The genetics of maternal care: Direct and indirect genetic effects on phenotype in the dung beetle *Onthophagus taurus*

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While theoretical models of the evolution of parental care are based on the assumption of underlying genetic variance, surprisingly few quantitative genetic studies of this life-history trait exist. Estimation of the degree of genetic variance in parental care is important because it can be a significant source of maternal effects, which, if genetically based, represent indirect genetic effects. A major prediction of indirect genetic effect theory is that traits without heritable variation can evolve because of the heritable environmental variation that indirect genetic effects provide. In the dung beetle, *Onthophagus taurus*, females provide care to offspring by provisioning a brood mass. The size of the brood mass has pronounced effects on offspring phenotype. Using a half-sib breeding design we show that the weight of the brood mass females produce exhibits significant levels of additive genetic variance due to sires. However, variance caused by dams is considerably larger, demonstrating that maternal effects are also important. Body size exhibited low additive genetic variance. However, body size exerts a strong maternal influence on the weight of brood masses produced, accounting for 22% of the nongenetic variance in offspring body size. Maternal body size also influenced the number of offspring produced but there was no genetic variance for this trait. Offspring body size and brood mass weight exhibited positive genetic and phenotypic correlations. We conclude that both indirect genetic effects, via maternal care, and nongenetic maternal effects, via female size, play important roles in the evolution of phenotype in this species.

The evolution of parental care has been the subject of intense study (1, 2). Most empirical research has focused on the evaluation of costs of care to parents, the benefits accrued by offspring, and the inevitable conflict between parents and their offspring over how much care should be provided (1). Surprisingly little attention has been paid to the underlying genetic variation in parental care that is required for its evolution and assumed by theoretical treatments of the subject (3). Although studies of lactation in agricultural animals (4–6) and nesting behavior in laboratory mice (7, 8) show that these aspects of maternal care can respond to artificial selection, few studies have examined the quantitative genetics of parental care from an evolutionary perspective (9, 10).

The amount of genetic variation for parental care has important evolutionary implications. In many organisms, the environment provided by parents determines the environmental conditions experienced by progeny, thus altering the traditional genotype-phenotype relationship (11–14). When there is variation in the quality of the environment being provided by parents in the form of parental care, and this variation reflects genetic differences among parents, indirect genetic effects can exist (11, 15). Thus, environmental effects derived from parental variation should be viewed as ''inherited environments'' because, while they constitute environmental effects in the offspring generation, the phenotypes in the parental generation producing these environmental effects could be heritable (11, 16). Both theoretical models (16–19) and empirical studies (20–24) have suggested that indirect genetic effects can have far-reaching evolutionary consequences (11).

Most research on indirect genetic effects has focused on the effects of the environment provided by the mother to her offspring, collectively referred to as maternal effects (25). Maternal care can thus represent a major cause of maternal effects (1, 12). The mechanisms through which maternal effects are transmitted across generations are highly variable across taxa and among traits within taxa (12–14, 26–29). One important life-history trait known to facilitate the transfer of maternal effects across generations is propagule size (28, 30). This transfer arises because propagule size is simultaneously both a maternal and offspring trait; propagules are produced by the mother but also provide the initial resources that determine offspring size (31). In a number of animal species, propagule size covaries positively with maternal size (see references in refs. 30, 32, and 33) and this variation in propagule size often has pronounced effects on the adult fitness of offspring (34–38). As such, maternal variation in both body size and propagule size can be transmitted across multiple generations (24, 25). Despite the importance of maternal effects to offspring fitness, few studies have quantitatively assessed levels of genetic variation in maternal effects (37, 39–41) and thus their potential to respond to natural selection and their ability to act as indirect genetic effects remains largely unknown.

