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

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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 prenatal 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.

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. Grandparental 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

Two weeks after emergence as adults, a son and daughter from each *F*1 brood were paired with an unrelated stock beetle *Nicrophorus vespilloides* at 20°C under a 15L:9D cycle and fed organic beef mincemeat, 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 (*F*2) larvae were weighed prior to being placed with their parent and individually at dispersal from the carcass, giving a measure of mass gain. Dunging, dispersal from the carcass and emergence were recorded for all offspring, in order to provide values for the duration of the prepupal dispersal ‘wandering’ and pupation phases.

Two weeks after emergence as an adult, a son and daughter from each *F*1 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 (*F*2) larvae hatched, a brood the same size as that cared for by the *F*1 parents in its family was given to the *F*2 parents. The same life-history traits were recorded as for *F*1 offspring. Data were collected from 19 families comprising a son and daughter from each *F*1 maternal and paternal care brood caring for *F*2 offspring (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 using the Satterthwaite approximation. When investigating parental effects of the P1 generation on *F*2 offspring, life-history traits parents provided care one at a time therefore a nested cross-factorial 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 on *F*2 offspring life-history traits were investigated using a factorial design because *F*2 sons and daughters of paternal and maternal care broods provided care to their offspring and therefore a three-factor fully cross-factorial model was used [15].
Grandparental effects in *N. vespilloides* J. E. Lock

**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 modelled 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).

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>Effect</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><em>F</em>_{1,75,256} = 0.084, <em>p</em> = 0.773</td>
<td><em>F</em>_{1,85,441} = 0.584, <em>p</em> = 0.447</td>
<td><em>F</em>_{1,76,288} = 0.064, <em>p</em> = 0.800</td>
</tr>
<tr>
<td>Brood order</td>
<td><em>F</em>_{1,75,256} = 0.030, <em>p</em> = 0.864</td>
<td><em>F</em>_{1,85,441} = 0.536, <em>p</em> = 0.466</td>
<td><em>F</em>_{1,76,288} = 1.825, <em>p</em> = 0.181</td>
</tr>
<tr>
<td>Parental gender × brood order</td>
<td><em>F</em>_{1,75,256} = 0.540, <em>p</em> = 0.465</td>
<td><em>F</em>_{1,85,441} = 2.313, <em>p</em> = 0.132</td>
<td><em>F</em>_{1,76,288} = 2.930, <em>p</em> = 0.091</td>
</tr>
<tr>
<td>Family (parental gender × brood order)</td>
<td><em>F</em>_{4,404} = 18.716, <em>p</em> &lt; 0.001</td>
<td><em>F</em>_{5,363} = 4.143, <em>p</em> &lt; 0.001</td>
<td><em>F</em>_{4,476} = 7.101, <em>p</em> &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).
species [8]. In this study, the interaction between grandparental gender (prenatal effect) and parental gender (postnatal effect) varies among families. They are unlikely to have a prenatal effect because burying beetles are opportunistic breeders, so females invest optimally in all eggs [17]. They may have a grandpaternal prenatal effect through the eggs of their daughters, delaying the wandering duration. It is unclear why parental gender does not have an effect on pupation duration and mass gain of F1 offspring but does on F2 offspring. This may also be due to the multi-transgenerational effects of uniparental care.

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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Table 2. Summary of three-factor fully crossed-factor randomized block 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></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>Grandparental Gender</td>
<td></td>
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<tr>
<td>Parental Gender</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Family</td>
<td></td>
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<tr>
<td>Grandparental Gender × Parental Gender</td>
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<td>Grandparental Gender × Parental Gender × Family</td>
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<td>Grandparental Gender × Parental Gender × Family</td>
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</tbody>
</table>

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 wandering phase (days) (2012) N. vespilloides. In this study, the interaction between grandparental gender and parental gender (b) duration of the pupation phase (days) (2012) N. vespilloides. Parental and grandparental gender are fixed effects and family is a random effect. Significant effects are highlighted in bold.


