Transgenerational effects of parent and grandparent gender on offspring development in a biparental beetle species

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Parental effects on offspring life-history traits are common and increasingly well-studied. However, the extent to which these effects persist into offspring in subsequent generations has received less attention. In this experiment, maternal and paternal effects on offspring and grand-offspring were investigated in the biparental burying beetle Nicrophorus vespilloides, using a split-family design. This allowed the separation of prenatal and postnatal transgenerational effects. Grand-parent and parent gender were found to have a cumulative effect on offspring development and may provide a selection pressure on the division of parental investment in biparental species.

Keywords: maternal effect; biparental care; burying beetle

1. INTRODUCTION

Maternal effects occur when the phenotype of the mother, through the environment she provides, influences the phenotype of her offspring over and above the direct effect of transmitted genes [1]. Maternal effects can be important for evolutionary change in response to directional selection [2], leading to rapid between-population divergence [1]. They also aid evolutionary adaptation to a variable environment due to their high level of phenotypic plasticity [3]. There is also evidence that the maintenance of maternal effects in subsequent generations (transgenerational maternal effects) may be important for rapid species adaptation through developmental plasticity [4].

Transgenerational effects have been suggested to be mediated through epigenetic regulation or egg composition [5]. In order to test this it is necessary to disentangle prenatal and postnatal effects. Prenatal effects typically reflect cytoplasmic factors in the mother’s egg, for example, the amount of yolk, hormones and mRNAs [6]; they will therefore include epigenetic effects. Postnatal effects occur due to differences in resource provisioning by parents, natural variation in the treatment of offspring by parents of different genotypes and previous experience of parental care [7]. The interaction between prenatal and postnatal transgenerational effects may also be adaptive [8].

In the burying beetle Nicrophorus vespilloides both parents cooperate to raise the brood, providing biparental care [9]. Under laboratory conditions, both females and males are equally competent single parents [9–14]. I was therefore able to separate pre- and postnatal transgenerational effects in first and second generation offspring.

2. MATERIAL AND METHODS

(a) Study organism and rearing conditions

Beetles were from an outbred laboratory population derived from 100 females and 57 males trapped in Japanese beetle traps baited with rotting beef steak and hung from the trees in Sunbank Wood in the Mersey Valley, UK, in August 2003. Prior to experimental use, all adult beetles were housed as individuals in clear plastic containers (17 × 12 × 6 cm) at 20 °C under a 15L:9D cycle and fed decapitated mealworms (Tenebrio) twice a week.

(b) Experimental procedures

All beetles used were aged two to three weeks post-emergence as adults, to control for age effects on parental care and offspring fitness. As offspring, parental generation (P1) individuals had received biparental care. Thirty-eight adult P1 virgin pairs were placed in clear plastic containers containing 2 cm depth of soil. Each pair was provided with a mouse, as weighing between 15 and 20 g (Livefoods Direct, Sheffield, UK) 2 h before the dark cycle began in the laboratory, the time at which beetles typically search for carrion in the wild. After 48 h eggs were collected and placed in a Petri dish on damp filter paper and incubated until the larvae hatched. The larvae were placed in a clean plastic box; this was decided at random. The other parent was moved to an individual box. The next day the Petri dish was checked twice for newly hatched larvae. Broods of 8–12 larvae were placed in the carcass. The larvae and parent waited until they dispersed from the carcass at the end of the parental care period. Meanwhile, the parent who had been removed from the carcass was fed organic beef mince, alternated with decapitated mealworms every 3 days, so that it experienced a change in diet quality similar to that of its partner.

Upon dispersing from its brood, the parent who had provided care was fed the same diet as its partner for one week. The male and female were then re-paired. They were again provided with a carcass, and eggs collected 48 h later, however, the roles of the parents were reversed, this allowed separate male and female care of full-sib offspring. The second parent was given the same number of offspring as the first. Therefore, each family comprised a brood that received maternal care and a brood that received paternal care. All first filial generation (F1) broods were weighed prior to being placed with their parent and individually at dispersal from the carcass, giving a measure of mass gain. Data on duration of hatching, dispersal, pupation and emergence were recorded for all offspring, in order to provide values for the duration of the preupal dispersive ‘wandering’ and pupation phases.

