–offspring genotype combination is assigned an index value for each of these eight effects. The matrix in Table 4 defines the genetic design matrix of the model ($\mathbf{S}_{MO}$), which gives the relationship between the maternal–offspring genotype classes and the genetic effects being estimated. This can be formalized by defining the vector of maternal–offspring genotypic values ($\mathbf{G}_{MO}$) (average phenotypes of offspring as a function of their genotype and the genotype of their mother) with the genotypes arrayed as in the first column in Table 4 (*i.e.*, the vector contains eight values, which are the mean phenotypes of the genotype combinations in that column, in the order shown in Table 4) and the vector of genetic effects ($\mathbf{E}_{MO}$) as the row of coefficients in Table 4: $E^{T}_{MO} = [R, a_o, d_o, i_o, a_m, d_m, c_{mo}, dd_{mo}]$, where T denotes the transpose. Thus, it is the genetic design matrix ($\mathbf{S}_{MO}$) that links the vector of genotypic values ($\mathbf{G}_{MO}$) to the vector of genetic effects ($\mathbf{E}_{MO}$):

$$\mathbf{G}_{MO} = \mathbf{S}_{MO} \cdot \mathbf{E}_{MO}. \tag{3}$$

This design matrix can be inverted to solve for the definition of the genetic effects in terms of the genotypic values, *i.e.*, $\mathbf{E}_{MO} = \mathbf{S}^{-1}_{MO}\mathbf{G}_{MO}$, and therefore the inverse of the genetic design matrix defines the solution for the genetic effects as a function of the average phenotypes (*i.e.*, genotypic values) associated with each of the maternal–offspring genotype combinations. The inverse of the genetic design matrix is shown in Table 5. The rows can be read as a set of contrasts based on the genotypic values.

Table 4 The statistical cross-fostering design matrix

Combination	Coefficients							
	R	a_o	d_o	i_o	a_m	d_m	c_{mo}	dd_{mo}
$M_{11}O_{11}$	1	0	$-\frac{1}{2}$	0	0	$-\frac{1}{2}$	1	$\frac{1}{4}$
$M_{11}O_{21}$	1	0	$\frac{1}{2}$	0	1	$-\frac{1}{2}$	0	$-\frac{1}{4}$
$M_{12}O_{11}$	1	1	$-\frac{1}{2}$	0	0	$\frac{1}{2}$	0	$-\frac{1}{4}$
$M_{12}O_{21}$	1	0	$\frac{1}{2}$	−1	0	$\frac{1}{2}$	0	$\frac{1}{4}$
$M_{12}O_{12}$	1	0	$\frac{1}{2}$	1	0	$\frac{1}{2}$	0	$\frac{1}{4}$
$M_{12}O_{22}$	1	−1	$-\frac{1}{2}$	0	0	$\frac{1}{2}$	0	$-\frac{1}{4}$
$M_{22}O_{12}$	1	0	$\frac{1}{2}$	0	−1	$-\frac{1}{2}$	0	$-\frac{1}{4}$
$M_{22}O_{22}$	1	0	$-\frac{1}{2}$	0	0	$-\frac{1}{2}$	−1	$\frac{1}{4}$

The rows are labeled to reflect the corresponding maternal–offspring genotype combination, as in Table 3 [given as $M_{ij}O_{kl}$, where M_{ij} is the unordered genotype of the mother (with *i* and *j* being the two alleles she has at the focal locus) and O_{kl} is the ordered genotype of the offspring (with again, *k* and *l* being the two alleles that the offspring has at the focal locus]. The columns labeled "Coefficients" correspond to the index scores used in a regression model to fit the intercept or reference point for the model (R), the three direct genetic effects (a_o, d_o, and i_o), and the two maternal effects (a_m and d_m). The last two columns correspond to terms that we do not interpret here because they do not correspond to independent effects of the maternal and offspring genomes. The first of these, c_{mo}, is the confounded effect (see text) and the second, *dd*, is an interaction between the dominance effects in mothers and their offspring (see *Discussion*)

From the values in Table 5 we can see that, as expected under the statistical cross-fostering framework, the additive direct effect is based on the homozygous offspring of heterozygous mothers and is defined formally as half the difference between the average phenotypes of these two classes,

$$a_o = \frac{1}{2}(M_{12}O_{11} - M_{12}O_{22}), \tag{4}$$

while the additive maternal effect is based on the heterozygous offspring of homozygous mothers,

$$a_m = \frac{1}{2}(M_{11}O_{21} - M_{22}O_{12}). \tag{5}$$

This again highlights the constraint that the additive maternal effect is confounded with the imprinting effect in the offspring because the two classes in this contrast differ in the parent of origin of the alleles (O_{21} *vs.* O_{12}). Likewise, the imprinting effect is based on a contrast between the reciprocal heterozygote offspring from heterozygous mothers:

$$i_o = \frac{1}{2}(M_{12}O_{12} - M_{12}O_{21}). \tag{6}$$

In contrast to additive and parent-of-origin effects, however, it is clear from Table 5 that the dominance effects under this model are actually based on all of the genotypic values—*i.e.*, they are not restricted to a subset of classes in the two contrasts. This is the primary difference between this unified orthogonal model and the conceptual approach to statistical cross-fostering described above. The dominance direct effect is defined as the difference between the average phenotypic value of heterozygous pups and that of homozygous pups,

$$d_o = \frac{1}{4}\left(\begin{array}{l}[M_{11}O_{21} + M_{12}O_{21} + M_{12}O_{12} + M_{22}O_{12}] \\ -[M_{11}O_{11} + M_{12}O_{11} + M_{12}O_{22} + M_{22}O_{22}]\end{array}\right), \tag{7}$$

and dominance maternal effects are defined as the difference between the average phenotypic value of pups with heterozygous mothers and that of pups with homozygous mothers:

$$d_m = \frac{1}{4}\left(\begin{array}{l}[M_{12}O_{11} + M_{12}O_{21} + M_{12}O_{12} + M_{12}O_{22}] \\ -[M_{11}O_{11} + M_{11}O_{21} + M_{22}O_{12} + M_{22}O_{22}]\end{array}\right). \tag{8}$$

The genetic design matrix also yields two terms that cannot be attributed to direct or maternal effects. The first of

Table 5 The inverse of the genetic-effect design matrix shown in Table 4

	Genotypic values							
Genetic effects	$M_{11}O_{11}$	$M_{11}O_{21}$	$M_{12}O_{11}$	$M_{12}O_{21}$	$M_{12}O_{12}$	$M_{12}O_{22}$	$M_{22}O_{12}$	$M_{22}O_{22}$
R	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$	$\frac{1}{8}$
a_o	0	0	$\frac{1}{2}$	0	0	$-\frac{1}{2}$	0	0
d_o	$-\frac{1}{4}$	$\frac{1}{4}$	$-\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$-\frac{1}{4}$	$\frac{1}{4}$	$-\frac{1}{4}$
i_o	0	0	0	$-\frac{1}{2}$	$\frac{1}{2}$	0	0	0
a_m	0	$\frac{1}{2}$	0	0	0	0	$-\frac{1}{2}$	0
d_m	$-\frac{1}{4}$	$-\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$-\frac{1}{4}$	$-\frac{1}{4}$
c_{mo}	$\frac{1}{2}$	0	0	0	0	0	0	$-\frac{1}{2}$
dd_{mo}	$\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$-\frac{1}{2}$	$-\frac{1}{2}$	$\frac{1}{2}$

The inverse of the genetic-effect design matrix in Table 4 gives the solution to the vector of genetic effects from the linear model. The columns of the matrix are labeled with the genotypic effect and the rows are labeled with the corresponding genetic effect.

these we call the confounded effect, c_{mo}, which is a contrast between the two classes of homozygous offspring of homozygous mothers,

$$c_{mo} = \frac{1}{2}(M_{11}O_{11} - M_{22}O_{22}). \tag{9}$$

It accounts for the cells where the additive direct and maternal effects are fully confounded, and hence this contrast is included only to make the genetic design matrix of full rank. That is, this term could be significant when either the direct or the maternal effect or both direct and maternal effects are present, and it can be nonsignificant even when they are both present because they may be of opposite sign and cancel out each other (*e.g.*, a positive maternal effect of $a_m = +Q$ and a negative direct effect of $a_o = -Q$ could make the phenotypes of the two classes of homozygous offspring of homozygous mothers phenotypically identical).

The genetic design matrix also includes a single interaction term, dd_{mo}, which is an interaction between the dominance direct and maternal effects. It is conceptually analogous to dominance-by-dominance epistasis (Cheverud 2000), except that the interactions are between the maternal and offspring genomes (Wolf 2001) and the interactions occur within a locus, rather than between loci (Wade 1998). It corresponds to a contrast between the combinations where the mother and her offspring have the same genotype (ignoring parent-of-origin of alleles) and where they have different genotypes:

$$dd_{mo} = \frac{1}{4}\begin{pmatrix} [M_{11}O_{11} + M_{12}O_{21} + M_{12}O_{12} + M_{22}O_{22}] \\ -[M_{11}O_{21} + M_{12}O_{11} + M_{12}O_{22} + M_{22}O_{12}] \end{pmatrix}. \tag{10}$$

As with the confounded effect, c_{mo} (Equation 9), the dominance interaction effect, dd_{mo}, is included primarily because it allows us to create a full rank orthogonal model. Finally, note that the reference point for this model is simply the grand mean (*i.e.*, is the average of the eight maternal–offspring genotype classes).

The effects in the genetic-effects vector, $\mathbf{E}_{MO}$, can be estimated empirically by regression using the coefficients shown in the genetic-effects design matrix (Table 4) as the independent variables and individual phenotypic values (*i.e.*, the trait values measured for individuals, not genotype classes) as the dependent variables (Cheverud 2000, 2006). That is, although we define the effects above in terms of average phenotypes of the maternal–offspring genotype classes, the effects can be estimated using individual phenotypes rather than the average values of each of the genotype classes. The regression model that yields estimates of the seven genetic effects and the reference point in the model is given as

$$\begin{aligned} P_{j(x)} = R &+ a_o X_{a_o(x)} + d_o X_{d_o(x)} + i_o X_{i_o(x)} \\ &+ a_m X_{a_m(x)} + d_m X_{d_m(x)} \\ &+ c_{mo} X_{c_{mo}(x)} + dd_{mo} X_{dd_{mo}(x)} \\ &+ r_j, \end{aligned} \tag{11}$$

where $P_{j(x)}$ is the phenotypic value of individual j with maternal–offspring genotype combination x (where x identifies which of the eight maternal–offspring combinations the individual has), $X_{Z(x)}$ is the genotypic index value (from Table 4) of parameter Z for that maternal–offspring genotype combination (where Z is one of the seven genetic effects being estimated), and r_j is the residual from the model for individual j. The form of the model (Equation 11) is analogous to the model used in Wolf *et al.* (2011) to examine prenatal and postnatal maternal effects, except that the model presented herein is based on an explicit orthogonal model structure that yields estimates of genetic effects corresponding to the specific forms given in Equations 4–10.

To demonstrate the use of this statistical cross-fostering scheme we present an empirical study using an experimental population of mice. We test for direct and maternal effects on size and growth traits, using marker data. We then use several identified loci to illustrate the phenotypes of individuals as a function of their genotype and the genotype of their mothers.

