

**Maternal effects, inheritance and evolution  
of life history and behaviour in  
*Nicrophorus vespilloides***

A thesis submitted to the University of Manchester for the  
degree of Doctor of Philosophy in the Faculty of Life  
Sciences

2005

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Abstract of Thesis submitted by Judith Elizabeth Lock  
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In many animal species parents display parental care behaviours to increase the survival of their offspring. As a consequence of parental care parents contribute directly to the phenotype of their offspring through their inherited genotype but also indirectly, through the parental care environment, causing maternal effects on offspring phenotypic traits. I used a quantitative genetic approach to investigate variation in parental care and the resultant maternal effects on offspring phenotype in the burying beetle, *Nicrophorus vespilloides*.

In chapter 1 I explored variation in parental care using a cross-fostering design. I found a correlation between parental care and offspring begging, even when parents and offspring did not share an environment, due to the cross fostering. This means that the genetic influences on parenting and begging are inherited together, so that they evolve together.

In chapter 2 I investigated the influence maternal age of variation in parental care by cross-fostering offspring to females of either the same or different age of their mother. I found an interaction between prenatal and postnatal maternal effects that allows mothers to adjust their maternal effects as they age. Therefore, if offspring were reared by a female of a different age to their mother their fitness was lowered.

I used a split-family design in chapter 3 to compare the genetic variation in the parental care behaviours of females and males and their contributions to variation in offspring traits. Estimates of Resemblance between parents and offspring were found to be influenced by the sex of the parent. Comparisons of resemblance estimates from shared and unshared environments also indicated a negative parental effect. However, most estimates of the coefficients of maternal (m) and paternal (f) effects did not support this, as they were very small. There was a negative maternal effect on larval provisioning behaviour. This may also be due to an interaction between prenatal and postnatal maternal effects.

Chapter 4 also used a split family design to separate postnatal variation in parental care from prenatal maternal effects. To investigate grandparental effects we continued the experiment to a third generation to investigate whether parental effects persist as grandparental effects. The sex of the parent was found to have no effect on offspring, however, grandparent sex did have an effect. This was again due to an interaction between prenatal and postnatal parental effects and a delay by one generation in the expression of prenatal grandpaternal effects, compared to grandmaternal effects

In this thesis I have examined the importance of parent-offspring interactions and maternal effects. I found that variation in the parental care environment causes variation in maternal effects. As a result, parents are able to adapt the phenotype of their offspring by varying the care they provide, maximising the fitness of their offspring.

## **Declaration**

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## General Introduction

When animals provide parental care, part of the environment the offspring experiences depends on the quality of care they receive in the form of parental care. Genetic variation among mothers can cause variation in maternal performance and, therefore, in the environment of the offspring (Cheverud & Moore 1994, Moore et al 1997, Peripato & Cheverud 2002). Hence, genes that determine the environment provided by the mother indirectly determine the phenotype of their offspring (Moore et al 1998), a phenomenon termed indirect genetic effects (Moore et al 1997, Moore et al 1998).

Indirect genetic effects (IGEs) occur when the genetically influenced phenotype of one individual acts as the environment for another (Moore et al 1997, Wade 1998, Wolf et al 1998). Most data on indirect genetic effects comes from studies of mother-offspring interactions and the consequent maternal effects on offspring phenotype (Cheverud 1984, Cheverud & Moore 1994, Moore et al 1998, Wolf 2000). However, IGEs can arise from the environment provided by a relation and the resulting effects on the phenotype of the offspring can be called kin effects (Cheverud 1984). Nonetheless, the term maternal effect is traditionally used as it is well established in the literature (Cheverud & Moore 1994).

