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Maternal Effects as the Cause of Parent-of-Origin Effects That Mimic Genomic Imprinting

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ABSTRACT

Epigenetic effects are increasingly recognized as an important source of variation in complex traits and have emerged as the focus of a rapidly expanding area of research. Principle among these effects is genomic imprinting, which has generally been examined in analyses of complex traits by testing for parent-of-origin-dependent effects of alleles. However, in most of these analyses maternal effects are confounded with genomic imprinting because they can produce the same patterns of phenotypic variation expected for various forms of imprinting. Distinguishing between the two is critical for genetic and evolutionary studies because they have entirely different patterns of gene expression and evolutionary dynamics. Using a simple single-locus model, we show that maternal genetic effects can result in patterns that mimic those expected under genomic imprinting. We further demonstrate how maternal effects and imprinting effects can be distinguished using genomic data from parents and offspring. The model results are applied to a genome scan for quantitative trait loci (QTL) affecting growth- and weight-related traits in mice to illustrate how maternal effects can mimic imprinting. This genome scan revealed five separate maternal-effect loci that caused a diversity of patterns mimicking those expected under various modes of genomic imprinting. These results demonstrate that the appearance of parent-of-origin-dependent effects (POEs) of alleles at a locus cannot be taken as direct evidence that the locus is imprinted. Moreover, they show that, in gene mapping studies, genetic data from both parents and offspring are required to successfully differentiate between imprinting and maternal effects as the cause of apparent parent-of-origin effects of alleles.

PARENT-of-origin-dependent effects (POEs) comprise a range of genetic and epigenetic phenomena modulating different complex traits such as individual growth and development (Hager and Johnstone 2003, 2006), cognitive abilities (Isles and Wilkinson 2000), and several human diseases such as Prader-Willi and Angelman syndromes and obesity (Nicholls 2000; Constancia *et al.* 2004; Dong *et al.* 2005; Morison *et al.* 2005). A number of recent genomewide linkage studies have investigated POEs on complex traits and identified several quantitative trait loci (QTL) with putative genomic imprinting effects (De Koning *et al.* 2000; Lindsay *et al.* 2002; Mantey *et al.* 2005). Similarly, evidence for genomic imprinting effects on cognitive abilities in humans has been reported from family correlation studies (Goos and Silverman 2006). However, in most of these studies further analysis has not been conducted to determine whether the detected POE is actually caused by genomic imprinting or other factors. The assumption that differences between reciprocal heterozygotes [*i.e.*, those that obtain, for example, their A_1 allele from their father (A_1A_2) as opposed to those that receive this allele from the mother (A_2A_1)] are equivalent to imprinting effects is found in other treatments as well (Hall 1997; Morison and Reeve 1998; Goos *et al.* 2007).

While some gene mapping studies have attempted to locate imprinted loci by detecting differences between the reciprocal heterozygotes, other genetic effects can also lead to the same phenotypic pattern. Maternal genetic effects (hereafter referred to as "maternal effects"), the effect of a mother's genotype on the expression of traits in her offspring via the maternally provided environment (Falconer 1965; Mousseau and Fox 1998), can also lead to differences between reciprocal heterozygotes. This occurs because homozygous mothers can give rise only to one of the reciprocal heterozygotes. For example, A_1A_1 mothers can produce only A_2A_1 (given as paternal/maternal) heterozygotes while A_2A_2 mothers can produce only A_1A_2 heterozygotes. Genomic imprinting can create the same pattern of effects through monoallelic or differential expression of the two alleles at a locus (Bartolomei and Tilghman 1997; Hayward *et al.* 1998; Reik and Walter 2001). Differences in methylation status between parental alleles can cause this uniparental pattern of gene expression, whereby

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the imprint is erased and reset during gametogenesis according to the sex of the parent (Lewis and Reik 2006; Wood and Oakey 2006).

The distinction between genomic imprinting and maternal effects has considerable implications both for research on the genetic basis of individual development and cognitive abilities as well as for our understanding of the evolutionary dynamics associated with them (Santure and Spencer 2006). This distinction will also be crucial for identifying the underlying genes causal to such effects. For example, loci may show significant differences between reciprocal heterozygotes in a genome scan (*e.g.*, a QTL study) but have no corresponding parent-of origin-dependent pattern of gene expression if the apparent POE is caused by a maternal-effect locus expressed by mothers but not their offspring. This may be particularly paradoxical if, for instance, the maternal-effect gene is expressed only in females, yet appears to show a POE in males despite the fact that they never express the gene. Furthermore, the evolutionary dynamics of traits affected by maternal effects are different from those affected by genomic imprinting since the response to selection in the case of maternal effects may show, for example, time lags and evolutionary inertia caused by the response to selection in previous generations (Kirkpatrick and Lande 1989; Pearce and Spencer 1992; Cheverud and Wolf 2008). Maternal-effect loci are exposed to selection both as a result of their direct effects on the mother's phenotype (and possibly their direct effects on their offspring's phenotype due to pleiotropy) and their indirect effects on their offspring's phenotype, as in evolution by kin selection (Cheverud 1984). This pattern of selection leads to different rates of evolution and levels of polymorphism for maternal-effect loci compared to direct-effect loci (Wade *et al.* 2008). Furthermore, several models have been developed explaining the evolution of genomic imprinting (Wilkins and Haig 2003; Wolf and Hager 2006) that make specific assumptions about the nature of imprinted loci and, therefore, the confounding of maternal-effect and imprinted loci will impede analyses of these models. Finally, Santure and Spencer (2006) have shown that maternal effects and genomic imprinting also have distinct effects on the phenotypic similarity among relatives.

