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. Grandparent 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 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.

(b) Experimental procedure
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 carcass, 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. The parents were moved to plastic boxes with the carcass 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 were kept 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 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, allowing for 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 for parental and offspring in the parental generation were recorded for all offspring, in order to provide values for the duration of the prepupal dispersive ‘wandering’ and pupation phases.

The 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 were recorded as for F1 offspring. Data were collected from 19 families comprising a son and daughter from each F1 parental and paternal care brood for F2 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 PASW 17 using the Satterthwaite approximation. When investigating parental effects of the F1 generation on F2 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 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-factorial 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
effects are highlighted in bold.
The impact of grandparental gender on offspring life-
history traits changes with the gender of the parent
Parental gender had a main effect on duration of pupa-
and brood order had no effect on any of the three F1
The interaction between prenatal and postnatal
maternal care paternal care maternal care paternal care
male father
F1 daughter unrelated
maternal care
unrelated
male
F2
grandchildren
grandchildren
grandchildren
grandchildren
grandchildren
grandchildren
Figure 1. Pedigree structure for each experimental family.
Table 1. Summary of nested cross-factor 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 on F2 offspring life-history traits. Previously a
Previous studies on burying beetles [9–14] have found
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
vided care to their parent (table 2 and figure 2a).
Parental gender had a main effect on duration of pupa-
tion 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).

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 off-
spring were modelled together. The highest order
interaction, the three-way interaction between grandpar-
ental 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).

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 vari-
ation 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
This may also be due to the father: this is the grandparent gender. F2 offspring also received care from either their mother or father (figure 1): this is grandparental parental gender. Grandparental (P1) gender had an effect on the F2 offspring trait (a) duration of the prepupal, non-feeding wandering phase of development. F1 parental gender effects on the F2 offspring traits (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></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>5.230, p = 0.034</td>
<td>2.232, p = 0.152</td>
<td>0.087, p = 0.772</td>
</tr>
<tr>
<td>parental gender</td>
<td>0.004, p = 0.953</td>
<td>0.007, p = 0.001</td>
<td>0.001, p = 0.001</td>
</tr>
<tr>
<td>family</td>
<td>2.532, p = 0.149</td>
<td>0.954, p = 0.567</td>
<td>2.853, p = 0.445</td>
</tr>
<tr>
<td>grandparental gender</td>
<td>0.247, p = 0.625</td>
<td>0.135, p = 0.717</td>
<td>0.095, p = 0.761</td>
</tr>
<tr>
<td>parental gender</td>
<td>1.450, p = 0.219</td>
<td>1.754, p = 0.121</td>
<td>0.860, p = 0.624</td>
</tr>
<tr>
<td>grandparental gender</td>
<td>0.536, p = 0.902</td>
<td>0.526, p = 0.909</td>
<td>0.465, p = 0.943</td>
</tr>
<tr>
<td>gender × parental</td>
<td>6.243, p &lt; 0.001</td>
<td>2.262, p = 0.003</td>
<td>6.784, p &lt; 0.001</td>
</tr>
<tr>
<td>gender × family</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 prenatal effects by one generation relative to postnatal effects, influencing 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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