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2 weeks after emergence as an adult, a son and daughter from each F1 brood were paired with an unrelated stock beetle 2 h before the dark cycle and provided with a mouse of between 15 and 20 g. Eggs were collected into Petri dishes 48 h later. The stock beetle was removed when the eggs were collected and the experimental beetle stayed with the carcass. When the second filial generation (F2) larvae hatched, a brood the same size as that cared for by the P1 parents in its family were given to the F1 parents. The same life-history traits on F2 offspring were recorded (figure 1).

Statistical analyses were carried out using PASW 17. Parental and grandparental effects were tested using unrestricted mixed model analyses of variance. Denominator degrees of freedom were calculated by PASW 17 using the Satterthwaite approximation. When investigating parental effects of the P1 generation on F1 offspring, life-history traits parents provided care one at a time therefore a nested cross-factor model was used [15], with family nested in the treatment factor of brood order which had two levels: mother first and father second, or father first and mother second. Parental and grandparental effects of F1 offspring life-history traits were investigated using a factorial design because F1 sons and daughters of paternal and maternal care broods provided care to their offspring and therefore a three-factor fully cross-factor model was used [15],
that F2 spent longer wandering if their grandfather had
gender had a main effect on wandering duration, such
ental gender, parental gender and family, had an effect
interaction, the three-way interaction between grandpar-
the F2 offspring life-history traits. There was one main
supplementary material). At lower orders, the two-
impact of grandparental gender on offspring life-
offspring life-history traits. Previously a
principal gender and brood order are fixed effects and family is a random effect. Significant
effects are highlighted in bold.

Table 1. Summary of nested cross-factored mixed model analyses of variance investigating parental effects on three F1
offspring life-history traits. P1 parental gender and brood order are fixed effects and family is a random effect. Significant
effects are highlighted in bold.

<table>
<thead>
<tr>
<th></th>
<th>duration of wandering phase</th>
<th>duration of pupation phase</th>
<th>mass gain during parental care period</th>
</tr>
</thead>
<tbody>
<tr>
<td>parental gender</td>
<td>(F_{1,75.256} = 0.084, p = 0.773)</td>
<td>(F_{1,85.441} = 0.584, p = 0.447)</td>
<td>(F_{1,76.288} = 0.064, p = 0.800)</td>
</tr>
<tr>
<td>brood order</td>
<td>(F_{1,75.256} = 0.030, p = 0.864)</td>
<td>(F_{1,85.441} = 0.536, p = 0.466)</td>
<td>(F_{1,76.288} = 1.825, p = 0.181)</td>
</tr>
<tr>
<td>parental gender × brood order</td>
<td>(F_{1,75.256} = 0.540, p = 0.465)</td>
<td>(F_{1,85.441} = 2.313, p = 0.132)</td>
<td>(F_{1,76.288} = 2.930, p = 0.091)</td>
</tr>
<tr>
<td>family (parental gender × brood order)</td>
<td>(F_{3,464} = 18.716, p &lt; 0.001)</td>
<td>(F_{5,363} = 4.143, p &lt; 0.001)</td>
<td>(F_{4,476} = 7.101, p &lt; 0.001)</td>
</tr>
</tbody>
</table>

with parental and grandparental gender as fixed effects and family as a random effect (statistical models in the electronic supplementary material).

3. RESULTS
The highest order interaction, family nested in parental gender crossed with brood order, had an effect on all three life-history traits (table 1). There was variation among families in their response to the interaction between parental gender and brood order (electronic supplementary material). At lower orders, the two-factor interaction and main effects of parental gender and brood order had no effect on any of the three F1 offspring life-history traits (table 1).