While the mechanisms of imprinting and maternal genetic effects are clearly recognized as being distinct, it has not been explored whether the two could yield identical patterns of phenotypic effects at individual loci, thus leading to a confounding of genomic imprinting and maternal effects in genome scans. In this article, we first use a simple single-locus model to show how maternal genetic effects can result in patterns that mimic the sorts of parent-of-origin-dependent effects of alleles expected under genomic imprinting. We then use the model to illustrate how the two (maternal effects and genomic imprinting) can be distinguished by using genotypic data from parents and offspring. Finally, we use an empirical investigation of QTL affecting weight and growth traits in mice to illustrate the model results (*i.e.*, how maternal effects can mimic but also be distinguished from genomic imprinting).

MATERIALS AND METHODS

Genetic model: We consider a simple single-locus model with two alleles to illustrate how maternal effects can cause an apparent parent-of-origin-dependent effect at a given locus that mimics various patterns of genomic imprinting (described below). We refer to these as "apparent parent-of-origin-dependent effects" because the expression of the locus does not actually depend on the parent-of-origin of alleles. Rather, in the model the locus is assumed to have a maternal effect (additive and/or dominance) on the trait of interest and may also show a direct effect on the same trait due to pleiotropy. Although we use a two-allele model for simplicity, the general results apply to any system where genetically variable parents produce offspring for whom parent-of-origin of alleles can be assigned. Therefore, these results do not apply to an F_2 population created from an intercross of a pair of inbred lines since all individuals have genetically identical F_1 parents and, as a result, there can be no phenotypic variation in the F_2 attributable to genetically based maternal effects. Because the detection and characterization of parent-of-origin-dependent effects depend critically on the distinction between reciprocal heterozygotes, the use of outbred populations or crosses between more than two lines may aid in the detection and characterization of parent-of-origin-dependent effects.

We assume a single locus with two alleles, *L* and *S* (to match the large and small alleles from the LG/J and SM/J lines used in our empirical example), with frequencies p and q, respectively. We assume random mating in a population that conforms to Hardy–Weinberg equilibrium genotype frequencies (which matches the population we use in the QTL analysis). We further assume that the locus has a maternal effect on the phenotype of her offspring when expressed by mothers. The additive maternal-effect genotypic value of the locus is denoted, a_m (where the subscript m is used for all terms that correspond to the maternal effect of the locus) such that offspring of *LL* mothers have the average phenotype $+a_m$ and those of *SS* mothers have the average phenotype $-a_m$ (the expected phenotypes for each possible maternal–offspring genotype combination are shown in Table 1). We denote the dominance maternal-effect genotypic value as d_m, which is the difference between the average phenotype of the offspring of heterozygous mothers (*LS* or *SL*) and the midpoint of the average phenotypes of the offspring of the two homozygous mothers. For simplicity, the two types of heterozygous mothers (*LS vs. SL*) are grouped together since we assume that the parent-of-origin of alleles does not affect the maternal trait responsible for the maternal effect on offspring phenotype. This assumption has no effect on the model results (*i.e.*, the results are identical regardless of the assumption about the imprinting state of the locus in mothers) and, therefore, the model results shown here apply equally well to cases where a locus has an imprinted effect on the maternal trait.

To examine the appearance of parent-of-origin-dependent effects we keep track of allelic parent-of-origin in the offspring and distinguish the two classes of heterozygotes. In the notation for offspring genotype (*e.g.*, *LS*), the first allele refers to the paternally inherited copy and the second to the maternally inherited copy. The locus may also have a direct effect (*i.e.*, the individuals' own genotypes affect their own phenotypes), where the effect of the locus is given by the additive (a_o),

TABLE 1

Expected genotypic values as a function of the ordered offspring genotypes (*i.e.*, ordered by the parent-of-origin of alleles, with the first allele indicating the paternally inherited copy and the second the maternally inherited copy) and the unordered maternal genotypes when variation in offspring traits is caused by maternal and direct effects