Dung beetles belonging to the genus *Onthophagus* provision offspring before hatching (42). During reproduction, females remove portions of dung and pack them into the blind end of tunnels excavated beneath the dung pad. A single egg is deposited into an egg chamber, which is then sealed; one egg and its associated dung provision constitutes a brood mass and represents the entire resource base that is available to a larvae during development (42). In previous studies of *Onthophagus taurus* we have shown that larger females construct heavier brood masses (43) and that brood mass weight is a major determinant of offspring size (43, 44). Moreover, offspring size is directly related to reproductive success in males (45) and survival and fecundity in females (46). As such, maternal effects have large effects on offspring phenotype in *O. taurus* (43) that are expected to persist through adult life to influence offspring fitness. In this article, we use a half-sib breeding design to directly quantify the relative contributions of genetic and nongenetic maternal effects to variation in the body size and levels of maternal care provided by daughters.

Materials and Methods

Source and Maintenance of Parents. *O. taurus* were collected from cattle pastures in Margaret River, Western Australia by using

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baited pitfall traps (see ref. 47). Beetles were maintained in the laboratory at 25°C for 2 weeks with constant access to cow dung. Four hundred females were randomly selected from this population and established in independent breeding chambers (PVC piping 25 cm in length × 6 cm in diameter), three-quarters filled with moist sand and 250 ml of cow dung, and maintained for 1 week. Chambers were sieved and brood masses were collected. Brood masses were buried in moist sand in individual plastic containers (9 cm × 9 cm × 5 cm) and maintained at 25°C until emergence. On emergence, beetles were sexed and maintained for 2 weeks in single-sex populations with constant access to cow dung. The pronotum widths of all beetles were measured by using digital calipers.

Breeding Design. We mated each of 20 sires to 10 randomly selected virgin females. Each sire was placed with his dams into an individual container (30 cm × 30 cm × 10 cm), three-quarters filled with moist sand, provided with 250 ml of cow dung, and left for 1 week to mate. Mated dams were established individually in breeding chambers supplied with 250 ml of homogenized cow dung and maintained for 1 week. Dung was homogenized with a cement mixer to reduce any differences in maternal care and/or offspring size that might arise because of differences in dung quality. Chambers were sieved and brood masses were collected. Excess sand was removed from brood masses with a dissecting probe and each brood mass was individually weighed to the nearest 0.01 mg with an electronic balance. Each brood mass was buried in an independent container and maintained under identical conditions to the parents until emergence.

On emergence, six daughters per dam were randomly selected and their pronotum widths were measured. Daughters were provided with fresh sand and 50 ml of cow dung and maintained in their original containers for 1 week. Each daughter was then paired with a randomly selected virgin male, provided with fresh sand and cow dung, and maintained for another week to ensure all daughters were mated. The virgin males were bred from 400 field females collected from the same Margaret River population as parentals and established and reared concurrently with experimental families. The body sizes of males were measured before mating to control for any effects that mate size may have on brood mass weight.

After mating, each daughter was established in an individual breeding chamber, supplied with 250 ml of homogenized dung, and maintained for 2 weeks. Chambers were sieved and brood masses were collected. Excess sand was removed from brood masses, and they were dried to a constant weight at 60°C. Dry weights were measured to reduce variance in brood mass weight caused by differences in soil and/or dung moisture. After drying, any remaining sand was removed and the total weight and number of brood masses produced by each daughter were recorded. Mean brood mass weight was calculated by dividing the total weight of brood masses by the number of brood masses produced.

Genetic Analyses. Our nested breeding design yielded data for both full-sib (dams nested within sires) and half-sib (among sires) families, permitting the estimation of the causal components of variance and covariance that are required for the calculation of heritability and genetic correlations (48). Some dams and female offspring failed to produced brood masses. Unequal numbers of offspring and dams per sire were accounted for by calculating values of $k_1 = 3.883$ offspring/dam, $k_2 = 4.499$ dams/sire, and $k_3 = 34.250$ offspring/sire, after Becker (49). Standard errors for the half-sib heritabilities were calculated as the variance of the variance components for an unbalanced breeding design (49). As we measured the pronotum widths of both parents and the level of care provided by mothers and daughters, we could also estimate heritability by using parent-offspring regression (48). To estimate the heritability of brood mass weight from a mother-daughter regression, we measured the relationship between wet and dry brood mass weight for 120 randomly selected brood masses (dry weight = 0.402 wet weight + 0.029, $r^2 = 0.93$, $F_{1,119} = 1461.30$, $P = 0.0001$) and used this to convert the wet brood mass weight of dams to a dry weight. Heritabilities and their associated standard errors were estimated by the method of intra-sire regression (48).

