

The mother-in-law effect

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Individuals often derive considerable evolutionary benefit from manipulating others. In the majority of cases, manipulation involves direct interactions between individuals. In the dung beetle, *Onthophagus taurus*, females mated with large males provide more resources to their offspring. Here, we demonstrate, however, that this may result in manipulation that extends across generations: the care that a mother provides to a developing son influences the parental effort of his mate (the mother's daughter-in-law (DIL)). Maternal care associated with constructing heavier brood masses has previously been shown substantially to influence offspring size, male mating success and female survival and fecundity in this species. The mother-in-law effect that we document here is, however, the ability to produce large sons from relatively lighter brood masses. Our results demonstrate not only that females are able to manipulate the parental effort of DILs that they do not directly encounter, but that provisioning relatively lighter brood masses may have evolutionary benefits that trade off against the considerable benefits of producing heavy brood masses.

Keywords: manipulation; mother-in-law effect; parental care; maternal effect; dung beetle; *Onthophagus taurus*

1. INTRODUCTION

Individuals often derive considerable evolutionary advantages from manipulating the behaviour of others (Trivers 1974). Such manipulation usually involves direct interactions between individuals, as is the case in sexual conflict (e.g. Holland & Rice 1999; Chippindale *et al.* 2001) and parent-offspring conflict (e.g. Kilner *et al.* 1999; Agrawal *et al.* 2001). However, in systems where maternal effects are large and are transmitted across multiple generations, the potential exists for individuals to manipulate others without direct interaction.

In Onthophagine dung beetles, females pack portions of dung into the blind end of tunnels that they have excavated beneath a dung pad, and then lay a single egg into an egg chamber and seal it. This egg and dung provision, collectively termed a brood mass, contains all the resources available to a larva during its development. In *Onthophagus taurus*, the weight of the brood mass is a major determinant of offspring size (Hunt & Simmons 1997, 2000, 2002), which is, in turn, directly related to reproductive success in males (Hunt & Simmons 2001) and survival and fecundity in females (Hunt *et al.* 2002). Consequently, maternal effects have far-reaching evol-

utionary implications in this species (Hunt & Simmons 2002).

Recently, Kotiaho *et al.* (2003) showed that females mated to large major males construct significantly heavier brood masses, construct more of them and survive for longer than females mated to small males. Here, we randomly assigned mates to 740 sons, reared in a full-sib/half-sib breeding design, to test whether mothers exploit this male size effect to manipulate indirectly the parental effort of their daughter-in-law (DIL).

2. MATERIAL AND METHODS

(a) Breeding design

We collected *O. taurus* from cattle pastures at Margaret River, in southwestern Western Australia, using baited pitfall traps. Four hundred randomly selected females were established in independent breeding chambers (PVC piping 25 cm in length and 6 cm in diameter). Their progeny were used as the parental stock for this genetic experiment.

We placed 20 sires with 10 randomly selected virgin females in a plastic container (30 cm × 30 cm × 10 cm) for one week to mate. Each mated dam was then placed in an independent breeding chamber with 250 ml of homogenized cow dung. After one week, chambers were sieved and brood masses collected. Excess sand was removed using a dissecting probe. Each brood mass was individually weighed to the nearest 0.01 mg with an electronic balance and buried in moist sand in an independent container (9 cm × 9 cm × 5 cm).

On emergence, a total of six sons per dam were randomly drawn and their pronotum widths and a horn lengths were measured. Pronotum width was measured using digital calipers and horn length using an eyepiece graticule in a dissecting microscope. Each offspring was maintained for one week in its original container until mature and then paired for one week with a randomly assigned virgin female. These virgin mates were F₁ progeny bred from 400 field-collected females from the same Margaret River population, reared concurrently with experimental families. The pronotum widths of virgin females were measured prior to mating so that the effects of mate size on the weight and number of brood masses produced could be accounted for.

After mating, each son's mate was established in an independent breeding chamber with 250 ml of homogenized dung, and maintained for two weeks. Brood masses were collected, excess sand removed and brood masses 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 son's mate were recorded.

We obtained the mean weight and number of brood masses produced for the mates of 740 sons. This is only 62% of the number expected from the breeding design (20 sires × 10 dams/sire × 6 sons/dam × 1 DIL/son = 1200 DILs) due to random cases of dam mortality, dams that failed to produce six sons and DILs that failed to produce brood masses. The frequency of DILs failing to produce brood masses was independent of sire (d.f. = 19, $\chi^2 = 0.03$, $p = 1.00$) and of dam within sire (d.f. = 153, $\chi^2 = 47.36$, $p = 1.00$).