Materials and Methods

Experimental population

We use an experimental population that was derived from a cross between inbred lines of mice that were originally derived by artificial selection for either large (the LG/J line) or small (the SM/J line) body weight at 60 days of age (Goodale 1938; MacArthur 1944; Chai 1956). These lines had been inbred for >120 generations prior to the crossing, making them essentially devoid of within-strain genetic variation. We use the F_2 generation of the line cross as our parental generation and their F_3 progeny as the "offspring" generation (Kramer *et al.* 1998; Vaughn *et al.* 1999). The population was created by mating 10 SM/J males to 10 LG/J females, producing 52 F_1 individuals. The F_1 animals were randomly mated to produce 510 F_2 animals. Approximately 400 of these F_2 animals were randomly mated (~200 males and 200 females) to create the F_3 population, so the mothers of these F_3 individuals are our focal set for analyses of maternal effects. The F_3 population contains a total of 1632 F_3 individuals in 200 full-sibling families (of which 1552 were genotyped). Half litters were reciprocally cross-fostered at random between pairs of females that gave birth on the same day (Wolf *et al.* 2002) so only some families were cross-fostered. In this study we limit our focus to those mice that were not cross-fostered to understand how one might study maternal effects in the absence of experimental manipulation (in Wolf *et al.* 2011 we use the cross-fostered population to examine how experimental cross-fostering can be used to identify pre- and postnatal maternal effects, but do not develop the statistical cross-fostering framework therein). Therefore, our focal population is the 937 F_3 individuals from 194 families who were not cross-fostered. Pups were weaned at 21 days of age and randomly housed with 3 or 4 other same-sex individuals. Previous quantitative genetic analysis suggested that maternal effects contribute a large component of variation for the first few weeks of age in this population (Kramer *et al.* 1998), but this previous study was unable to differentiate genetic from nongenetic maternal effects and accounted only for postnatal maternal-effect variation.

All animals were weighted weekly from 1 week through 10 weeks of age, using a digital scale with an accuracy of 0.1 g. For consistency with previous work on this population, sex differences in body size and those associated with differences in birth and weaning litter sizes were removed prior to analyses.

Genotypes

Details of the genotype data used in all analyses are given in Wolf *et al.* (2008b). Briefly, all F_2 and F_3 individuals were genotyped at 353 autosomal single-nucleotide polymorphism (SNP) loci, using the Illumina Golden-Gate assay, with an average map distance between markers in the F_2 generation of 4 cM. A complete list of the markers and their physical and recombinational map positions are given in Supporting Information, Table S2.

To link the experimental population to the model presented above we assign the A_1 allele to the LG/J line and the A_2 allele to SM/J. Allele frequencies at all loci are close to the expected frequency of 0.5 and all genotypes occur in approximately Hardy–Weinberg proportions (conforming to p_1^2, $2p_1p_2$, and p_2^2 for the A_1A_1, A_1A_2, and A_2A_2 genotypes).

QTL analysis

We used a linear mixed-models framework to estimate direct and maternal effects associated with marker loci, using the index values in Table 4 in a regression model analogous to Equation 11. This was done by assigning the appropriate index values at each locus on the basis of the SNP genotype of the mother and her offspring. The model shown in Equation 11 was fitted as a mixed model with the fixed effects corresponding to the genotypic index values and with the mother as a random (classification) effect. Therefore, Equation 11 corresponds to the fixed-effects part of the model, but the implementation in our experimental population included an additional random effect. The random effect accounts for the residual relatedness of siblings, who share alleles at other loci and also share common environmental effects, which together produce a phenotypic autocorrelation of siblings that inflates the apparent significance of genetic effects (Lynch and Walsh 1998; Wolf *et al.* 2008b). This mixed model was fitted using maximum likelihood in the Mixed Procedure of SAS (SAS version 9.1; SAS Institute, Cary, NC). We used a 2-d.f. test that simultaneously tested the a_m and d_m effects together to produce an overall test for maternal effects at a locus. Denominator degrees of freedom were generated by the Satterthwaite approximation (see Littell *et al.* 2006), which uses the variance structure of the model (*i.e.*, the structure of the random effects) to determine the degrees of freedom. In this model, the Satterthwaite approximation essentially determines the effective sample size for each fixed effect (Ames and Webster 1991; Keselman *et al.* 1999; Faes *et al.* 2009) on the basis of family structure, and consequently, the degrees of freedom for the maternal-effect terms are close to the number of mothers while the degrees of freedom for the direct-effect terms are close to the total number of F_3 individuals (*i.e.*, the number of offspring) (Wolf *et al.* 2011). Probabilities from all significance tests were converted to logarithmic probability ratios [LPR = $-\log_{10}$(probability)], which are analogous to the LOD scores that are commonly

reported. Proportions of variance explained by QTL (R^2) were estimated by calculating the genetic variance contributed by a locus as

$$V_g = \frac{1}{2}a_o^2 + \frac{1}{4}d_o^2 + \frac{1}{2}i_o^2 + \frac{1}{2}a_m^2 + \frac{1}{4}d_m^2 + \frac{1}{2}(a_m a_o + a_m i_o) \quad (12)$$

and dividing this variance by the total phenotypic variance. Only significant effects at a locus were included in this calculation to avoid artificial inflation.

To facilitate the identification of QTL locations, we used multivariate versions of the model in Equation 11 (see Hager *et al.* 2009). Weight measurements were divided into three sets, which correspond to different growth phases (Kramer *et al.* 1998): (1) preweaning weights (weeks 1–3); (2) postweaning weights, where there is rapid weight gain (weeks 4–6); and (3) adult weights, when there is slow growth (weeks 7–10). The multivariate model was fitted using the framework described by Fry (2004), modified to include a multivariate QTL effect. In this model, the weekly weights are treated as repeated measures of a "weight" trait and QTL effects are fitted to this vector of weight traits. Week is included in this model to account for variation in weight through time. The correlation between weekly weights measured within individuals was modeled using the Toeplitz autoregressive structure (Kincaid 2005), which approximates the temporally autocorrelative structure of the weight traits (Kramer *et al.* 1998). Denominator degrees of freedom were determined using the Kenward–Roger approximation, which is analogous to the Satttherthwaite approximation but is preferred for repeated measures designs (Kenward and Roger 1997; Schaalje *et al.* 2001).

Maternal-effect QTL (meQTL) locations were first identified using the LPR values from the multivariate models, with the maximum LPR value on a chromosome above the threshold value (see below) taken as evidence of a meQTL on that chromosome. However, once a locus was identified, the location was refined (using the results from the multivariate model) by examining the individual patterns of the additive and dominance maternal effects; if the locus had only one form of maternal effect, then the meQTL position was taken as the location of the LPR peak for the one significant effect alone. This is because random variation in the other term in the model can displace the overall peak away from the peak for the significant effect, but the best evidence for the meQTL location is assumed to be the position that maximizes the fit for the significant effect. We also examined the multivariate genome scan for the possibility of multiple peaks on a chromosome, which could appear as either two or more peaks in the overall test for maternal effects or a difference in the position of the peaks for individual additive and dominance maternal effects underlying a single peak. We found no cases of the former (multiple distinct peaks on a chromosome), and so the analysis of multiple distinct peaks is not discussed further. However, we found a case where one region of a chromosome had distinct separate peaks for additive and dominance maternal effects. In this case, we ran a model that included the maternal effects of both loci to determine whether there was support for a two-locus model. Support for two meQTL was determined using a likelihood-ratio test, with the full model containing the meQTL effects at their individual locations and the reduced model containing a single meQTL at the overall peak location. The difference in the -2 log likelihoods of the two models (reduced model minus full model) is approximately chi-square distributed with the number of degrees of freedom corresponding to the number of additional terms in the full model (*i.e.*, the number of terms dropped in the reduced model). The multiple-QTL model was accepted when it had a significantly better fit than the reduced single-QTL model.

Confidence intervals were defined as a one-LPR drop (on the basis of the multivariate model), which is analogous to the commonly used one-LOD drop (Lynch and Walsh 1998). Maternal-effect loci were named following the convention of *mebsX.Y* (indicating **m**aternal **e**ffects on **b**ody **s**ize), where *X* is the chromosome number and *Y* is the locus number on that chromosome to distinguish between multiple QTL on a chromosome.

Whenever maternal-effect loci with additive effects were identified, we examined whether those loci had imprinting effects of similar magnitude but of opposite sign, which was taken as evidence that the maternal effect appeared because of the occurrence of an imprinting effect (see above and Table 3). Because a locus could potentially show both an imprinting effect and a maternal effect, we devised a test to determine whether the appearance of an additive maternal effect could be attributed to the presence of the imprinting effect, with the possibility that the locus may show both a maternal and an imprinting effect. We took the parameter estimate for the imprinting effect (i) from the analysis of direct effects (where the parent-of-origin effect is not confounded with any other effects) and removed (using the linear model) the variation attributable to the imprinting effect from the heterozygous offspring with homozygous mothers. We then reran the analysis of maternal effects as above and tested whether the maternal-effect terms were still significant after being "corrected" for the imprinting effect. This is a very conservative analysis that assumes that the imprinting effect estimated in the analysis of direct effects is the best estimate of the imprinting effect since it is not confounded with any other effects and then removes this variation from the genotypes where the two effects are confounded, thereby removing the confounding.

Significance testing

Previous analyses have demonstrated that the distribution of significance tests associated with direct effects produced by the mixed model behaves as expected under the null model (Wolf *et al.* 2008b). However, maternal-effect terms in the model are pseudoreplicated because there are, on average,

Table 6 Maternal effects of meQTL

	meQTL	*mebs2.1*[a]	*mebs3.1*	*mebs7.1*[a]	*mebs7.2*	*mebs8.1*[a]	*mebs17.1*[a]	*mebs18.1*[a]	R^{2b}
cM		44.5	25.1	57.0	76.5	48.9	9.0	0.0	
Mb		75.9	53.1	122.7	145.0	98.2	20.4	3.7	
C.I. (Mb)		69.2–82.2	46.1–63.8	117.6–134.5	122.7–145.0	83.2–114.5	8.4–61.3	3.7–34.0	
Traits	Early	$\mathbf{+a_m}^c$		$\mathbf{+d_m}$		$\mathbf{-d_m}$	$\mathbf{-a_m}^i$	$\mathbf{-a_m}$	
	Mid	$\mathbf{+a_m}^c$	$+a_m$	$\mathbf{+d_m}$		$\mathbf{-d_m}$	$\mathbf{-a_m}^i$	$\mathbf{-a_m}$	
	Late	$\mathbf{+a_m}^c$	$\mathbf{+d_m}$	$\mathbf{+d_m}$	$\mathbf{+a_m}$	$-d_m$	$-a_m{}^i$	$\mathbf{-a_m}$	
	Week 1	$\mathbf{+a_m}$		$\mathbf{+d_m}$		$\mathbf{-d_m}$	$\mathbf{-a_m}^i$	$\mathbf{-a_m}^c$	25.1
	Week 2	$\mathbf{+a_m}$		$\mathbf{+d_m}$		$-d_m$	$-a_m{}^i$	$\mathbf{-a_m}$	26.9
weaning	Week 3	$\mathbf{+a_m}^c$		$\mathbf{+d_m}$		$\mathbf{-d_m}$	$\mathbf{-a_m}^i$	$-a_m$	28.0
	Week 4	$\mathbf{+a_m}^c$	$+a_m$	$+d_m$		$\mathbf{-d_m}$	$\mathbf{-a_m}^i$	$\mathbf{-a_m}$	36.3
	Week 5	$\mathbf{+a_m}^c$	$+d_m$	$+d_m$	$+a_m$	$\mathbf{-d_m}$	$\mathbf{-a_m}^i$	$-a_m{}^i$	22.6
	Week 6	$\mathbf{+a_m}^c$	$+a_m+d_m$	$\mathbf{+d_m}$	$+a_m$	$-d_m$	$\mathbf{-a_m}^i$	$\mathbf{-a_m}^i$	21.8
	Week 7	$\mathbf{+a_m}^c$	$+a_m\mathbf{+d_m}$	$\mathbf{+d_m}$	$\mathbf{+a_m}$	$-d_m$	$\mathbf{-a_m}^i$	$-a_m{}^i$	22.2
	Week 8	$\mathbf{+a_m}^c$	$\mathbf{+d_m}$	$\mathbf{+d_m}$	$+a_m$	$-d_m$	$\mathbf{-a_m}^i$	$\mathbf{-a_m}^i$	21.8
	Week 9	$\mathbf{+a_m}$	$\mathbf{+d_m}$	$\mathbf{+d_m}$	$\mathbf{+a_m}$	$-d_m$	$-a_m{}^i$	$\mathbf{-a_m}^i$	20.2
	Week 10	$\mathbf{+a_m}$	$\mathbf{+d_m}$	$\mathbf{+d_m}$	$\mathbf{+a_m}$	$-d_m$		$\mathbf{-a_m}^i$	20.6