	Offspring genotype				
	LL	*SL*	*LS*	*SS*	$\bar{z}_{\text{maternal}}$
Maternal genotype					
LL	$+a_m + a_o$ (p^3)	$+a_m + d_o - i_o$ (p^2q)	$\mathbf{+a_m + d_o + i_o}$ **(0)**	$\mathbf{+a_m - a_o}$ **(0)**	$+a_m + a_o p$ $+ d_o q - i_o q$
LS, SL	$d_m + a_o$ (p^2q)	$d_m + d_o - i_o$ (pq^2)	$d_m + d_o + i_o$ (p^2q)	$d_m - a_o$ (pq^2)	$+ d_m + \frac{1}{2}d_o$ $\frac{1}{2}(a_o + i)(p - q)$
SS	$\mathbf{-a_m + a_o}$ **(0)**	$\mathbf{-a_m + d_o - i_o}$ **(0)**	$-a_m + d_o + i_o$ (pq^2)	$-a_m - a_o$ (q^3)	$-a_m - a_o q$ $+ d_o p + i_o p$
$\bar{z}_{\text{offspring}}$	$a_m p + d_m q$ $+ a_o$	$a_m p + d_m q$ $+ d_o - i_o$	$-a_m q + d_m p$ $+ d_o + i_o$	$-a_m q + d_m p$ $- a_o$	

The average phenotypes of individuals (offspring) with each of the four ordered genotypes ($\bar{z}_{\text{offspring}}$) are given at the bottom of the table. Also shown in the right-hand column is the average phenotype of the offspring of mothers ($\bar{z}_{\text{maternal}}$) with each of the three unordered maternal genotypes. The frequencies of the maternal–offspring genotype combinations are given in parentheses, where p and q are the frequencies of the L and S alleles, respectively. Note that four maternal–offspring combinations (boldface) cannot occur (*i.e.*, have zero frequency) under Mendelian inheritance and, therefore, do not contribute to the means. a_m, the additive maternal-effect genotypic value; d_m, the dominance maternal-effect genotypic value; a_o, d_o, and i_o, the additive, dominance, and imprinting direct-effect genotypic values.

dominance (d_o), and parent-of-origin (i_o) genotypic values (where the subscript o is used to indicate terms corresponding to direct effects on the offspring's own phenotype). These direct-effect genotypic values are defined from the genotypic values (the average phenotypes) of the four ordered genotypes (*i.e.*, genotypes where the order of alleles reflects their parent-of-origin as described above): $\overline{LL}, \overline{LS}, \overline{SL}, \overline{SS}$. The additive genotypic value is defined as half the difference between the average phenotype of the homozygote genotypes, $(\overline{LL} - \overline{SS})/2$; the dominance genotypic value is defined as the difference between the average heterozygote phenotype and the average homozygote phenotype, $[(\overline{LS} + \overline{SL})/2] - [(\overline{LL} + \overline{SS})/2]$; and the parent-of-origin genotypic value is defined as half the difference between the average phenotypes of the reciprocal heterozygotes, $(\overline{LS} - \overline{SL})/2$. These direct-effect genotypic values correspond to those defined in the model of genetic effects used in the QTL analysis below (see Equation 3).

The offspring phenotypes are given as a function of maternal and offspring genotypes in Table 1 for the general model in which we include both direct and maternal effects. Table 1 also gives the average phenotype expected for each of the four ordered offspring genotypes and the average offspring phenotype associated with each of the three unordered maternal genotypes (*i.e.*, the latter are the means of the offspring produced by each of these types of mothers), both calculated as the frequency-weighted average of the cells in a given row or column. Any specific combination of direct and maternal effects at any specific allele frequencies can be examined using the equations in Table 1.