Results

In total, 994 daughters were reared from 176 full-sib families (20 sires and 176 dams). Of these, pronotum width and brood mass weight data were collected for 696 daughters. Overall egg-to-adult survivorship was high in the experiment (85.39%), thus reducing the likelihood of inadvertent selection biases. There was no evidence that sires indirectly influenced the size of the brood masses produced by dams ($F_{19,695} = 1.29$, $P = 0.20$). Similarly, the size of a daughter's mate (included as a covariate) did not influence the size of the brood mass she produced ($F_{1,694} = 0.19$, $P = 0.66$). Thus mate size was excluded from all further analyses.

Significant sire effects were detected for brood mass weight but not for pronotum width (Table 1). In contrast there were significant dam effects for both traits. Accordingly, the estimate of narrow sense heritability (caused by sires) was significant only for brood mass weight whereas the broad sense heritabilities (caused by dams, which includes genetic and maternal effects) were significant for both pronotum width and brood mass weight (Table 1). In general, there were large asymmetries in variances caused by sires and dams; the broad sense heritabilities were considerably greater than the narrow sense heritabilities (Table 1).

Asymmetries in genetic variance components between sires and dams can arise when estimates caused by dams are inflated by maternal effects (48). If we assume that dominance and epistasis are negligible, the extent to which dam variances exceed sire variances should largely reflect nongenetic maternal effects because, apart from the brood mass provided by the mother, all offspring were reared individually and thus experienced environmental conditions that were unique to each individual. Where maternal effects have been measured, they are typically much greater than dominance or epistatic effects, making this assumption reasonable (14). The causal components of variance in Table 2 suggest that 22% of the variance in daughters' pronotum width was caused by a common environmental factor, which in our case can represent only the maternal effect. In contrast, the maternal effect on daughters' brood mass weight was only 5%.

There were significant phenotypic correlations between brood mass weight and pronotum width within and between generations. The weight of brood masses produced by a female depended on her body size for both parental ($r = 0.211$, $df = 172$, $P = 0.005$) and offspring generations (mean of offspring/dam, $r = 0.400$, $df = 172$, $P < 0.001$), and offspring body size itself depended on the size of brood mass provided by mothers (mean of offspring/dam, $r = 0.631$, $df = 172$, $P < 0.001$). Thus, female body size, which had low underlying additive genetic variance (Tables 1 and 2), appears to exert a strong maternal effect on the size of offspring by the weight of brood masses produced.

There was a significant broad sense heritability (dam effect) but no narrow sense heritability (sire effect) on the number of brood masses produced (Table 1). There was a phenotypic relationship between the number of brood masses produced and female size ($\beta = 3.36 \pm 0.98$, $F_{1,694} = 11.85$, $P = 0.0006$). However, quantitative genetic analysis suggests that this is a strictly nongenetic maternal effect (Tables 1 and 2).

Nested analysis of covariance returned positive covariances between brood mass weight and pronotum width for both sires and dams (Table 3). The genetic correlation caused by sires was

Table 1. Nested ANOVA, observational components of variance, and heritability estimates for daughters' pronotum width, brood mass weight, and brood mass number in *O. taurus*