The map position in both centimorgans (cM) and megabases (Mb) along with the confidence interval (C.I.) (in Mb) and the effects on each of the 10 weekly weight measurements are given for each locus. The rows labeled "Early," "Mid," and "Late" give the significance values from the multivariate tests. Significance tests are in LPR units (where LPR = $-\log_{10}[p]$). Entries in boldface type are significant using the chromosome-level significance threshold. Additive maternal effects that appear to be attributable to an imprinting effect are marked with a superscript "i". The estimates of all effects and the significance values for all tests are provided in Table S1. The proportions of phenotypic variance accounted for by the set of loci (R^2) on each of the weekly weight traits are also included.

[a] Loci also show significant maternal effects in the analyses of Wolf *et al.* (2011).

[b] Does not include variation from apparent additive maternal effects that were attributed to imprinting effects (*i.e.*, those with a superscript "i").

[c] Entries are significant using the genome-wide threshold.

about four pups from each mother that were included in the analysis, and therefore, each maternal genotype appears in the linear equation about four times. We emphasize that this pseudoreplication is not a shortcoming of the analysis, but rather is a feature of the framework and is easily addressed by adjusting the denominator degrees of freedom of the model to reflect the true level of replication (the individual mothers). This pseudoreplication in the design is accounted for by the use of the Satterthwaite approximation for the denominator degrees of freedom, but could also be approximated by adjusting the denominator degrees of freedom to reflect the number of individual mothers in the analysis (Wolf *et al.* 2011).

Significance thresholds were determined on the basis of the number of tests in a Bonferroni correction for familywise error, using the Šidák equation, $1 - 0.95^{1/n}$, where n is the number of tests in the family. We calculated both a genome-wise and separate chromosomewise thresholds, using the effective number of markers method as described by Li and Ji (2005). This method uses the correlation between markers to estimate the number of independent tests on each chromosome and over the whole genome (since correlated tests are not independent, using M markers results in a threshold based on M_{eff} because the M markers do not count as M independent tests). Because more recombination events have accumulated in the F_3 genotypes compared to the F_2 (*i.e.*, there is less linkage disequilibrium in the F_3 generation), the number of independent tests is lower for the maternal-effect tests compared to the direct-effect tests. The chromosomewise thresholds are used for QTL discovery because they have been shown to increases the discovery of true positives while avoiding the representation of false positives (Chen and Storey 2006). Significance thresholds are given in Table S3. Once a maternal-effect locus was identified, we used a pointwise threshold (*i.e.*, LPR = 1.3 for $P = 0.05$) to determine which individual effects were significant at that locus.

Results

The orthogonal model of statistical cross identified seven loci showing significant maternal effects on offspring body size (Table 6). Two of these loci, *mebs*17.1 and *mebs*18.1, had significant additive maternal effects and imprinting effects (Table 7) that are of opposite signs, suggesting that the maternal effect may have appeared because of the presence of imprinting. However, the patterns are complex and somewhat ambiguous. For *mebs*17.1, the imprinting effect is strongest at week 2 and is no longer significant after week 5, while the additive maternal effect peaks at week 6. At week 2 the magnitude of the additive maternal effect is nearly identical to that of the imprinting effect (standardized $a_m = -0.260$ and $i_o = 0.255$), strongly suggesting that the two arise from an imprinting effect. The temporal pattern would suggest that the locus has an imprinting pattern early and a maternal effect later in life; however, when the imprinting-effect variation is removed, the additive maternal effect is no longer significant at any age (*i.e.*, partialling the imprinting effect even when it is not significant later in life still results in a nonsignificant additive maternal effect). Consequently, the conservative conclusion is that the appearance of a significant additive maternal effect can be attributed to the

Table 7 Direct effects of meQTL

	meQTL	*mebs2.1*	*mebs3.1*	*mebs8.1*	*mebs17.1*	*mebs18.1*	R^2
Traits	Week 1				$+i_o$		0.82
	Week 2	$+d_o$	$+d_o$		$\mathbf{+i_o}^a$		5.15
weaning	Week 3	$+d_o$			$\mathbf{+i_o}$		2.59
	Week 4	$+d_o$	$+d_o$		$+i_o$		3.54
	Week 5		$+d_o$		$+i_o$	$+i_o$	3.34
	Week 6		$+d_o$				0.81
	Week 7			$-i_m$		$+i_o$	1.81
	Week 8			$-i_m$		$+i_o$	1.84
	Week 9			$-i_m$		$+i_o$	2.10
	Week 10	$+a_o$				$+i_o$	1.67

The effects on each of the 10 weekly weight measurements are given for each locus. Significance tests are in LPR units (where LPR = $-\log_{10}[p]$). Entries in boldface type are significant using the chromosome-level significance threshold. The estimates of all effects and the significance values for all tests are provided in Table S1 and genomic locations in Table 4. The proportions of phenotypic variance accounted for by the set of loci (R^2) on each of the weekly weight traits are also included.

[a] Significant using the genome-wide threshold.

presence of the imprinting effect. We follow this conclusion in the presentation of the results, but we note that this same locus was previous identified by Wolf *et al.* (2011) as a maternal-effect locus with a persistent additive postnatal maternal effect similar to the one observed for *mebs*17.1. Because the postnatal maternal effects identified by Wolf *et al.* (2011) were detected as the effect of the genome of the foster mother on the phenotypes of her foster pups, they could not have been caused by genomic imprinting (since the nurse mothers and their foster pups were unrelated). Furthermore, this conclusion is supported by the results from Hager *et al.* (2008), who identified a locus (*Wtmge*17.1) at this same genomic region as a maternal-effect locus that appears to have an imprinting effect. Therefore, prior evidence suggests that this locus is indeed a maternal-effect locus, but the data used herein do not allow us to differentiate a maternal effect at this locus from an imprinting effect that mimics a maternal effect.

For *mebs*18.1, there is a strong additive maternal effect in the first few weeks of age (weeks 1–4; see Table 8 for an example of week 1 weight for this locus), which cannot be attributed to an imprinting effect (both because the imprinting effect is not significant and removing the imprinting effect from the maternal effect does not result in a nonsignificant additive maternal effect), but at week 5 a significant imprinting effect appears and from that age onward it appears to account for the additive maternal effect (*i.e.*, partialling the imprinting effect from the additive maternal effect makes the maternal effect nonsignificant). Thus, our conclusion is that there is support for both an imprinting and a maternal effect at *mebs*18.1, which are temporally separate.

Overall, the temporal pattern of maternal effects varies greatly across loci, but all loci have significant maternal effects on several weight measurements (Table 6). This may be partly due to the use of a multivariate model to identify loci, where we use an overall test for effects on a growth phase to identify loci that are, therefore, likely to be pleiotropic across the growth phase. Interestingly, the traits with the fewest significant effects are the three preweaning weights (weeks 1–3), which are each affected by the same set of five of the seven loci. No locus had effects limited to early, preweaning weights and only one locus (*mebs*18.1) had an effect that peaked before weaning (with a very strong peak effect at week 1; see Table 8). Two loci (*mebs*3.1 and *mebs*7.2) had effects limited to postweaning weights and both had effects that peaked very late (at weeks 7 and 10, respectively). Interestingly, four loci show effects through all of the weekly weight measures (although the late effects of *mebs*18.1 appear to be caused by an imprinting effect), demonstrating that maternal effects that appear early can be persistent through development. The effect estimates, significance values, and percentages of variance explained by the meQTL are provided in Table S1.

The overall percentage of the phenotypic variance explained by the maternal effects of the meQTL (Table 6) peaks at week 4 (the week after weaning). After week 4 the percentage of variance explained declines slowly, but the meQTL continue to explain ~20% of the variance through week 10. The additive maternal effect of *mebs*2.1 on week 4 weight explains the most variance of any locus on any trait (nearly 11% of the variance).

The meQTL have relatively minor direct effects (Table 7), and there is a particular paucity of additive effects, with only one locus showing a significant additive direct effect on a single weight trait. Two loci (*mebs*2.1 and *mebs*3.1) show overdominance direct effects, while three others (*mebs*8.1, *mebs*17.1, and *mebs*18.1) show significant imprinting effects. In the case of these imprinting effects, the appearance of a significant direct effect could be potentially biased since the imprinting effects contributed to the significance of the maternal effect.

Discussion

Our conceptual framework, which we call statistical cross-fostering, opens the possibility of identifying and characterizing maternal-effect loci in natural and experimental populations in which cross-fostering or other manipulations have not been done or are not possible. This is an important advancement because, although maternal effects are likely

Table 8 Examples of additive and dominance maternal effects

	Offspring genotype				
	LL	*SL*	*LS*	*SS*	Maternal litter average
A. Expected week 1 weights from the mixed model (in standardized units) of offspring as a function of their genotype and the genotype of their mothers at *mebs*18.1[a]					
Maternal genotype					
LL	–0.15	–0.28	—	—	–0.21
LS,SL	–0.06	–0.05	–0.11	–0.25	–0.12
SS	—	—	0.41	0.28	0.34
Offspring average	–0.10	–0.17	0.15	–0.01	
B. Expected week 4 weights from the mixed model (in standardized units) of offspring as a function of their genotype and the genotype of their mothers at *mebs*8.1[b]					
Maternal genotype					
LL	0.15	0.07	—	—	0.11
LS,SL	–0.14	–0.19	–0.43	–0.30	–0.26
SS	—	—	0.29	0.15	0.22
Offspring average	0.00	–0.06	–0.07	–0.07	

Table cells with an em dash indicate combinations of material and offspring genotypes that cannot occur under Mendelian inheritance.
[a] This locus has a strong additive maternal effect (standardized $a_m = -0.36$ from the linear model, but it is calculated as -0.35 from these cell means) and no direct effect.
[b] This locus has a strong dominance maternal effect (standardized $a_m = -0.43$ from the linear model, but it is calculated as -0.49 from these cell means) and no direct effect.

to be a major component of the genetic architecture of many traits, they are largely ignored in studies of genetic architecture. Consequently, we currently have a very poor understanding of their contribution to genetic variation and as a result, we have a very biased view of the genetic basis of phenotypic variation. The statistical cross-fostering framework accomplishes the separation of maternal effects from the direct effects of the individual's own genome by using a set of offspring genotypes that are uncorrelated to the genotypes of their mothers, thereby removing the confounding effects of the two genomes. Conceptually, this process could be achieved by limiting the analysis of maternal effects at a given locus to a set of genetically identical offspring coming from genetically variable mothers while limiting the analysis of direct effects to offspring from genetically identical mothers (Figure 1). We implement this approach using an orthogonal model (Table 4) that achieves the statistical cross-fostering design by removing the confounded combinations (*i.e.*, homozygous mothers having homozygous offspring) from the analysis of additive direct and maternal effects, while using the entire data set in a single model-fitting process. We have applied this framework to an analysis of maternal genetic effects in an experimental population of mice and identified a set of six maternal-effect QTL that explain a major component of phenotypic variation in body size at all ages.