The apparent POEs caused by maternal effects can lead to several different patterns of genotypic values for the ordered genotypes. These patterns can be characterized by the relationship between the additive genotypic value (a_o), dominance genotypic value (d_o), and parent-of-origin genotypic value (i_o). It is important to keep in mind that, for this analysis, these patterns mimic POEs due to genomic imprinting but are in fact caused by maternal effects or a combination of maternal and direct effects. We divide these patterns conceptually into three categories (discussed in R. Hager, J. Cheverud, C. Roseman and J. Wolf, unpublished results), parental expression, polar dominance, and bipolar dominance, reflecting the pattern of genomic imprinting they mimic. In *parental expression* the two genotypes sharing the same allele of identical parent-of-origin (maternal or paternal) have the same phenotype, which results in either a paternal or a maternal expression pattern. For the case of maternal expression we expect the genotypic value to be determined solely by the maternally inherited allele such that $\overline{LL} = \overline{SL}$, $\overline{SS} = \overline{LS}$, and $a_o = -i_o$. This contrasts with paternal expression where we expect the genotypic value to be determined by the paternally inherited allele such that $\overline{LL} = \overline{LS}, \overline{SS} = \overline{SL}$, and $a_o = i_o$. In both cases, we expect $d_o = 0$, thus, $d_o/i_o = 0$ and $a_o/i_o = -1$ or 1 for maternal and paternal expression, respectively. *Polar dominance* refers to the pattern where the phenotype of one of the reciprocal heterozygotes differs from that of the other three ordered genotypes, all of which have the same phenotype (*i.e.*, are not significantly different from each other). In this case, $\overline{LL} = \overline{SS}$ and both of the homozygotes are also equal to either $\overline{LS}$ or $\overline{SL}$. When the divergent heterozygote is larger than the other genotypes the locus shows polar overdominance and when it is smaller it shows polar underdominance (*cf.* the callipyge locus in sheep; Cockett *et al.* 1996; Georges *et al.* 2003). In its canonical form (whether the locus be over- or underdominant), we expect $d_o = i_o$ while $a_o = 0$, thus $d_o/i_o = 1$ and $a_o/i_o = 0$. Finally, a bipolar dominance pattern occurs when the phenotypes of the two reciprocal heterozygotes differ from each other (*i.e.*, $\overline{LS} \neq \overline{SL}$) while the two homozygotes have the same phenotype (*i.e.*, $\overline{LL} = \overline{SS}$). In its canonical form, we expect i_o to be significant and $a_o = d_o = 0$; thus $d_o/i_o = 0$ and $a_o/i_o = 0$.

The case of an additive maternal effect without any direct effect or maternal dominance effect is illustrated in Table 2a, using a hypothetical numerical example for simplicity. The genotypic values are entirely dependent on the additive maternal-effect genotypic value ($a_m = 2$) with all direct effects (a_o, d_o, and i_o) and the dominance maternal genetic effect

TABLE 2

Expected maternal and offspring genotypic values for four scenarios that mimic various patterns of genomic imprinting

	Offspring genotype				
	LL	*SL*	*LS*	*SS*	$\bar{z}_{\text{maternal}}$
a.					
Maternal genotype					
LL	2	2	**2**	**2**	2
LS, SL	0	0	0	0	0
SS	**−2**	**−2**	−2	−2	−2
$\bar{z}_{\text{offspring}}$	1	1	−1	−1	
b.					
Maternal genotype					
LL	0	2	**2**	**4**	1
LS,SL	−2	0	0	2	0
SS	**−4**	**−2**	−2	0	−1
$\bar{z}_{offspring}$	−1	1	−1	1	
c.					
Maternal genotype					
LL	1	2	**2**	**3**	1.5
LS,SL	−1	0	0	1	0
SS	**−3**	**−2**	−2	−1	−1.5
$\bar{z}_{offspring}$	0	1	−1	0	
d.					
Maternal genotype					
LL	1	3	**3**	**3**	2
LS,SL	−1	1	1	1	0.5
SS	**−3**	**−1**	−1	−1	−2
$\bar{z}_{offspring}$	0	2	0	0	

In all cases the locus has no true imprinting effect (*i.e.*, $i_o = 0$). Table format is identical to Table 1. (a) Maternal-effect locus mimicking maternal expression. The pattern is caused by an additive maternal effect ($a_m = 2$) with $p = q = 0.5$. (b) Maternal-effect locus mimicking paternal expression. Here, an additive maternal effect ($a_m = 2$) shows the same magnitude of negative pleiotropic effect as the direct additive effect ($a_o = -2$). (c) Maternal-effect locus mimicking bipolar dominance imprinting that is caused by an additive maternal effect ($a_m = 2$) and a weaker negative pleiotropic additive direct effect ($a_o = -1$). (d) Maternal-effect locus mimicking polar overdominance. Here, the maternal effect ($a_m = 2$) shows a weaker negative pleiotropic than additive effect ($a_o = -1$). In addition, there is a dominance direct effect ($d_o = 1$). In all cases, the allele frequency is set to $p = q = 0.5$. It should be noted that in all cases the allele frequency does not affect the pattern of genotypic values (although it does affect the actual values themselves). Boldface values correspond to maternal–offspring genotype combinations that cannot exist under Mendelian inheritance.

(d_m) being set to zero. In this example both alleles are at equal frequency (*i.e.*, $p = q = 0.5$). Note that even without any direct imprinting effect ($i_o = 0$), there is a difference between reciprocal heterozygote offspring (apparent $i_o = -1$). Also, without any direct additive effect ($a_o = 0$), there is a difference between offspring homozygotes (apparent $a_o = 1$). Since each of the reciprocal heterozygotes resembles the homozygote carrying the same maternal allele (*i.e.*, $\overline{LL} = \overline{SL}$ and $\overline{SS} = \overline{LS}$), this maternal genetic effect mimics maternal expression imprinting ($a_o/i_o = -1$). While this example has equal allele frequencies, the result that additive genotypic maternal effects mimic maternal expression is independent of the allele frequency. This results in an apparent imprinting effect of half the difference between reciprocal heterozygotes, or

$$i_o = -a_m/2 \quad (1)$$

with additive maternal effects alone or

$$i_o = (a_m + d_m(p - q))/2 \quad (2)$$

with additive and dominance maternal effects, showing that dominance maternal effects can also produce a pattern of POEs, the value of which depends on the frequencies of the alleles in the population. Therefore, when performing an analysis using the parent-of-origin of alleles to look for genomic imprinting effects, significant positive results may actually be due to maternal effects.