Source	*df*	MS	*F* ratio	Variance	% of total	Sib $h^2 \pm$ SE	Parent-offspring $h^2 \pm$ SE
Pronotum width							
Sire	19	0.14629	1.24	1.565×10^{-4}	0.20	0.008 ± 0.070	-0.020 ± 0.069
Dam[Sire]	154	0.13015	2.09***	1.750×10^{-2}	21.91	0.876 ± 0.195	$0.279 \pm 0.186^{\dagger}$
Progeny	522	0.06221		6.221×10^{-2}	77.89		
Total	695	0.07987		7.987×10^{-2}	100.00		
Brood mass weight							
Sire	19	0.11693	2.10**	1.632×10^{-3}	3.36	0.134 ± 0.091	
Dam[Sire]	154	0.05854	1.37**	4.034×10^{-3}	8.31	0.332 ± 0.165	0.219 ± 0.111*
Progeny	522	0.04288		4.288×10^{-2}	88.33		
Total	695	0.04863		4.854×10^{-2}	100.00		
Brood mass number							
Sire	19	46.8207	0.69	−0.872	−1.65	-0.066 ± 0.037	
Dam[Sire]	154	72.6513	1.54**	6.541	12.36	0.494 ± 0.044	0.014 ± 0.165
Progeny	522	47.2514		47.251	89.29		
Total	695	53.6843		52.921	100.00		

To account for unequal sample sizes of offspring per sire, the error term for sires was calculated by using Satterthwaite's approximation: 0.8238 MS(Dam[Sire]) + 0.1762 MS(Error). Hypothesis testing of sib heritabilities based on $MS_S/MS_{D[S]}$, as recommended by Lynch and Walsh (50). The parent-offspring heritability caused by sires were calculated from linear regression by using midoffspring values calculated across the dams of each sire ($df = 19$). The parent-offspring heritability caused by dams was calculated by intrasire regression (48). Significance testing was based on Student's *t*. ***, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$; †, $P = 0.075$.

outside the theoretical limit, as expected because of the low heritabilities (50). Moreover, standard errors were characteristically high (47).

Discussion

Traditionally, the common environment provided by mothers has been viewed as a troublesome source of environmental resemblance to overcome in quantitative genetic experiments (48). Our results demonstrate that although body size in *O. taurus* exhibits low additive genetic variance, maternal effects greatly increase the degree of phenotypic resemblance between mothers and daughters. Moreover both nongenetic maternal effects (the influence of female size on brood mass weight) and indirect genetic effects caused by additive genetic variance in brood mass weight contribute to variation in offspring phenotype (Table 1). Therefore, although previous studies have concluded that body size is not heritable (51), the conditions necessary for cross-generational transmittance of body size do exist in *O. taurus* (11). Our study provides empirical evidence demonstrating that indirect genetic effects can promote evolutionary change in traits that exhibit low additive genetic variance.

Both theoretical models (16–19) and empirical studies (20–24) suggest that maternal effects may impede or accelerate responses to selection and can generate large time lags in evolutionary responses to selection, even after selection has been relaxed (17, 19). In *O. taurus*, the presence of a genetically based maternal effect coupled with a positive phenotypic correlation between pronotum width and brood mass weight is thus likely to facilitate the evolution of body size across generations. There should be a selective advantage for increased body size, given the positive relationship between female size and brood mass number found in this study, and the effect of body size on male reproductive success (45). In general, maternal effects may represent an important source of phenotypic variation. This is particularly true for species like *O. taurus* that provide substantial levels of parental care to their offspring (24, 41, 52, 53).

We have identified one source of maternal effect in our study, the amount of provisions provided by the mother in the brood mass. Other maternal effects also may contribute to the common environmental variation. One possibility is the transmission of maternal effects through egg size. Maternal size is often positively related to egg size in animals (see reviews by refs. 30, 32, and 33). In the seed beetle (*Collosobruchus maculatus*) offspring phenotype is mediated through two discrete maternal effects: a mother's egg-laying decisions and the size of the egg she produces (24). The number of eggs laid on a seed is inversely related to offspring size although the effects of larval competition and the positive phenotypic correlation between maternal size and egg size (37) means that these smaller progeny will produce smaller eggs and offspring maturing at a smaller body size (24). Selection experiments show that these maternal effects result in the rapid divergence of offspring body size across

Table 2. Causal components of variance and coefficients of variation (52) in daughters' pronotum width, brood mass weight, and brood mass number