Although the statistical cross-fostering approach opens up new avenues of research on maternal genetic effects, it is important to understand that to achieve this separation of maternal and direct effects requires us to impose assumptions on the analysis. This is because the missing maternal–offspring genotype combinations are necessarily absent from the analysis. We discuss these assumptions and limits, but we strongly emphasize that these are imposed by biological constraints and are not simply statistical limitations. Despite these limits, the ability to detect and characterize maternal-effect loci in systems where it would not otherwise be possible to separate maternal and direct genetic effects should provide significant insights into the genetic basis of phenotypic variation.

Empirical analysis of meQTL

Our statistical cross-fostering approach identified six maternal-effect loci with pleiotropic effects on body weight (see Table 6), demonstrating that maternal effects can be successfully identified without experimental manipulation. Two of these loci have maternal effects limited to the phases of growth after weaning (which occurred at week 3 of age). Overall, the maternal-effect loci explain the most variance in the traits around weaning, with a peak at week 4. This pattern differs from that observed in this population for postnatal maternal effects using variance partitioning in an experimental cross-fostering design (Kramer *et al.* 1998), where the proportion of variance explained by the foster mother peaked at week 2 and showed a clear decline by week 4. However, it is similar to the overall pattern of maternal genetic effects observed using a reciprocal crossing experiment (Jarvis *et al.* 2005), which, like our study, combines pre- and postnatal effects and matches the pattern for the sum of pre- and postnatal effects identified in Wolf *et al.* (2011). Four of the loci have effects that extend through to 10 weeks of age, which is somewhat counterintuitive given that maternal care and all contact with the mothers ended at weaning (week 3). Indeed, two of these loci have effects that are not significant until after weaning, suggesting that early maternal influences may affect developmental and physiological processes that are manifest after the end of actual maternal care. For most of these loci, the effects on adult traits are relatively strong, and together they account for >20% of the variance in body weight from week 5 to week 10. Two loci have additive maternal effects on week 9 weight, which is approximately the target of selection in the

LG/J and SM/J strains (Goodale 1938; MacArthur 1944; Chai 1956), with the sign of effect matching the direction of divergence between these lines (*i.e.*, the allele derived from LG/J has a positive maternal effect), suggesting that these maternal effects may have contributed to the evolved difference in week 9 weight between these strains.

Although the absence of cross-fostering prevents us from separating the maternal effects into pre- and postnatal components, the previous results of Wolf *et al.* (2011) provide some insights into the likely origin of the effects for several loci. Four of the seven loci detected here (*mebs*2.1, *mebs*7.1, *mebs*17.1, and *mebs*18.1) map very close to and have similar patterns of effect to loci identified using experimental cross-fostered pups by Wolf *et al.* (2011). The results from Wolf *et al.* (2011) suggest that *mebs*2.1 and *mebs*18.1 have prenatal effects, while *mebs*7.1 and *mebs*17.1 have postnatal effects. Interestingly, in our analysis we were unable to support the hypothesis that *mebs*17.1 is a maternal-effect locus because the pattern of effect could be explained by the presence of genomic imprinting at this locus. However, the results from Wolf *et al.* (2011) strongly suggest that this locus does indeed have a maternal effect since the effect detected there was postnatal and, therefore, was not confounded with genomic imprinting (see below). This conclusion is further supported by the results from Hager *et al.* (2008), where they concluded that this same locus has a maternal effect that creates an apparent imprinting effect. In reexamining the results from Wolf *et al.* (2011) we also find that, although not significant at the threshold used for QTL discovery in that study, *mebs*8.1 appears to have a dominant prenatal maternal effect on body weight from week 6 to week 10 that is significant at the $P < 0.05$ level (with LPR values ranging from 1.38 to 1.88 for these traits). Thus, the level of overlap between the loci identified here and those detected in our previous work on maternal effects in this population is surprisingly good given that the effect sizes are relatively small and the two analyses used different groups of pups and different statistical methods. This overlap allows us to conclude that three of the seven loci (*mebs*2.1, *mebs*8.1, and *mebs*18.1) arise from prenatal effects and two (*mebs*7.1 and *mebs*17.1) from postnatal effects. This suggests that, because maternal effects can potentially arise from these two very different sources (pre- *vs.* postnatal), one should consider using experimental cross-fostering at birth in conjunction with our statistical cross-fostering model (which allows for the identification of prenatal effects without prenatal cross-fostering) to identify the origin of maternal effects.

The loci detected here do not correspond to the maternal-effect loci detected in another study by Wolf *et al.* (2002) that used cross-fostered pups, where the litter means were treated as traits of the nurse mother (so only postnatal effects were examined, and they combined the offspring into a single measure). Although one locus (*mebs*7.1) does map close to one of the loci identified by Wolf *et al.* (2002), the patterns of effect are incompatible (with the sign of the dominance effect being opposite). The difference in the results of these studies is likely caused by the much lower power of the Wolf *et al.* (2002) study, where individual variation and among-litter variation were not accounted for in the model. That study also used a much more diffuse marker map and a smaller number of mothers, which further reduced power.

The maternal-effect loci we identified generally show weak direct effects (Table 7), which is somewhat surprising given that maternal effects tend to be genetically correlated to direct effects (Roff 1997). Previous analyses focused on identifying direct effects for these same traits (Wolf *et al.* 2008b; Hager *et al.* 2009) and did not identify any of these same loci, which is not surprising given that the direct effects we identified here are small. The one exception is *mebs*17.1, which appears to have a strong imprinting effect in our analysis, but this locus was not previously identified as an imprinted locus because, again, the imprinting effect was attributed to a maternal effect (Hager *et al.* 2008; Wolf *et al.* 2011). Analyses of body composition traits in these same animals (Cheverud *et al.* 2008) did identify direct effects for two of these loci (*mebs*3.1 and *mebs*7.1), both of which showed an imprinting effect (see *Bwi*3.1 and *Bwi*7.1 in Cheverud *et al.* 2008).

Confounding with imprinting effects

Additive maternal genetic effects are partially confounded with genomic imprinting effects because of the correlation between genotype of the mother and the parent of origin of alleles in the offspring (Hager *et al.* 2008; Wolf and Wade 2009). This is clear from Table 3, where the imprinting effect (i_o) is fully confounded with the additive maternal effect (a_m) in heterozygous offspring, meaning that a locus may appear to have an additive maternal effect when an imprinting effect is present if we limit the analysis of maternal effects to heterozygous offspring. This occurs because, although the two different homozygous mothers produce heterozygous offspring that are genetically identical at a locus, they are not *epigenetically* equivalent because they necessarily differ in the parent of origin of the two alleles (*i.e.*, the two types of mothers produce the alternative forms of the reciprocal heterozygotes). This possibility can be accounted for because the analysis of direct effects allows one to identify an imprinting effect independent of the maternal effect and thereby determine when maternal effects appear only because of the presence of the imprinting effect. The confounding is essentially asymmetrical, where additive maternal effects are confounded with imprinting effects, but imprinting effects can be examined independent of maternal effects. This is because heterozygous mothers can have either type of heterozygous offspring, but homozygous mothers can produce only one of the two types of reciprocal heterozygotes. Therefore, one can estimate the imprinting effect in the heterozygous offspring of heterozygous mothers (Hager *et al.* 2008), where the difference between reciprocal heterozygotes must be caused by a imprinting effect, and

then remove this effect from the heterozygotes produced by homozygous mothers and determine whether there are still significant additive maternal effects.

In our analysis of meQTL we found evidence for two cases where the additive maternal effect was caused by an imprinting effect at the locus. For one locus, *mebs17.1*, the apparent additive maternal effect at all ages appears to be caused by an imprinting effect. Although the additive maternal effect at this locus is significant from week 1 to week 9 of age and the imprinting effect is not significant after week 5, the imprinting effect is still large enough to account for the apparent maternal effect. However, as we noted above, the results from Wolf *et al.* (2011; see also Hager *et al.* 2008) suggest that this locus does indeed have a maternal effect, despite the fact that we could not distinguish the maternal effect from an imprinting effect here. For the second locus, *mebs*18.1, the pattern appears to be clearer, where the locus has a very strong additive maternal effect from week 1 to week 4, but from week 5 onward, the appearance of an additive maternal effect can be accounted for by the presence of an imprinting effect. The co-occurrence of genomic imprinting and maternal effects is perhaps not surprising given that several imprinted loci have been shown to affect both growth and parental behavior traits [*e.g.*, Peg3 and Grb10 (Li *et al.* 1999; Charalambous *et al.* 2010)].

It is important to keep in mind that this analysis requires one to be able to infer the parent of origin of alleles to identify imprinting effects (Wolf *et al.* 2008a). Parent-of-origin information can be determined using marker genotype data combined with pedigree information to infer haplotype configurations using various algorithms. In the simplest case, one can genotype the mother, the father, and their offspring to track alleles within families. In our analysis, this was done using the combined genotype data from linked markers from the parents and siblings of an individual in an algorithm that finds the most likely haplotype configuration for the entire set (Li and Jiang 2005). In populations where this cannot be achieved, one can still identify maternal-effect loci, but there will be a chance that any loci with additive maternal effects could be explained by the occurrence of an imprinting effect at that locus (Casellas *et al.* 2009).

Potential for nonadditivity

An important assumption of analyses that separate maternal and direct effects is the assumption that the two genomes have independent effects that are additive (Wade 1998). However, it is possible that the two genomes interact, where the maternal effect depends on the offspring genotype and vice versa (Gavrilets 1998; Wade 1998; Wolf 2001). For example, if heterozygous offspring respond differently from homozygous offspring to the maternal genotype at the focal locus, then the maternal effects characterized will not apply to the entire population of offspring. Such nonadditivity is analogous to an epistatic interaction between the maternal and the offspring genomes (Wade 1998; Wolf 2001), but within a locus, and means that the effects cannot be cleanly separated into independent influences of the two genomes (Cheverud and Wolf 2009). The missing maternal–offspring genotype combinations make some types of interaction effects confounded with the direct and maternal effects detected using the statistical cross-fostering design, and therefore it is important to recognize the potential for such nonadditivity in the interpretation of effects.