One way to separate these effects is to restrict the analysis to offspring of heterozygous mothers, since maternal effects (due to either dominance or additive effects) do not contribute to differences between these offspring. This is illustrated in Table 1 where the difference between the reciprocal heterozygotes born of heterozygous mothers is $2i_o$, containing no maternal genetic effect terms. In the example in Table 2a (where $i_o = 0$ and $a_m = 2$), both types of heterozygous offspring of heterozygous mothers have the same expected phenotype (zero) while the two types of heterozygous offspring of homozygous mothers are distinct. Thus, restricting an analysis to the offspring of heterozygous mothers can be used to control the confounding of direct and maternal genetic effects which is an inevitable consequence of Mendelian inheritance.

In addition to mimicking maternal expression, when both maternal effects (a_m and d_m) and direct additive (a_o) and dominance (d_o) effects occur at a locus but imprinting effects are absent, the patterns of ordered genotypes can mimic any other type of genomic imprinting: paternal expression, bipolar dominance, or polar dominance. The conditions under which each of these patterns will appear can be determined using the average phenotypes of the four ordered genotypes, which are given in the last row of Table 1 (labeled $\bar{z}_{\text{offspring}}$). These values are simply the expected mean phenotype for each of the genotypes. A locus will appear to show paternal expression (Table 2b) as the result of an additive maternal effect in combination with an additive direct effect of similar magnitude, but of opposite sign (*i.e.*, $a_m = -a_o$). A similar scenario leads to the appearance of bipolar dominance imprinting (Table 2c), except in this case the magnitude of the direct effect is half the maternal effect ($a_m = -2a_o$). Such scenarios involving a negative relationship between the direct and maternal effects at a locus may be quite common as the appearance of negative genetic correlations (and presumably negative pleiotropy) between direct and maternal effects may be relatively common (see, *e.g.*, Roff 1997, Table 7.5, p. 251). Finally, an apparent polar dominance imprinting pattern (Table 2d) can be caused when a direct dominance effect (d_o) of similar magnitude as the direct additive effect (a_o) cooccurs with the maternal effect. With positive dominance ($d_o > 0$; Table 2d) a pattern mimicking overdominance imprinting will appear while with negative dominance ($d_o < 0$) a pattern mimicking polar underdominance imprinting will appear.

Importantly, Table 1 also illustrates that, just as maternal effects can mimic genomic imprinting effects, the opposite is also true; actual genomic imprinting effects can masquerade as maternal effects if an analysis is focused on maternal effects rather than genomic imprinting. This is due to the fact that *SS* mothers cannot have *SL* heterozygous offspring and that *LL*

TABLE 3

Patterns of genotypic values for the five maternal-effect QTL

	QTL				
	Wtmge5.1	*Wtmge6.1*	*Wtmge12.1*	*Wtmge17.1*	*Wti18.1*
Chromosome	5	6	12	17	18
Location	15.99	74.87	20.69	8.99	8.20
Coordinate	32,651,323	147,333,176	50,976,889	20,436,833	22,236,323
Traits					
wk 1					**Overd (+*i*)**
wk 2				Overd (+*i*)	**Overd (+*i*)**
wk 3			Bipolar (−*i*)	Overd (+*i*)	Overd (+*i*)
wk 4			**Bipolar (–*i*)**		Mat
wk 5	Mat		**Overd (–*i*)**		Bipolar (+*i*)
wk 6	Mat		**Overd (–*i*)**	**Bipolar (+*i*)**	
wk 7			**Overd (–*i*)**		
wk 8			**Overd (–*i*)**		
wk 9			**Overd (–*i*)**		
wk 10			**Overd (–*i*)**		
Growth 1–2		**Mat**			
Growth 1–6	**Mat**		**Overd (–*i*)**		
Growth 3–10			**Overd (–*i*)**		