	Pronotum width			Brood mass weight			Brood mass number		
	Variance	%	CV	Variance	%	CV	Variance*	%	CV
V_A	6.26×10^{-4}	0.78	0.50	6.53×10^{-3}	13.45	6.30	0	0	0
V_{Ec}	1.73×10^{-2}	21.72	2.65	2.40×10^{-3}	4.95	3.82	6.54	12.16	14.99
V_{Ew}	6.19×10^{-2}	77.50	5.01	3.96×10^{-2}	81.60	15.51	47.25	87.84	40.29
V_P	7.99×10^{-2}	100.00	5.69	4.85×10^{-2}	100.00	17.16	53.79	100.00	42.99

V_A, additive genetic variation; V_{Ec}, variation caused by common environment; V_{Ew}, residual environmental variation; V_P, total phenotypic variation; calculated assuming zero dominance and epistasis after Falconer and Mackay (47). CV, coefficient of additive genetic variance, calculated according to Houle (63).
*Negative variance set to zero.

Table 3. Genetic covariances (genetic correlations ± SE) between daughters' pronotum width, brood mass weight, and brood mass number between traits

	Pronotum width	Brood mass weight	Brood mass number
Pronotum width		1.31×10^{-3}	9.69×10^{-3}
		(2.579 ± 9.740)	n/a
Brood mass weight	3.77×10^{-3}		12.30×10^{-3}
	(0.155 ± 0.229)		n/a
Brood mass number	15.46×10^{-3}	29.15×10^{-3}	
	(-0.029 ± 0.099)	(-0.076 ± 0.148)	

n/a, Genetic correlations can not be calculated because of negative variance caused by sires (Table 1). Sire (narrow sense) estimates above the diagonal, dam (broad sense) estimates below the diagonal.

generations that persist even when selection has been relaxed (24). A second possibility is the influence of maternal effects on egg quality. In insects, yolk proteins present in the egg provide the primary source of energy for the offspring before feeding is initiated (54) and may vary according to maternal diet (55, 56). Unfortunately, little is known about the influences of egg size and/or quality on offspring phenotype in *O. taurus* and further experiments should focus on these potential maternal effects.

More generally, our results provide quantitative genetic analysis of maternal care and its influence on offspring phenotype. A recent study of burrower bugs, *Sehirus cinctus*, used a cross-fostering design that implied genetic variation for maternal provisioning rates that were negatively correlated with variation in offspring solicitation (10). Two studies have examined the inheritance of paternal care by using father-son regressions (57, 58). In both species, nongenetic paternal effects mediated through paternally derived nutritional effects (56) or the cultural transmission of feeding rates (58) cannot be excluded as a source of phenotypic resemblance between fathers and their sons. Finally, Savalli and Fox's study (59) suggested that genetic variance in ejaculate size (which contributes nutritionally to eggs produced by the female) may be linked to the X chromosome because there was no variation caused by sires in their half-sib analyses. Nevertheless, their result is equally consistent with nongenetic maternal effects. Moreover, both Savalli and Fox's (59) and Sakaluk and Smith's (57) studies focused on a form of parental investment, ejaculate traits, that are primarily subject to selection by sperm competition with the nutritional value of the ejaculate representing a secondary function (60). Although paternal effects undoubtedly exist in *O. taurus*, with major males known to assist females during the provisioning of a brood mass (43, 61), such effects were excluded in our experimental design. Our quantitative genetic analysis demonstrates that maternal care can respond to selection, a finding that has widespread implications for studies of parental care (see ref. 33).

Our observed heritability for maternal care was markedly lower than previous estimates for behavioral traits, which average around 0.30 (reviewed in ref. 62). The finding that coefficients of additive genetic variance were greater for brood mass weight than pronotum width are in agreement with Houle's notion (63) that life history traits, on average, have higher additive genetic variances than morphological traits. Higher additive genetic variances for fitness traits are expected if a greater number of genetic and/or environmental effects influence the expression of these traits (63). Indeed, maternal care in onthophagines has been shown to vary with numerous environmental factors, including dung quality (64) and soil moisture (65). This finding suggests that considerable plasticity still remains in the expression of maternal care in this species, a fact reflected in the large residual variation for brood mass weight found in our study.

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