The patterns and implications for the effect of nonadditivity can be clearly seen by drawing an analogy between the

Table 9 Relationship between two-locus epistasis and the maternal-effect model

	Locus B		
	B_1B_1	B_1B_2	B_2B_2
A. Two-locus epistasis with unordered genotypes[a]			
Locus A			
A_1A_1	$+a_A + a_B + aa$	$+a_A + d_B + ad$	$+a_A - a_B - aa$
A_1A_2	$+d_A + a_B + da$	$+d_A + d_B + dd$	$+d_A - a_B - da$
A_2A_2	$-a_A + a_B - aa$	$-a_A + d_B - ad$	$-a_A - a_B + aa$
	Offspring genotype		
	A_1A_1	A_1A_2	A_2A_2
B. Maternal-effect model[b]			
Maternal genotype			
A_1A_1	$+a_m + a_o + aa_{mo}$	$+a_m + d_o + ad_{mo}$	—
A_1A_2	$+d_m + a_o + da_{mo}$	$+d_m + d_o + dd_{mo}$	$+d_m - a_o - da_{mo}$
A_2A_2	—	$-a_m + d_o - ad_{mo}$	$-a_m - a_o + aa_{mo}$

[a] With two-locus epistasis with unordered genotypes, epistasis can be measured as the interaction between the additive (a_A and a_B) and dominance effects of the two loci (d_A and d_B). There are four forms of epistasis corresponding to the additive-by-additive (aa), additive-by-dominance (ad), dominance-by-additive (da), and dominance-by-dominance (dd) interaction effects (Cheverud 2000).

[b] With the maternal-effect model, the cells are maternal–offspring genotype combinations at a single locus (Wade 1998; Wolf and Hager 2006), so the interactions are between the additive (a_m) and dominance (d_m) maternal effects with the additive (a_o) and dominance (d_o) direct effects at the same locus. There are two missing genotype combinations (dashes) because mothers that are homozygous for one allele cannot have offspring that are homozygous for the other allele.

maternal–offspring model that includes interactions between the maternal and offspring genomes (Table 9A) and a classic model of two-locus epistasis, where interactions are between two different loci within the same individual (Table 9B) (Cheverud 2000). In the two-locus epistasis model (Table 9A) there are four main effects (additive and dominance effects of the two loci) and four epistatic interactions (pairwise interactions of the additive and dominance effects of the two loci). These effects can be estimated in a regression framework using the phenotypic information from the two-locus genotypes (Cheverud 2000; Álvarez-Castro and Carlborg 2007). However, with the maternal–offspring model, the two missing cells necessarily make some effects confounded, meaning all of the effects cannot be estimated independently in a regression model. The additive-by-additive maternal–offspring interaction effect (*i.e.*, the interaction between the additive direct and additive maternal effects; denoted aa_{mo}) is confounded with the additive maternal (a_m) and direct (a_o) effects and in our framework contributes to the confounded effect (c_{mo}) and consequently is not estimated independently (Table 5). Under the statistical cross-fostering approach, the additive-by-dominance (ad_{mo}) and dominance-by-additive (da_{mo}) maternal–offspring interaction effects are confounded with the additive maternal and direct effects, respectively (compare Figure 1 to Table 9B). This constraint is imposed on the analysis as essentially the assumption that heterozygous offspring are representative of all offspring when estimating additive maternal effects and that the offspring of heterozygous mothers are representative of all offspring when estimating additive direct effects. Although this constraint complicates the interpretation of the direct and maternal effects, it still means that, when there is a significant additive maternal effect, the offspring phenotype depends on the genotype of its mother, so there is some form of context-dependent maternal effect (Cheverud and Wolf 2009) and the maternal effect estimated applies only to the subpopulation of heterozygous offspring. The only interaction effect that can be estimated independently is the dominance-by-dominance (dd_{mo}) maternal–offspring interaction effect (Equation 10), which is a measure of the degree to which the dominance maternal effect depends on the offspring's genotype and vice versa.

Although the confounding of nonadditive effects representing interactions between the maternal and offspring genomes at a locus can make the interpretation of maternal effects problematic, it is important to keep in mind again that it is a constraint imposed by the nature of the problem, not the model.

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Detecting Maternal-Effect Loci by Statistical Cross-Fostering

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Files S1-S3

Supporting Data

Files S1-S3 are available for download in compressed folders at
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Table S1 Additional information about the significant *me*QTL.

Listed are the *me*QTL name (Locus), the traits affected by the locus (with Early, Mid and Late corresponding to the multivariate trait sets described in the text), the chromosome (Chr),marker name (Marker) at the *me*QTL position along with its physical position (Mb), confidence interval (in Mb)) based on mouse genome build 36 (www.ensembl.org) and F_2 map position (cM). Significant tests (expressed as LPR) are provided for the overall maternal effect test (ME) and the five individual genetic terms in equation (11) (a_m, d_m, a_o, d_o, and, i_o). Loci where the maternal effect appears to be caused by the presence of an imprinting effect are indicated by a 'Y' in the column labeled 'ME caused by i_o?'. The significance tests are followed by estimates of the effects, which are indicated by the effect name (a_M, d_M, a, d, or, i) and are expressed in both raw and standardized (STD) units along with their standard errors (s.e.). Each effect is followed by the percentage of the phenotypic variance (% V_p) accounted for by each individual term and for the total direct or maternal effect. For clarity, only values associated with significant tests are included for clarity with empty cells indicated by a '–'.

			Position				Significance tests (LPR)								ME caused
							Maternal effects			Direct effects			Conf.	Interact.	by i_o?
Locus	Traits	Chr	Marker	Phys Pos (Mb)	Conf. Int. (Mb)	Map Pos (cM)	ME	a_M	d_M	a_O	d_O	i_O	c_{mo}	dd_{mo}	
mebs2.1	Early	2	rs3713848	75.86	69.18-	44.49	3.19	3.53	0.55	0.29	0.83	0.63	-	-	-
	Mid				88.19		3.98	4.59	0.05	0.54	0.91	0.46	-	-	-
	Late						2.55	3.15	0.06	1.29	0.02	0.14	-	-	-
	week 1						2.45	2.44	-	-	-	-	-	-	-
	week 2						2.75	3.12	-	-	1.51	-	-	-	-
	week 3						3.21	3.70	-	-	1.41	-	-	-	-
	week 4						4.02	4.61	-	-	1.40	-	-	-	-
	week 5						3.29	3.89	-	-	-	-	-	-	-
	week 6						3.07	3.66	-	-	-	-	-	-	-
	week 7						2.89	3.50	-	-	-	-	-	-	-
	week 8						3.03	3.65	-	-	-	-	-	-	-
	week 9						2.56	3.16	-	-	-	-	-	-	-
	week 10						2.15	2.70	-	1.64	-	-	-	-	-
mebs3.1	Early	3	rs6335414	53.14	46.15-	25.05	0.42	0.59	0.37	0.19	1.01	0.11	-	-	-
	Mid				63.79		1.82	1.62	1.15	0.21	1.65	0.29	-	-	-
	Late						2.52	1.00	2.51	0.95	1.00	0.06	-	-	-

	week 3						-	-	-	-	-	-	1.33	-	-
	week 4						-	1.47	-	-	1.56	-	-	-	-
	week 5						1.68	-	1.43	-	1.60	-	1.41	-	-
	week 6						2.31	1.44	1.89	-	1.48	-	-	-	-
	week 7						2.80	1.36	2.49	-	-	-	-	-	-
	week 8						2.78	-	2.70	-	-	-	-	-	-
	week 9						2.38	-	2.50	-	-	-	-	-	-
	week 10						2.20	-	2.39	-	-	-	-	-	-
mebs7.1	Early	7	CEL-7_116160192	122.73	115.93-134.52	57.04	2.11	0.13	2.63	0.96	0.67	0.06	-	-	-
	Mid						2.18	0.69	2.34	1.60	0.02	0.25	-	-	-
	Late						2.96	1.38	2.55	2.39	0.33	0.67	-	-	-
	week 1						1.90	-	2.36	-	-	-	-	-	-
	week 2						1.73	-	2.22	-	-	-	-	-	-
	week 3						2.14	-	2.53	-	-	-	-	-	-
	week 4						1.82	-	2.06	-	-	-	-	-	-
	week 5						1.97	-	2.04	-	-	-	-	-	-
	week 6						2.10	-	2.18	-	-	-	-	-	-
	week 7						2.34	-	2.22	-	-	-	-	-	-
	week 8						2.63	-	2.51	-	-	-	-	-	-
	week 9						2.72	-	2.58	-	-	-	-	-	-
	week 10						3.33	-	2.69	-	-	-	-	-	-
mebs7.2	Early	7	rs6216320	145.00	122.73-145.00	76.45	0.85	0.55	0.94	0.03	1.04	0.29	-	-	-
	Mid						0.83	1.03	0.43	0.21	0.65	0.04	-	-	-
	Late						2.52	2.51	0.85	0.75	0.40	0.69	-	-	-
	week 1						-	-	-	-	-	-	-	-	-
	week 2						-	-	-	-	-	-	-	-	-
	week 3						-	-	-	-	-	-	-	-	-
	week 4						-	-	-	-	-	-	-	-	-
	week 5						-	1.40	-	-	-	-	-	-	-
	week 6						1.36	1.69	-	-	-	-	-	1.43	-

	week 7						1.97	2.17	-	-	-	-	-	2.06	-
	week 8						2.07	2.10	-	-	-	-	-	1.92	-
	week 9						2.63	2.54	-	-	-	-	-	2.20	-
	week 10						2.85	2.67	-	-	-	-	-	1.55	-
mebs8.1	Early	8	rs3705695	98.26	83.23-114.52	48.85	2.19	0.60	2.36	0.02	0.76	0.05	-	-	-
	Mid						2.56	0.80	2.58	0.84	0.18	0.64	-	-	-
	Late						1.71	0.67	1.82	0.82	0.35	1.45	-	-	-
	week 1						1.96	-	2.35	-	-	-	-	-	-
	week 2						1.43	-	1.87	-	-	-	-	-	-
	week 3						2.09	-	2.13	-	-	-	-	-	-
	week 4						2.89	-	3.06	-	-	-	-	-	-
	week 5						2.45	-	2.64	-	-	-	-	-	-
	week 6						1.88	-	1.87	-	-	-	-	-	-
	week 7						1.53	-	1.65	-	-	1.56	-	-	-
	week 8						1.51	-	1.74	-	-	1.59	-	-	-
	week 9						1.65	-	1.67	-	-	1.65	-	-	-
	week 10						1.77	-	1.87	-	-	-	-	-	-
mebs17.1	Early	17	CEL-17_21229557	20.44	8.41-61.29	8.99	2.43	2.37	1.30	0.50	1.07	2.64	-	-	-
	Mid						2.73	3.15	0.72	0.07	0.52	1.56	-	-	-
	Late						1.43	1.80	0.23	0.19	0.43	0.53	-	-	-
	week 1						2.23	2.36	-	-	-	1.32	-	-	Y
	week 2						1.86	1.77	-	-	-	3.59	1.68	-	Y
	week 3						2.17	2.10	-	-	-	2.73	2.09	-	Y
	week 4						2.55	2.47	-	-	-	1.89	2.04	-	Y
	week 5						2.05	2.56	-	-	-	1.92	1.67	-	Y
	week 6						2.50	3.11	-	-	-	-	-	-	Y
	week 7						2.05	2.51	-	-	-	-	-	-	Y
	week 8						1.51	1.96	-	-	-	-	-	-	Y
	week 9						1.38	1.77	-	-	-	-	-	-	Y
	week						-	-	-	-	-	-	-	-	-