QTL names are given as *WtmgeX.Y* to indicate that the effects are on weight or weight-related traits (*Wt*) and to show a maternal genetic effect (*mge*), with the first number (*X*) denoting the chromosome and the second (*Y*) denoting the QTL number on that particular chromosome. The chromosome number, location (given as F_2 locations in centimorgans), and genome coordinates (in base pair units, based on mouse genome build 36; http://www.ensembl.org) are given for each QTL. Patterns are listed as follows: Mat, maternal expression; Bipolar, bipolar dominance; and Overd, polar overdominance. In the cases of bipolar and polar overdominance, the sign of *i* is given in parentheses to indicate the observed pattern. Boldface type shows the patterns for which the maternal effect was significant at the chromosomewide level. Non-boldface type shows patterns that were significant at the locus level in a protected test (see MATERIALS AND METHODS). Shown are the 10 weekly weights and three growth traits (*e.g.*, growth 1–2 is weight gain from week 1 to week 2, etc.). Values of effects and exact significance values are given in supplemental Table 1 at http://www.genetics.org/supplemental/.

mothers cannot have *LS* offspring. As a result, a correlation exists between maternal genotype and the parent-of-origin of alleles in their progeny and, therefore, genomic imprinting can lead to a difference between the average phenotypes ($\bar{z}_{\text{maternal}}$) of the offspring of *LL* and *SS* mothers. With all effects set to zero except the direct imprinting effect, the apparent additive maternal effect, a_m, equals ($i_o/2$). Thus, in the absence of maternal genetic effects there would still appear to be a maternal effect caused by genomic imprinting. In the case of actual maternal expression of a locus (where $a_o = -i_o$), the additive maternal-effect genotypic value, which is half the difference between the homozygotes, would appear to have the value $\frac{1}{2}(a_o - i_o)$, which is equivalent to the value of i_o in an analysis of the ordered offspring genotypes. Clearly, just as maternal genetic effects can mimic those expected for genomic imprinting, the opposite is also true.

QTL analysis: *Animal husbandry and phenotypes:* The details of the strains used, breeding design, and general animal husbandry can be found elsewhere (VAUGHN *et al.* 1999; WOLF *et al.* 2002). Briefly, we used the 382 F_2 and 1632 F_3 animals from an intercross between the two inbred mouse strains, large (LG/J) and small (SM/J), which were originally created by selection for either large or small body weight at 60 days of age (CHAI 1956). Mice were weighed weekly from 1 week of age to week 10. From these weekly weights we also created a set of growth variables corresponding to weight change over time (Table 3). These size and growth traits were all corrected for sex differences.

Genotyping and QTL analysis: All F_2 and F_3 individuals were genotyped at 353 SNP loci across all 19 autosomes by Illumina (San Diego). Ordered haplotypes of the F_3 animals were reconstructed using the "block-extension algorithm" option in the program Pedphase (LI and JIANG 2003a,b), which infers haplotype configurations using the pedigree information. This haplotype reconstruction method produces a set of maternally and paternally derived chromosomal haplotypes for all individuals. Therefore, the parental origin of each allele is inferred for all alleles at all loci on a chromosome, regardless of whether the individual is homozygous or heterozygous at a particular locus.

We denote the four ordered genotypes *LL*, *LS*, *SL*, and *SS*, where the first allele refers to the paternally derived allele and the second to the maternally derived copy. Each genotype was assigned an index score for the additive (a_o), dominance (d_o), and parent-of-origin (i_o) genotypic values (where the subscript "o" denotes that these are direct effects of the F_3 "offspring" genotype on its own phenotype—these are contrasted later with the maternal effect of the F_2 mothers' genotypes on their F_3 offsprings' phenotypes) corresponding to

$$\begin{bmatrix} \overline{LL} \\ \overline{LS} \\ \overline{SL} \\ \overline{SS} \end{bmatrix} = \begin{bmatrix} 1 & 1 & 0 & 0 \\ 1 & 0 & 1 & 1 \\ 1 & 0 & 1 & -1 \\ 1 & -1 & 0 & 0 \end{bmatrix} \begin{bmatrix} r_o \\ a_o \\ d_o \\ i_o \end{bmatrix}, \tag{3}$$

where r_o is the "reference point," which, in this model, corresponds to the midpoint between the two homozygotes. QTL were identified using canonical correlation, as implemented in the SAS Cancorr procedure (SAS Institute, Cary, NC), to fit