Grandparental and parental gender effects on F2 offspring were modeled together. The highest order interaction, the three-way interaction between grandparental gender, parental gender and family, had an effect on all three F2 offspring life-history traits (table 2). The impact of grandparental gender on offspring life-history traits changes with the gender of the parent but more so for some families than for others (electronic supplementary material).

There were no two-way interaction effects on any of the F2 offspring life-history traits. There was one main effect on each life-history trait (table 2). Grandparental gender had a main effect on wandering duration, such that F2 spent longer wandering if their grandfather had provided care to their parent (table 2 and figure 2a). Parental gender had a main effect on duration of pupation and also on mass gain, with offspring spending longer pupating and gaining more mass when cared for by their mother (table 2). The pupation duration of F2 offspring was longest when their mother was cared for by their grandmother (figure 2b). The largest F2 larval mass gain during the parental care period also occurred when their mother had received care from their grandmother (figure 2c).

4. DISCUSSION
Both models testing for parental and grandparental effects found highest order effects when family was included as a factor. This indicates inter-family variation in their response to maternal and paternal care. However, accounting for brood order and family effects, P1 parental gender was found to have no effect on F1 offspring life-history traits, which is in agreement with previous studies on burying beetles [9–14].

Parental gender and grandparental gender had main effects on F2 offspring life-history traits. Previously a negative correlation between the duration of wandering and pupation phases in N. vespilloides has been found, with fitter burying beetle larvae spending less time wandering and more time pupating [16]. In this study, F2 offspring with the longest pupation duration and largest mass gain received care from their mother, and she had previously received care from her mother. Prepupal wandering duration was also shortest if their grandmother had provided care. Thus, accounting for inter-family variation, persistent direct care from females is best.

The interaction between prenatal and postnatal maternal effects has been found to be adaptive in this
Grandparental effects in N. vespilloides J. E. Lock

Figure 2. Grandparental and parental gender effects in F2 burying beetle offspring on life-history traits. Data shown as means ± standard error bars. The split-family design meant that F1 offspring received care from either their mother or father: this is the grandparent gender. F2 offspring also received care from either their mother or father (figure 1): this is parental gender. Grandparental (P1) gender had an effect on the F2 offspring trait (a) duration of the prepupal, non-feeding care period, (b) duration of the pupal stage, (c) mass gain during the larval stage were largest when as offspring F1 individuals had been cared for by their mother.

Table 2. Summary of three-factor fully crossed-factored mixed model analyses of variance investigating grandparental and parental effects on three F2 generation offspring life-history traits. Parental and grandparental gender are fixed effects and family is a random effect. Significant effects are highlighted in bold.

<table>
<thead>
<tr>
<th>Effect</th>
<th>F-value</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>grandparental gender</td>
<td>$F_{1,18.141} = 5.230, p = 0.034$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>parent gender</td>
<td>$F_{1,18.393} = 0.004, p = 0.953$</td>
<td></td>
<td></td>
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<tr>
<td>family</td>
<td>$F_{1,18.5161} = 2.532, p = 0.149$</td>
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<td></td>
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<tr>
<td>grandparental gender × parental gender</td>
<td>$F_{1,18.205} = 0.247, p = 0.625$</td>
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<td></td>
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<tr>
<td>grandparental gender × family</td>
<td>$F_{1,18} = 1.450, p = 0.219$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>parental gender × family</td>
<td>$F_{1,18} = 0.536, p = 0.902$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>grandparental gender × parental gender × family</td>
<td>$F_{1,8.391} = 6.243, p &lt; 0.001$</td>
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In the wild, 85 per cent of burying beetle broods are cared for biparentally [18], so consistent direct female care would occur across generations. Under biparental conditions, there are marked gender-specific differences in the time parents allocate to different parental care behaviours, with females spending more time provisioning larvae (direct care) and males spending more time maintaining the carcass (indirect care) [13,19]. Multi-transgenerational effects may therefore provide selection pressures for the division of biparental care behaviours, seen across a wide range of taxa, from insects to humans.

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