	10														
mebs18.1	Early	18	rs13483183	3.68	3.68-47.82	0.00	2.18	2.34	0.56	0.27	0.06	0.09	-	-	-
	Mid						2.16	2.64	0.17	0.36	0.02	0.95	-	-	-
	Late						1.68	2.23	0.02	1.15	0.08	1.51	-	-	-
	week 1						3.78	3.67	-	-	-	-	1.46	-	-
	week 2						1.52	1.92	-	-	-	-	1.53	-	-
	week 3						1.39	1.62	-	-	-	-	1.42	-	-
	week 4						2.09	2.51	-	-	-	-	1.94	-	-
	week 5						1.33	1.82	-	-	-	1.31	1.40	-	Y
	week 6						1.56	2.08	-	-	-	-	-	-	Y
	week 7						1.43	1.96	-	-	-	1.48	-	-	Y
	week 8						1.68	2.22	-	-	-	1.37	-	-	Y
	week 9						1.56	2.11	-	-	-	1.71	-	-	Y
	week 10						1.76	2.32	-	-	-	1.44	-	-	`

Maternal effect estimates

Locus	Traits	a_m est.	a_m (STD) s.e	a_m (STD) est.	a_m s.e	a_m $\%V_p$	d_m	d_m est.	d_m (STD) s.e	d_m (STD) est.	d_m s.e	Total $\%V_p$	Total $\%V_p$ maternal
mebs2.1	Early	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	0.24	0.08	0.32	0.11	4.97	-	-	-	-	-	-	4.97
	week 2	0.36	0.11	0.41	0.12	8.42	-	-	-	-	-	-	8.42
	week 3	0.63	0.17	0.43	0.11	9.29	-	-	-	-	-	-	9.29
	week 4	0.94	0.22	0.47	0.11	10.98	-	-	-	-	-	-	10.98
	week 5	0.85	0.22	0.39	0.10	7.47	-	-	-	-	-	-	7.47
	week 6	0.91	0.24	0.36	0.10	6.39	-	-	-	-	-	-	6.39
	week 7	0.98	0.27	0.34	0.09	5.90	-	-	-	-	-	-	5.90
	week 8	1.10	0.29	0.35	0.09	6.11	-	-	-	-	-	-	6.11
	week 9	1.09	0.32	0.32	0.09	4.96	-	-	-	-	-	-	4.96
	week 10	1.06	0.34	0.29	0.09	4.23	-	-	-	-	-	-	4.23
mebs3.1	Early	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-	-	-
	week 4	0.44	0.20	0.22	0.10	2.34	-	-	-	-	-	-	2.34
	week 5	-	-	-	-	-	-	0.53	0.25	0.24	0.11	2.85	2.85
	week 6	0.46	0.22	0.18	0.09	1.66	-	0.69	0.27	0.27	0.11	3.67	5.33
	week 7	0.49	0.24	0.17	0.09	1.48	-	0.89	0.30	0.31	0.11	4.93	6.41
	week 8	-	-	-	-	-	-	1.03	0.33	0.33	0.10	5.33	5.33

	week 9	-	-	-	-	-	-	1.06	0.35	0.31	0.10	4.70	4.70
	week 10	-	-	-	-	-	-	1.11	0.38	0.30	0.10	4.60	4.60
mebs7.1	Early	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	0.28	0.10	0.37	0.13	6.72	6.72
	week 2	-	-	-	-	-	-	0.35	0.13	0.40	0.14	7.99	7.99
	week 3	-	-	-	-	-	-	0.61	0.20	0.42	0.14	8.70	8.70
	week 4	-	-	-	-	-	-	0.68	0.26	0.34	0.13	5.76	5.76
	week 5	-	-	-	-	-	-	0.65	0.25	0.29	0.11	4.35	4.35
	week 6	-	-	-	-	-	-	0.75	0.27	0.29	0.11	4.28	4.28
	week 7	-	-	-	-	-	-	0.82	0.29	0.29	0.10	4.12	4.12
	week 8	-	-	-	-	-	-	0.95	0.32	0.30	0.10	4.60	4.60
	week 9	-	-	-	-	-	-	1.04	0.34	0.30	0.10	4.54	4.54
	week 10	-	-	-	-	-	-	1.14	0.37	0.31	0.10	4.89	4.89
mebs7.2	Early	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-	-	-
	week 5	0.42	0.20	0.19	0.09	1.79	-	-	-	-	-	-	1.79
	week 6	0.52	0.22	0.20	0.09	2.07	-	-	-	-	-	-	2.07
	week 7	0.66	0.24	0.23	0.09	2.71	-	-	-	-	-	-	2.71
	week 8	0.72	0.27	0.23	0.09	2.59	-	-	-	-	-	-	2.59
	week 9	0.86	0.29	0.25	0.08	3.12	-	-	-	-	-	-	3.12

	week 10	0.96		0.31	0.26	0.08	3.42	-	-	-	-	-	-	3.42
mebs8.1	Early	-		-	-	-	-	-	-	-	-	-	-	-
	Mid	-		-	-	-	-	-	-	-	-	-	-	-
	Late	-		-	-	-	-	-	-	-	-	-	-	-
	week 1	-		-	-	-	-	-	-0.28	0.10	-0.37	0.13	6.80	6.80
	week 2	-		-	-	-	-	-	-0.32	0.13	-0.36	0.15	6.57	6.57
	week 3	-		-	-	-	-	-	-0.55	0.20	-0.38	0.14	7.10	7.10
	week 4	-		-	-	-	-	-	-0.86	0.26	-0.43	0.13	9.21	9.21
	week 5	-		-	-	-	-	-	-0.77	0.25	-0.35	0.11	6.10	6.10
	week 6	-		-	-	-	-	-	-0.69	0.28	-0.27	0.11	3.71	3.71
	week 7	-		-	-	-	-	-	-0.70	0.30	-0.25	0.11	3.01	3.01
	week 8	-		-	-	-	-	-	-0.79	0.33	-0.25	0.11	3.17	3.17
	week 9	-		-	-	-	-	-	-0.83	0.36	-0.24	0.10	2.88	2.88
	week 10	-		-	-	-	-	-	-0.96	0.38	-0.26	0.10	3.42	3.42
mebs17.1	Early	-		-	-	-	-	-	-	-	-	-	-	-
	Mid	-		-	-	-	-	-	-	-	-	-	-	-
	Late	-		-	-	-	-	-	-	-	-	-	-	-
	week 1	-0.22		0.07	-0.28	0.10	3.90	-	-	-	-	-	-	3.90
	week 2	-0.23		0.09	-0.26	0.11	3.37	-	-	-	-	-	-	3.37

	week 3	-0.41	0.15	-0.28	0.10	3.81	-	-	-	-	-	-	3.81
	week 4	-0.59	0.20	-0.29	0.10	4.29	-	-	-	-	-	-	7.72
	week 5	-0.60	0.20	-0.27	0.09	3.74	-	-	-	-	-	-	3.74
	week 6	-0.75	0.22	-0.30	0.09	4.36	-	-	-	-	-	-	4.36
	week 7	-0.74	0.25	-0.26	0.09	3.33	-	-	-	-	-	-	3.33
	week 8	-0.70	0.27	-0.22	0.09	2.45	-	-	-	-	-	-	2.45
	week 9	-0.71	0.29	-0.20	0.09	2.08	-	-	-	-	-	-	2.08
	week 10	-	-	-	-	-	-	-	-	-	-	-	-
mebs18.1	Early	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	-0.28	0.07	-0.36	0.10	6.60	-	-	-	-	-	-	6.60
	week 2	-0.25	0.10	-0.28	0.11	3.93	-	-	-	-	-	-	3.93
	week 3	-0.35	0.16	-0.24	0.11	2.89	-	-	-	-	-	-	2.89
	week 4	-0.61	0.20	-0.30	0.10	4.56	-	-	-	-	-	-	4.56
	week 5	-0.50	0.20	-0.23	0.09	2.55	-	-	-	-	-	-	2.55
	week 6	-0.60	0.23	-0.24	0.09	2.77	-	-	-	-	-	-	2.77
	week 7	-0.64	0.25	-0.22	0.09	2.53	-	-	-	-	-	-	2.53
	week 8	-0.76	0.27	-0.24	0.09	2.92	-	-	-	-	-	-	2.92

week 9	-0.80	0.30	-0.23	0.09	2.65	-	-	-	-	-	-	2.65
week 10	-0.90	0.32	-0.25	0.09	3.06	-	-	-	-	-	-	3.06

		Direct Effects																
		a_o	a_o (STD)	a_o	d_o	d_o (STD)	d_o	i_o	i_o (STD)	i_o	Total							
Locus	**Traits**	**est.**	**s.e**	**est.**	**s.e**	$\%V_p$	**est.**	**s.e**	**est.**	**s.e**	$\%V_p$	**est.**	**s.e**	**est.**	**s.e**	$\%V_p$	**$\%V_p$ direct**	
mebs2.1	Early	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Mid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Late	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 2	-	-	-	-	-	0.12	0.06	0.14	0.06	0.92	-	-	-	-	-	0.92	
	week 3	-	-	-	-	-	0.18	0.08	0.12	0.06	0.71	-	-	-	-	-	0.71	
	week 4	-	-	-	-	-	0.27	0.13	0.14	0.07	0.92	-	-	-	-	-	0.92	
	week 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 10	0.46	0.20	0.13	0.06	0.79	-	-	-	-	-	-	-	-	-	-	0.79	
mebs3.1	Early	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Mid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	Late	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 2	-	-	-	-	-	0.12	0.05	0.14	0.06	0.97	-	-	-	-	-	0.97	
	week 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 4	-	-	-	-	-	0.28	0.13	0.14	0.06	0.99	-	-	-	-	-	0.99	
	week 5	-	-	-	-	-	0.31	0.14	0.14	0.06	0.98	-	-	-	-	-	0.98	
	week 6	-	-	-	-	-	0.33	0.15	0.13	0.06	0.82	-	-	-	-	-	0.82	
	week 7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	week 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

	week 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mebs7.1	Early	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mebs7.2	Early	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	week 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mebs8.1	Early	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-	-0.39	0.18	-0.14	0.06	0.94	0.94
	week 8	-	-	-	-	-	-	-	-	-	-	-0.45	0.20	-0.14	0.06	1.01	1.01
	week 9	-	-	-	-	-	-	-	-	-	-	-0.50	0.22	-0.14	0.06	1.03	1.03
	week 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mebs17.1	Early	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-	0.10	0.05	0.13	0.06	0.82	0.82
	week 2	-	-	-	-	-	-	-	-	-	-	0.22	0.06	0.26	0.07	3.26	3.26
	week 3	-	-	-	-	-	-	-	-	-	-	0.29	0.09	0.20	0.06	1.98	1.98
	week 4	-	-	-	-	-	-	-	-	-	-	0.36	0.15	0.18	0.07	1.63	1.63
	week 5	-	-	-	-	-	-	-	-	-	-	0.39	0.15	0.18	0.07	1.57	1.57
	week 6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