(b) Genetic analysis

We estimated heritabilities and genetic/maternal correlations using restricted maximum likelihood (REML) in SPSS v. 10.0 and AS REML (see <http://www.vsn-intl.com/ASREML/>), respectively. All genetic analyses were performed with and without fitting maternal brood mass weight (i.e. the brood mass constructed by the dam that forms the environment for the developing son) as a covariate. Heritabilities of all traits and genetic/maternal correlations among son/DIL traits were estimated by fitting a standard nested model. The genetic/maternal correlations reported here are the correlations attributable to covariation among dams, and therefore contain additive genetic, maternal and some epistatic and dominance components (Lynch & Walsh 1998).

3. RESULTS

The number and weight of brood masses constructed by a DIL were positively correlated with her body size (brood mass number: $r = 0.16$, $p = 0.0001$, $n = 740$; brood mass weight: $r = 0.47$, $p = 0.0001$, $n = 740$). However, controlling for body size using multiple regression, the weight of brood masses constructed by a DIL was not

Table 1. Multiple regression analysis of the phenotypic effects of DIL body size, son's body size and the brood mass in which a son develops on the weight and number of brood masses that a DIL produces.

(The multiple regression is calculated using each son/DIL pair as an independent data point. Therefore, the degrees of freedom in each analysis is 3736. β , beta regression coefficient (\pm s.e.); t , test statistic; p , probability; VIF, variance inflation factor. Collinearities among predictor variables are well within the acceptable range (Quinn & Keough 2002).)

source	brood mass weight			brood mass number			collinearity statistics	
	β	t	p	β	t	p	tolerance	VIF
DIL's pronotum width	0.47 \pm 0.03	14.57	0.00	0.16 \pm 0.04	4.47	0.00	1.00	1.00
son's pronotum width	0.08 \pm 0.04	2.04	0.04	0.10 \pm 0.04	2.29	0.02	0.76	1.31
mother's brood mass weight	-0.04 \pm 0.04	-0.93	0.35	-0.09 \pm 0.04	2.14	0.03	0.76	1.32
r_{adj}^2	0.22	—	—	0.03	—	—	—	—
overall p	0.00	—	—	0.00	—	—	—	—

Table 2. Heritabilities and among-dam correlations between traits.

(The standard errors for heritability estimates and among-dam correlations are provided in normal text. Heritability estimates and genetic correlations in bold do not include a mother's brood mass weight as a covariate in their calculation, whereas values in normal text contain the covariate. h^2 , heritability; * significant at $p < 0.05$ level.)

source	correlation attributable to dam					
	heritability		sons		DIL	
	sire h^2	dam h^2	pronotum width	horn length	brood mass number	brood mass weight
sons						
pronotum width	0	1.32 \pm 0.20*	—	—	—	—
	0.15 \pm 0.10	0.77 \pm 0.16*	—	—	—	—
horn length	0	1.05 \pm 0.18*	0.91 \pm 0.23*	—	—	—
	0.06 \pm 0.07	0.72 \pm 0.15*	0.99 \pm 0.22*	—	—	—
DIL						
brood mass number	0	0.39 \pm 0.14*	0.54 \pm 0.21*	0.76 \pm 0.22*	—	—
	0	0.38 \pm 0.14*	0.56 \pm 0.19*	0.78 \pm 0.17*	—	—
brood mass weight	0	0.19 \pm 0.12	0.72 \pm 0.38	0.78 \pm 0.36*	0.59 \pm 0.47	—
	0	0.19 \pm 0.12	0.71 \pm 0.33*	0.81 \pm 0.32*	0.71 \pm 0.35*	—

influenced by brood mass number (brood mass number: $\beta = -0.001 \pm 0.001$, $t = 0.62$, $p = 0.54$; DIL body size: $\beta = 0.341 \pm 0.025$, $t = 13.86$, $p = 0.0001$).

DIL reproductive effort was not significantly correlated in univariate analyses with son's body size or with the weight of the brood mass in which he developed (son's body size: DIL brood mass weight, $r = 0.060$, $p = 0.103$; DIL brood mass number, $r = 0.092$, $p = 0.159$; mass of brood mass in which son developed: DIL brood mass weight, $r = 0.030$, $p = 0.418$; DIL brood mass number, $r = -0.034$, $p = 0.359$). However, controlling statistically for DIL body size using multiple regression analysis, we found that the mean weight of the brood masses that a DIL produced was positively correlated with son's body size (table 1). Surprisingly, DILs also constructed more brood masses when mated to a son that was large relative to the weight of the brood mass he developed in (table 1). Thus, a mother-in-law (MIL) may indirectly influence the weight of the brood masses made by her DILs by making larger sons, and the number of brood masses made by her DILs by making large sons from relatively lighter brood masses.