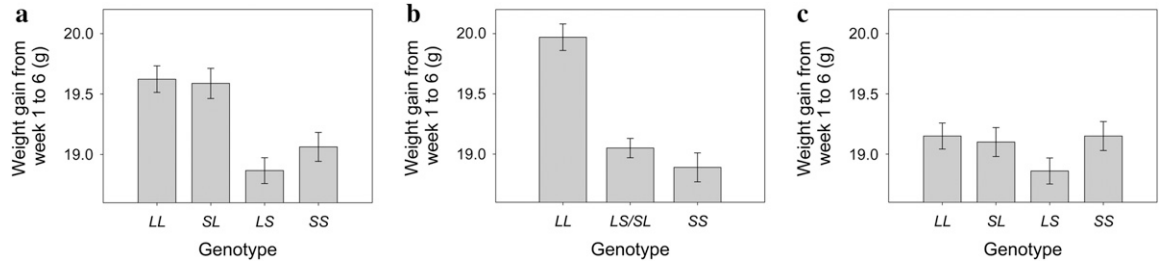

Figure 1.—Illustration of the direct effect, maternal effect, and overall genotypic values for the locus *Wtmge5.1*, which affects growth between weeks 1 and 6. (a) The overall genotypic values, calculated as the average phenotype of each of the four ordered genotypes, are shown with standard error bars. This pattern matches what would be expected for maternal expression, where the genotypes sharing their maternally derived allele have similar average phenotypes. (b) The average phenotypes of the offspring of the three unordered maternal genotypes. This pattern shows that offspring of *LL* mothers grow more than those of the other two types of mothers, both of which show similar average growth. (c) The genotypic values of the ordered genotypes are shown for offspring of heterozygous mothers in which any differences between phenotypes are not caused by maternal genetic effects because heterozygous mothers do not differ in these effects. In the absence of the maternal effect, the four ordered genotypes all show similar average growth, showing that the appearance of maternal expression was due to a maternal effect, not genomic imprinting.

a model with the additive, dominance, and parent-of-origin genotypic index scores as orthogonal predictors and the traits in question as response variables. Details of the use of canonical correlation for QTL analysis are given in Leamy *et al.* (1999) and Wolf *et al.* (2005). Using this model, we scanned the genome to generate a distribution of probabilities for the parent-of-origin (i_o) effect. These probabilities were transformed to a logarithmic probability ratio (LPR) comparable to the LOD scores typically seen in QTL analyses (LPR = $-\log_{10}$[probability]).

Because both genomic imprinting and maternal effects can result in the appearance of POEs in an analysis (*i.e.*, a difference between *LS* and *SL* heterozygotes), loci identified from the genome scan for significant POEs may be due to either phenomenon, and the causal origin of the apparent POE cannot be distinguished from the genome scan results alone. Two strategies can be used to separate these effects: (1) include genotype scores for both maternal genetic effects and imprinting effects in the model jointly and obtain the partial regression coefficient for each, holding the other constant or (2) restrict the sample to offspring of heterozygous mothers as there is no maternal genetic effect variation among these offspring. Here, we have chosen the second strategy, which is more powerful because it predicts that when a locus shows an apparent POE due to a maternal effect, the POE disappears when the analysis is limited to offspring from heterozygous mothers. It should also be pointed out that dominance maternal effects (d_m) are not confounded with imprinting in our population as it has approximately equal frequencies for both alleles at all loci.

Due to the family structure of the F_3 population we could not use standard resampling techniques to generate significance thresholds. Therefore, we used results from simulated F_3 populations that maintained the family structure while randomizing sets of genotypes. Following Chen and Storey (2006) we generated chromosomewide significance thresholds which were shown to yield overall the best results by increasing the discovery of true positives while at the same time reducing problems using the false discovery rate in genetic mapping studies.

RESULTS

In the genomewide scan for QTL affecting body weight and growth traits we detected a total of five loci located on chromosomes 5, 6, 12, 17, and 18 that show an apparent parent-of-origin-dependent effect due to maternal genetic effects. These loci displayed a diversity of patterns that resembled maternal expression, polar, and bipolar dominance imprinting (Table 3). The conclusion that the detected POEs were due to maternal effects rather than genomic imprinting was confirmed using the analysis restricted to offspring of heterozygous mothers (where the locus showed no POE in this group of animals, which includes ~800 individuals). A model including both direct and maternal additive and dominance effects further confirmed the existence of a maternal effect at these loci (supplemental Table 1 at http://www.genetics.org/supplemental/). QTL are named as *WtmgeX.Y* to indicate that the effects are on weight traits (*Wt*) and show a maternal genetic effect (*mge*). The first number (*X*) denotes the chromosome and the second (*Y*) denotes the QTL number on that particular chromosome (however, no chromosome has more than one QTL, but we keep this naming convention to match that used in our characterization of imprinted loci; R. Hager, C. Roseman, M. Cheverud and J. Wolf, unpublished results).