	week 9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
mebs18.1	Early	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	week 1	0.07	0.05	0.10	0.06	-	0.04	0.04	0.05	0.06	-	-0.03	0.04	-0.03	0.06	-	-
	week 2	0.03	0.06	0.04	0.07	-	0.03	0.05	0.03	0.06	-	-0.01	0.05	-0.01	0.06	-	-
	week 3	0.00	0.09	0.00	0.06	-	-0.01	0.08	-0.01	0.06	-	0.00	0.08	0.00	0.06	-	-
	week 4	0.03	0.14	0.02	0.07	-	0.01	0.13	0.01	0.06	-	0.18	0.13	0.09	0.07	-	-
	week 5	0.13	0.15	0.06	0.07	-	0.06	0.14	0.03	0.06	-	0.28	0.14	0.13	0.06	0.79	0.79
	week 6	0.20	0.16	0.08	0.06	-	0.02	0.15	0.01	0.06	-	0.28	0.16	0.11	0.06	-	-
	week 7	0.33	0.18	0.11	0.06	-	0.05	0.17	0.02	0.06	-	0.38	0.18	0.13	0.06	0.87	0.87
	week 8	0.39	0.21	0.12	0.07	-	-0.07	0.20	-0.02	0.06	-	0.40	0.20	0.13	0.06	0.82	0.82
	week 9	0.36	0.23	0.11	0.07	-	-0.04	0.21	-0.01	0.06	-	0.51	0.22	0.15	0.06	1.07	1.07
	week 10	0.43	0.24	0.12	0.07	-	-0.13	0.23	-0.04	0.06	-	0.48	0.23	0.13	0.06	0.88	0.88

		Confounded effect					dxd interaction effect				
		c_{mo}		c_{mo} (STD)		c_{mo}	dd_{mo}		dd_{mo} (STD)		dd_{mo}
Locus	Traits	est.	s.e	est.	s.e	$\%V_p$	est.	s.e	est.	s.e	$\%V_p$
mebs2.1	Early	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-
	week 5	-	-	-	-	-	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-
	week 9	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-
mebs3.1	Early	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-
	week 3	0.319156084	0.159759004	0.217361395	0.108803942	2.362298803	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-
	week 5	0.430634814	0.20760843	0.195622523	0.094309339	1.913408566	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-

	week 9	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-
mebs7.1	Early	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-
	week 5	-	-	-	-	-	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-
	week 9	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-
mebs7.2	Early	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-
	week 5	-	-	-	-	-	-	-	-	-	-
	week 6	-	-	-	-	-	0.6346165	0.304200506	0.248941458	0.119328945	3.098592464
	week 7	-	-	-	-	-	0.899135961	0.34224889	0.315552734	0.120112617	4.978676402
	week 8	-	-	-	-	-	0.972222593	0.386257831	0.308938282	0.122739207	4.77214309

	week 9	-	-	-	-	-	1.14250604	0.417607602	0.33054798	0.120821549	5.463098369
	week 10	-	-	-	-	-	0.985934934	0.448293211	0.269727501	0.122641975	3.637646235
mebs8.1	Early	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-
	week 2	-	-	-	-	-	-	-	-	-	-
	week 3	-	-	-	-	-	-	-	-	-	-
	week 4	-	-	-	-	-	-	-	-	-	-
	week 5	-	-	-	-	-	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-
	week 9	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-
mebs17.1	Early	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-
	week 1	-	-	-	-	-	-	-	-	-	-
	week 2	-0.217434845	0.093609929	-0.247743825	0.106658534	3.068850135	-	-	-	-	-
	week 3	-0.399823	0.149676143	-0.272299635	0.101937005	3.707354562	-	-	-	-	-
	week 4	-0.514453204	0.19592146	-0.255847583	0.097435553	3.272899299	-	-	-	-	-
	week 5	-0.451849339	0.195647712	-0.205259549	0.088875998	2.106574113	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-

	week 9	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-
mebs18.1	Early	-	-	-	-	-	-	-	-	-	-
	Mid	-	-	-	-	-	-	-	-	-	-
	Late	-	-	-	-	-	-	-	-	-	-
	week 1	-0.153817429	0.072326736	-0.199400349	0.093760353	1.988024965	-	-	-	-	-
	week 2	-0.207212845	0.094531447	-0.236096946	0.107708505	2.787088392	-	-	-	-	-
	week 3	-0.315438134	0.151518223	-0.214829284	0.103191555	2.307581059	-	-	-	-	-
	week 4	-0.49948854	0.196231861	-0.248405365	0.097589921	3.085261265	-	-	-	-	-
	week 5	-0.403367916	0.194839967	-0.183236113	0.088509067	1.678773652	-	-	-	-	-
	week 6	-	-	-	-	-	-	-	-	-	-
	week 7	-	-	-	-	-	-	-	-	-	-
	week 8	-	-	-	-	-	-	-	-	-	-
	week 9	-	-	-	-	-	-	-	-	-	-
	week 10	-	-	-	-	-	-	-	-	-	-

Table S2 The 353 SNP markers used in the study. Listed for each locus are: the chromosome (chr), marl
(Marker), F_2 map position in cM [Map Pos (cM)] and the physical position based on mouse genome build
(ensembl.org) [Phy. Pos. (bp)].

chr	Marker	Map Pos (cM)	Phys Pos (bp)
1	rs3683945	0.00	3,194,282
1	rs3697012	0.89	11,044,085
1	rs13475748	4.23	19,111,933
1	rs13475769	7.35	25,116,111
1	rs4137502	8.26	31,054,038
1	rs13475816	15.19	37,799,320
1	rs13475837	21.53	43,351,651
1	rs13475863	23.05	50,950,140
1	rs3653534	25.21	57,058,624
1	rs13475894	29.10	63,819,094
1	rs6288543	31.79	68,752,862
1	rs13475927	34.70	74,835,399
1	rs13475945	37.39	80,804,768
1	petM22381-169-2	40.40	87,020,297
1	rs6268443	42.66	93,290,397
1	rs3695980	43.26	99,427,298
1	rs3692731	44.27	105,881,357
1	mCV22824651	44.97	112,322,533
1	gnf01.117.970	45.98	118,059,128
1	rs6189020	48.46	124,494,167
1	rs6308228	49.56	131,196,731
1	rs6250257	53.23	137,526,917
1	rs6186115	55.59	144,639,080
1	rs3688042	55.89	151,188,966
1	gnf01.157.188	60.23	157,686,377
1	rs8256589	64.13	164,030,203
1	rs8242852	71.43	171,027,167
1	rs13476258	74.89	176,948,891
1	rs6157620	79.12	183,086,558
1	rs3654705	88.61	188,867,672
1	rs13476312	91.08	193,519,909
2	rs13476319	0.00	3,379,629
2	rs13476343	2.90	9,084,552
2	rs3688854	14.12	20,346,865
2	mCV23209429	22.65	31,212,156
2	rs6313371	24.91	39,603,320
2	rs13476473	27.38	46,078,428
2	rs3718711	33.12	56,536,504
2	rs3022886	37.46	62,575,883
2	rs8263587	40.92	69,177,796
2	rs3713848	44.49	75,862,390

2	mCV23002990	47.72	82,247,852
2	rs6314788	48.92	88,190,015
2	rs6252400	49.80	94,100,662
2	rs6406705	50.81	100,210,695
2	rs3715478	51.72	106,039,578
2	rs3681694	53.77	112,135,089
2	rs3725853	56.46	117,999,826
2	rs13476750	56.96	123,396,329
2	rs6318992	59.65	129,004,907
2	rs13476790	63.89	135,384,836
2	rs6303304	66.36	141,184,172
2	rs3701696	67.99	147,169,586
2	rs13476846	74.08	152,772,293
2	gnf02.161.674	76.23	158,197,749
2	rs6185704	81.15	164,694,179
2	rs3689258	84.82	169,280,554
2	rs8238755	94.92	174,162,904
2	rs6402916	97.07	182,308,436
3	rs13476950	0.00	3,789,309
3	rs13476970	1.73	10,024,340
3	rs13476992	3.57	16,629,527
3	rs13477017	7.91	22,361,195
3	rs3677132	9.96	27,735,912
3	rs4223864	13.30	32,752,595
3	gnf03.036.164	17.53	38,786,835
3	CEL-3_46558020	19.90	46,147,367
3	rs6335414	25.05	53,144,448
3	rs6363066	27.42	57,465,266
3	rs13477154	30.32	63,785,711
3	rs13477178	31.94	69,845,859
3	gnf03.073.308	34.20	75,963,473
3	rs13477230	36.88	82,906,933
3	rs13475064	39.35	89,023,753
3	rs13459185	43.80	95,820,465
3	gnf03.117.090	49.82	102,175,728
3	rs3671622	52.93	107,288,989
3	rs3684333	54.17	*?*
3	rs13477364	60.71	118,359,586
3	rs13477384	64.26	124,645,248
3	rs3658914	68.61	130,664,530
3	CEL-3_137067761	74.45	135,402,411
3	rs4224277	77.79	142,465,675
3	rs3722447	82.69	148,845,957
3	rs3695386	86.47	155,691,193
4	rs13477541	0.00	6,320,883
4	rs13477558	1.63	10,825,524

4	rs13477576	4.53	15,649,659
4	rs13477621	9.44	28,086,223
4	CEL-4_34055416	13.33	34,102,850
4	CEL-4_40541402	16.77	40,588,836
4	rs3663355	21.34	48,127,148
4	rs13477711	23.70	53,916,305
4	rs3671277	27.03	59,485,927
4	gnf04.062.327	29.93	65,807,438
4	rs13477773	31.66	72,789,249
4	CEL-4_78089985	33.50	78,876,961
4	rs13477816	37.28	84,728,023
4	rs13477831	38.79	88,355,456
4	rs13477854	40.10	95,212,056
4	rs32231654	42.79	101,499,103
4	rs3709496	45.91	107,207,797
4	rs3687391	47.95	113,741,177
4	rs3678308	53.32	120,184,273
4	rs13477968	57.78	126,841,948
4	rs13477996	61.67	134,467,043
4	rs3023025	73.17	142,594,845
4	rs13478051	76.73	149,937,635
4	rs3720634	82.57	154,897,136
5	rs13478093	0.00	3,974,781
5	mCV25284008	0.90	12,529,445
5	rs13478133	8.32	20,652,208
5	rs13478175	15.99	32,651,323
5	CEL-5_45872918	25.47	46,008,170
5	rs3711950	33.89	63,366,297
5	rs3691938	35.83	67,623,605
5	gnf05.069.163	40.40	73,713,675
5	rs13478355	43.63	82,093,009
5	rs3667067	44.13	88,174,349
5	rs3661241	45.76	97,050,706
5	rs6350578	49.44	105,471,990
5	rs3658755	52.02	111,954,844
5	rs13478487	56.93	118,538,063
5	rs13478509	59.72	124,754,775
5	rs6377710	68.01	131,612,906
5	rs6393330	75.29	137,849,985
5	mCV25009162	82.46	143,577,772
5	rs13478595	87.94	149,279,125
6	rs13478602	0.00	3,799,842
6	CEL-6_10519419	1.94	10,759,266
6	rs13478645	4.09	17,082,496
6	rs3710004	6.88	23,144,345
6	rs13478681	9.78	28,737,142