Sons' horn length and pronotum width exhibited large dam (within sire) variances, but low sire variances (table 2). Furthermore, the dam heritabilities for both phenotypic

measures were significantly reduced when maternal brood mass weight was included as a covariate in the analysis (table 2). Taken collectively, these results indicate a direct non-genetic maternal effect on a son's phenotype. Similarly, the number of brood masses constructed by DILs have large, significant dam variances and low sire variances (table 2). Similar asymmetries between dam and sire variances have previously been demonstrated for daughters' size and reproductive effort (Hunt & Simmons 2002) and can largely be explained by indirect non-genetic maternal effects. It is therefore reasonable to predict that the MIL effect is due to the direct influence of maternal brood mass weight on son's body size, and the indirect effects of son's body size on DIL reproductive effort. This explanation, however, is not complete. Although male size and DIL reproductive effort are positively correlated at the phenotypic level (table 1) and the dam's contributions to these traits are also significantly positively correlated (table 2), both effects still persist when the brood mass weight produced by a mother is controlled for by including it as a covariate in the genetic analysis. This suggests that brood mass weight is not the only significant source of maternal effects, particularly with regard to the MIL effect.

4. DISCUSSION

Our results provide the first demonstration, to our knowledge, of a non-genetic maternal effect on the parental effort (or any other attribute) of a non-relative. We propose that the mother's ability to manipulate the parental effort of their DILs is due to two important effects associated with the production of large sons. First, if females produce large sons from relatively lighter brood masses, DILs will produce significantly more brood masses. Second, sons that are large in absolute terms stimulate DILs to provision heavier brood masses. One way to make a large male is to provide a heavier brood mass. Although mothers undoubtedly benefit by producing large sons (Hunt & Simmons 2001), it is possible that the benefits of producing large offspring from relatively lighter brood masses and the costs to a mother of provisioning (Hunt *et al.* 2002) may together constrain the evolution of maternal care.

Our finding that the maternal heritabilities of son's phenotype and DIL reproductive effort remain substantial, even after controlling for the weight of the maternal brood mass, suggests that the maternal brood mass is not the only source of maternal effects in *O. taurus*. Undoubtedly, other maternal effects contribute to the MIL effect observed here. One possible way in which mothers may be able to produce large sons relative to the size of the brood mass they provide is by laying larger eggs. In the seed beetle, *Collosobruchus maculatus*, egg size is an important non-genetic maternal effect contributing to offspring phenotype (Fox & Savalli 1998). Maternal body size and the size of eggs that she produces are positively correlated, and offspring developing from larger eggs mature at a larger body size. Thus, body size is transmitted across generations via maternal effects mediated by egg size (Fox & Savalli 1998). Clearly, future experiments on *O. taurus* should focus more heavily on the importance of egg size and its potential as a source of maternal effects.

It has been speculated that male *O. taurus* manipulate the parental effort of their mates via seminal products contained in their ejaculate (Kotiaho *et al.* 2003). The positive effects of seminal products on female reproduction are well established in beetles and are often dose dependent (reviewed by Simmons 2001). In *O. taurus*, male ejaculate size is positively correlated with body size (Simmons *et al.* 1999) so that the MIL effect reported here may be mediated at least in part via male seminal products. If this is the case, we predict that there will be a trade-off between relative brood mass weight and the concentration and/or effectiveness of sons', as yet, unidentified seminal products.

In *Drosophila*, accessory gland proteins (Acps) contained in the seminal fluid function to stimulate and regulate female reproduction and frequently come at a cost to females (reviewed by Chapman 2001). It has been suggested that the X chromosome should be a 'hot spot' for sexually antagonistic genes governing Acp regulation (Rice 1984). If this is the case, it is possible that our inflated dam variances for DIL reproductive effort could be explained by the X-linked inheritance of seminal fluid products by sons. However, in *Drosophila*, all of the 75 currently mapped Acps reside on the autosomes (Chapman 2001), which makes this interpretation of the observed MIL effect in *O. taurus* seem unlikely. However, studies measuring the

inheritance of the MIL effect across multiple generations are required to partition formally the magnitude of X linkage (if any) and non-genetic maternal effects.

Our findings that mothers are able, via the care they provide to their sons, to manipulate the parental effort of unrelated daughters-in-law is further evidence that maternal effects have wide-ranging non-genetically transmitted evolutionary consequences (Fox & Savalli 1998; Francis *et al.* 1999; Rauter & Moore 2002). Furthermore, the fact that DIL reproductive effort is highest when mated to large sons that emerge from lighter, rather than heavier, brood masses demonstrates that maternal effects may consist of multiple components. These components have the potential to trade off and to constrain the optimum level of maternal care.

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