The loci *Wtmge5.1* and *Wtmge6.1* show a pattern that mimicked maternal expression such that the maternal effect results in a significant difference between genotypes differing in their maternally but not paternally derived alleles. An example of such apparent maternal expression is illustrated using the genotypic values of *Wtmge5.1* for growth between weeks 1 and 6 in Figure 1. We also detected two loci (*Wtmge12.1* and *Wtmge18.1*) whose effects caused an apparent polar dominance imprinting pattern in that only one of the two heterozygotes is significantly different from the other genotypes. *Wtmge12.1* showed polar overdominance of the *SL* genotype (*i* negative) for two growth traits and weight between weeks 5 and 10 while *Wtmge18.1* displayed polar overdominance of the *LS* genotype (*i* positive) for weeks 1–3 weight caused by significant maternal additive

genetic effects (supplemental Table 1). Polar overdominance was also found for weeks 2 and 3 body weight at *Wtmge17.1* due to maternal effects. This locus, however, displayed a bipolar pattern at week 6. This change in pattern at a given locus depending on the trait was also found for *Wtmge12.1*, which changed from a bipolar pattern at weeks 3 and 4 to polar overdominance at later ages and is evinced by an increase in the negative *d/i* ratio (see supplemental Table 1). The QTL on chromosome 18 (*Wtmge18.1*) changed its predominant pattern of polar overdominance to maternal expression at week 4 and to a bipolar pattern at week 5. Generally, these pattern changes reflect the quantitative nature of maternal effects and can be seen in the changing ratios of the additive (*a*) or dominance (*d*) effect in relation to the apparent parent-of-origin effect (*i*) (supplemental Table 1).

DISCUSSION

The key result of this article is that genomic imprinting and maternal genetic effects can both generate the same phenotypic patterns that appear as parent-of-origin-dependent effects on offspring traits. However, in the case of maternal-effect loci, these are only apparent parent-of-origin-dependent effects since the pattern is not *caused* by parent-of-origin-dependent gene expression. This is evinced by the results of our QTL analysis of complex traits in mice detecting five QTL with maternal effects that mimic imprinting patterns. These phenotypic patterns mimic not only the traditional imprinting pattern of maternal expression, but also more complex patterns such as bipolar and polar dominance imprinting. Furthermore, we found that such complex patterns can be caused by a combination of the direct additive and dominance effects of the offspring genotype and the indirect effects of maternal genotypes. Our findings suggest that prior genomewide mapping studies using differences between reciprocal heterozygotes to detect imprinting may have confounded imprinting effects with maternal effects. Similarly, the confounding may also have occurred in previous work on maternal effects such that reported genetic maternal effects may have been caused by genomic imprinting.

The importance of parent-of-origin-dependent effects in affecting complex trait variation is reflected in an increasing number of association and linkage mapping studies that include POEs in their analysis of complex traits (Dong *et al.* 2005; Mantey *et al.* 2005). The distinction between the causes of apparent parent-of-origin-dependent effects is crucial not only for studies aiming to ultimately locate underlying genes but also for our understanding of evolutionary dynamics. In particular, if the purpose of the research is to identify potential candidate regions in the genome that may be causal to a specific phenotype, *i.e.*, to explore the genetic basis of a given trait, mistaking a maternal for an imprinting effect might lead to an inappropriate focus in follow-up studies. For example, if a trait is clearly identified as being affected by imprinting one can attempt to isolate the gene by genomewide screens using differences in methylation status or other appropriate molecular methods, such as gene expression studies, to locate imprinted genes (see *e.g.*, Kaneko-Ishino *et al.* 1999; Luedi *et al.* 2005). However, if the trait is caused by a maternal effect, research should be focused on genes expressed by the mother that affect a focal trait in her offspring.

Among the more complex maternal-effect patterns we found three loci showing a pattern that mimicked polar dominance. This pattern is similar to the polar overdominance phenotype described for sheep caused by a mutation at the callipyge (*CLPG*) locus where the phenotype of one of the four possible genotypes is different from all others (Cockett *et al.* 1996; Georges *et al.* 2003). Interestingly, the *CLPG* mutation is caused by a single nucleotide substitution within an imprinted domain affecting several imprinted genes (Georges *et al.* 2003). Our results show that a complex phenotypic pattern such as polar overdominance can also be caused by maternal effects. In addition to the diversity of maternal-effect phenotypic patterns, we also found that maternal effects affected traits at different stages in development from as early as week 1 body weight to as late as week 10. As can be seen in Table 3, several maternal-effect loci affected both weekly weights as well as growth traits and persisted in time for up to 7 weeks (*Wtmge12.1*). Surprisingly, one QTL (*Wtmge6.1*) affected early growth from week 1 to 2 only but not weekly weights. During this early period offspring body weight increase is solely due to maternal provisioning and the conversion into body mass by the offspring's metabolism. Since this QTL seems to particularly affect the rate of weight gain it may be linked to maternal provisioning behavior.

Researchers are becoming increasingly aware that predictions of phenotypes as a direct function of genotypes are often simplified or even flawed because the trait in question is the result of interaction or joint direct and indirect effects (*e.g.*, Reifsnyder *et al.* 2005; Hager and Johnstone 2007). Our study has shown that both maternal genetic and direct genetic effects result in a specific phenotype that is different from the effects of the focal genotype alone and that particular consideration should be given to the distinction between genomic imprinting effects and maternal effects in future studies aiming to analyze either of the two effects.

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