6	gnf06.032.524	12.36	35,626,148
6	rs13478726	15.37	42,345,253
6	rs3684860	19.47	48,946,087
6	rs13478762	25.79	54,189,047
6	gnf06.058.959	27.52	60,050,421
6	rs8270116	29.25	65,105,646
6	rs13478839	34.28	77,829,431
6	rs13478882	38.73	89,740,126
6	gnf06.093.201	41.96	94,758,887
6	rs13478949	50.52	105,229,614
6	rs13478974	52.68	112,141,005
6	mCV23042866	54.62	117,194,262
6	rs3722157	58.30	122,444,202
6	rs13479024	62.19	127,614,936
6	rs6339546	64.45	133,981,207
6	rs6199136	70.18	140,483,280
6	rs6265387	74.87	147,333,176
7	CEL-7_12787527	0.00	19,225,941
7	rs13479174	7.43	29,731,663
7	rs6217275	13.28	36,135,464
7	rs3719256	19.25	44,406,689
7	gnf07.056.997	27.70	56,324,216
7	rs6296859	29.43	68,855,004
7	rs13479342	33.44	74,842,909
7	rs13479385	38.13	87,009,942
7	rs3683030	39.86	92,968,513
7	UT_7_90.803899	42.55	93,425,306
7	rs13459173	44.07	105,510,122
7	rs13479461	44.98	111,739,733
7	rs3656074	47.03	115,925,415
7	gnf07.120.460	53.59	117,630,799
7	CEL-7_116160192	57.04	122,731,498
7	rs3719258	67.13	134,524,389
7	rs6299045	73.87	140,005,209
7	rs6216320	76.45	144,991,090
8	rs6273176	0.00	7,850,079
8	rs6410533	4.22	14,301,441
8	rs13479628	7.35	18,316,424
8	CEL-8_25677705	13.19	25,625,193
8	rs13479769	33.90	56,099,587
8	rs3672639	35.84	64,113,928
8	rs13479811	37.16	69,982,295
8	UT_8_84.8318	42.64	83,229,904
8	rs3705695	48.85	98,245,251
8	rs6374927	61.90	114,518,266
8	rs6347490	69.69	119,458,949

8	rs13480023	77.87	125,648,980
8	rs3695597	80.35	131,819,988
9	rs13480065	0.00	9,252,350
9	gnf09.009.970	3.26	15,532,281
9	rs4227562	8.94	24,474,784
9	CEL-9_29909656	13.51	29,851,373
9	mCV25302097	18.42	36,389,664
9	rs3676086	21.43	42,592,846
9	rs13480180	26.12	48,172,738
9	rs3660104	31.73	54,782,339
9	rs13480227	35.51	60,848,809
9	rs3670579	37.77	66,705,910
9	rs13480273	42.45	73,069,886
9	rs3658458	44.81	80,089,158
9	rs6182207	47.60	87,235,216
9	rs13480351	50.83	94,088,160
9	gnf09.096.289	52.22	99,654,867
9	rs3711089	54.83	105,349,763
9	rs3723953	58.94	110,881,584
9	rs3669563	70.52	117,767,462
9	rs8254378	75.77	123,973,253
10	rs13480510	0.00	15,772,078
10	rs3679120	4.91	22,610,695
10	rs3023233	8.68	28,285,463
10	rs13459120	12.57	35,728,463
10	rs13480601	17.70	43,994,834
10	rs6164020	18.41	49,436,630
10	rs3682060	20.35	56,297,867
10	rs6312070	25.25	62,470,059
10	rs13480638	28.47	68,840,024
10	rs13480652	30.94	74,056,142
10	rs13480678	33.41	84,115,180
10	mCV25373751	38.54	90,680,515
10	rs3704401	43.79	98,672,238
10	rs3716443	46.26	104,916,467
10	rs6353445	49.16	111,098,211
10	rs3670118	55.95	116,797,981
10	rs13480797	61.08	121,330,793
10	rs6290842	66.22	128,518,968
11	rs13480835	0.00	3,286,216
11	rs3661631	3.22	9,286,553
11	rs3724116	5.48	14,664,727
11	rs3154937	11.90	21,522,897
11	rs6276300	13.85	27,200,063
11	rs3726288	16.22	32,716,003
11	rs3690160	21.95	36,868,781

11	gnf11.045.072	24.31	43,196,003
11	rs6326787	28.43	48,667,564
11	rs13481031	30.57	54,375,348
11	rs13481050	34.68	60,835,622
11	rs13481075	38.46	66,584,279
11	rs13481093	43.37	72,632,085
11	rs6381209	44.48	78,938,143
11	rs3661657	49.97	84,802,276
11	rs3688955	52.01	90,645,574
11	rs3710148	55.77	96,634,470
11	rs6180460	61.62	103,063,126
11	rs3683086	67.46	109,441,968
11	rs3662930	73.89	115,631,088
11	CEL-11_118234030	79.37	118,383,994
12	rs3699421	0.00	3,975,723
12	rs6209157	3.34	10,609,007
12	rs13481322	5.81	17,013,007
12	rs13481361	6.82	25,405,139
12	rs6223000	11.74	32,025,639
12	rs13481408	15.53	40,966,872
12	rs3709102	20.20	47,692,696
12	rs3670749	20.69	50,976,889
12	rs3662939	24.47	56,814,847
12	rs3686891	25.26	63,419,679
12	rs6335879	28.82	69,863,512
12	rs3682382	32.05	77,217,942
12	rs6263380	36.86	83,729,205
12	rs3711162	40.75	89,664,381
12	rs6207869	42.80	94,763,235
12	rs6184745	49.26	98,341,221
12	rs13481632	54.29	103,895,527
12	rs4229612	59.31	113,706,439
12	rs3679276	60.53	120,329,034
13	rs6215262	0.00	3,668,788
13	rs13481689	1.11	10,743,171
13	gnf13.016.175	3.05	18,339,106
13	CEL-13_25470193	5.63	25,570,134
13	rs4229685	10.67	35,290,378
13	rs6271232	15.71	41,896,501
13	rs6244558	18.61	47,298,225
13	rs3699522	20.76	52,031,398
13	gnf13.057.762	24.78	58,363,844
13	rs3718727	28.46	66,239,776
13	rs13481886	32.35	76,596,223
13	rs13481905	34.08	81,570,691
13	rs6296621	37.98	87,436,552

13	rs13481947	40.77	90,736,401
13	rs13481990	49.58	102,172,453
13	mCV24886326	53.58	106,844,497
13	rs13482028	57.91	117,370,793
13	rs3675054	61.14	120,533,918
14	rs3687889	0.00	18,479,914
14	rs3658866	2.56	21,747,331
14	rs13482143	15.90	36,987,366
14	rs13482174	19.57	44,917,612
14	rs8244195	20.37	50,561,306
14	rs13482206	23.59	54,206,615
14	rs3701623	32.14	67,879,239
14	rs6352085	35.70	74,362,227
14	rs3663148	37.75	81,549,269
14	rs3718262	39.17	92,413,488
14	rs6211694	50.67	103,092,227
14	rs3683221	51.58	106,966,046
14	rs3707842	57.19	118,774,157
14	rs13482416	60.09	123,897,764
15	rs13459176	0.00	3,229,130
15	rs13482431	5.03	11,369,635
15	rs13482461	7.93	18,989,437
15	rs13482486	9.98	25,834,974
15	rs6333696	13.77	32,230,149
15	rs3695416	15.83	38,595,274
15	CEL-15_44698021	18.09	44,621,103
15	rs3692040	21.66	51,130,382
15	rs6400804	23.70	56,869,671
15	rs4139476	25.96	62,973,141
15	rs13482635	30.19	69,530,236
15	rs13482654	35.12	76,776,858
15	rs4230908	38.13	83,210,799
15	rs3720931	44.21	89,581,344
15	rs13482726	54.47	95,950,747
15	rs13482741	58.03	101,104,558
16	rs4152386	0.00	3,658,583
16	rs4165119	9.08	20,048,857
16	rs6294027	12.40	25,370,478
16	rs4170074	18.02	31,869,305
16	rs4174469	21.58	37,877,542
16	rs4180126	25.47	44,439,523
16	rs3696661	30.27	51,453,712
16	rs6188665	34.62	65,343,226
16	rs4201178	39.07	71,985,529
16	rs4207452	42.41	77,426,457
16	rs4211731	44.45	83,401,918

16	rs4219239	52.22	91,720,510
16	rs3164088	55.34	96,696,753
17	rs3724616	0.00	3,000,665
17	rs3702484	4.78	8,407,212
17	rs3675740	7.36	14,492,285
17	CEL-17_21229557	8.99	20,436,833
17	rs6358703	12.21	28,506,799
17	gnf17.035.152	15.77	32,759,014
17	CEL-17_40073719	16.37	39,439,743
17	rs13483011	19.88	46,555,895
17	mCV22888090	31.56	61,294,637
17	rs6210797	36.94	67,976,068
17	rs13483110	42.79	76,067,016
17	rs3684732	53.21	81,302,791
17	rs13483159	60.39	88,028,720
18	rs13483183	0.00	3,682,469
18	rs13483200	1.84	10,108,718
18	rs13483226	5.51	16,414,943
18	rs6194744	8.20	22,236,323
18	rs3722205	10.78	28,065,456
18	UT_18_33.980716	13.04	34,018,979
18	rs6184541	21.45	47,816,646
18	gnf18.051.412	23.50	53,321,117
18	CEL-18_60214752	29.71	59,924,390
18	rs13483409	36.52	66,269,017
18	gnf18.069.928	40.09	71,877,091
18	rs3705890	44.32	77,151,914
18	rs13483466	51.85	82,589,970
19	rs3713033	0.00	4,924,597
19	rs6307076	5.03	14,252,840
19	rs3714482	26.97	28,637,227
19	rs13483601	32.11	34,651,093
19	mCV23121032	38.78	40,524,220
19	rs13483650	44.61	47,273,905
19	rs13483677	49.75	53,772,375
19	rs3711945	53.97	58,633,582

Table S3 Significance thresholds for maternal and direct effect tests. Genome-wise and individual chromosome-wise thresholds were determined by Bonferroni correction based on the effective number of markers. Maternal effect thresholds are based on the effective number of markers (M_{eff}) in the F_2 generation and the direct effect thresholds are based on the effective number of markers in the F_3 generation.

Chromosome	Maternal effects (F_2)		Direct effects (F_3)	
	Number of tests (M_{eff})	Threshold	Number of tests (M_{eff})	Threshold
1	9	2.19	11	2.33
2	8	2.25	10	2.29
3	7	2.19	9	2.25
4	6	2.14	9	2.25
5	7	2.07	8	2.19
6	7	2.14	8	2.19
7	5	2.14	7	2.14
8	7	1.99	7	2.14
9	6	2.14	8	2.19
10	7	2.07	7	2.14
11	6	2.14	8	2.19
12	6	2.07	7	2.14
13	5	2.07	7	2.14
14	6	1.99	6	2.07
15	5	2.07	6	2.07
16	5	1.99	6	2.07
17	4	1.99	6	2.07
18	4	1.89	5	1.99
19	9	1.89	5	1.99
(genome)	112	3.34	132	3.41