Maternal effects are ubiquitous due to prenatal and postnatal parental investment but are often overlooked in quantitative genetic studies (Cheverud & Moore 1994, Wade 1998, Moore et al 1998). Interactions between parents and offspring are usually investigated using a behavioural ecology approach, often investigating the conflict between parents and offspring. The close genetic relationship between parent and offspring creates conflict between parents and offspring (Trivers 1974) because natural selection acting on genes expressed in the young will lead to the evolution of behaviours where offspring make greater demands for parental resources than is optimal for the parent (Trivers 1972), so that

the maximum fitness of parents and individual offspring is at different levels of parental investment (Agrawal et al 2001). Due to this there is a trade-off between offspring number and offspring quality, dependent on parental care, and future reproductive success (Cheverud & Moore 1994, Wade 1998).

In this study I used integrated research into the genetics and evolution of parent-offspring interactions, particularly maternal effects. Using a quantitative genetic design, I ask specific questions about the evolutionary effects of parental care in my model system, the burying beetle, *Nicrophorus vespilloides*, an insect species that displays natural parental care behaviours under laboratory conditions.

Successful reproduction by *Nicrophorus* is dependent upon locating a mate and the carcass of a small vertebrate, which is required for food and as a nest for developing larvae (Beeler et al 1999). Carcass preparation involves removal of hair or feathers, shaping into a ball, covering with oral and anal secretions to delay decay, and chewing a hole in the top from which the larvae feed (Milne & Milne 1976). Once the carcass is prepared it is buried and the female lays eggs in the soil nearby. After hatching the larvae crawl into the hole on the top of the carcass, where they are cared for by their parents (Eggert et al 1998, Scott 1998, Beeler et al 1999, Eggert & Müller 2000, Jenkins et al 2000, Rauter & Moore 2001).

Burying beetle parents exhibit parental care behaviours to maximise the fitness of their brood on the valuable carcass resource (Scott 1998, Tallamy & Brown 1999), and to increase the development rate of the larvae (Rauter & Moore 2002). Parental care behaviours include: regurgitating carrion to the larvae; treating the carcass with antibacterial and proteolytic secretions and defending the carcass and brood (Jenkins et al 2000).

The effects of parental care on offspring (maternal effects) are important for variation in early offspring traits. Variation in parental care, due to variation in the genotype of the mother, produces variation in the environment experienced by offspring (Cheverud & Moore 1994). This causes an epistatic interaction between offspring and parent genotypes (Wade 1998), that results in a genetic coadaptation detected experimentally as genetic correlations between parental care and offspring begging behaviours (Wolf & Brodie 1998, Agrawal et al 2001). Chapter 1 of this thesis measures genetic correlations and components of social selection between two parental care behaviours and two offspring begging behaviours using a cross fostering design.

The cross-fostering method eliminates genetic and phenotypic sources of covariance between parents and the offspring for which they are caring. As a result parental care behaviours towards unrelated offspring were correlated with the begging behaviours of their actual offspring towards an unrelated parent. This covariance between parents and offspring therefore indicates a genetic coadaptation because they are not in a shared environment.

The cross-fostering experimental design was used again in chapter 2. In this experiment the effects of maternal age at first reproduction are investigated. Variation in the amount of care parents provide is due not only to its genotype but also the physical condition of the parents. Females were bred at either 2 or 6-weeks post-eclosion. Their offspring were then cross fostered to a female of either the same or other age group as their mother, and maternal age effects on parental care and offspring begging behaviours, and offspring life history traits were investigated. This is to examine the adaptive nature of maternal effects.

The genetic variation behind phenotypic variation in parental care between individuals was investigated in chapter 3. A split-family experimental design was employed to investigate maternal and paternal effects separately. Estimates of resemblance between parents and offspring are calculated from regressions between parents and offspring with a shared or unshared environment. Any difference between heritability values will be due to parental effects. The influence of parental effects was then further investigated by calculating matrices of the causal coefficients describing maternal (m) and paternal (f) effects.

The split-family experimental design is used in chapter 4. The design allows me to control for prenatal maternal effects and investigate postnatal parental effects of female and male parental care separately. I made the most of individual variation in parental care behaviours having postnatal parental effects on offspring. I also investigated the persistence of parental effects into the next generation as grandparental effects.

**1 Selection, inheritance and the  
evolution of parent-offspring  
interactions**

## 1.1 Introduction

Social interactions are a ubiquitous feature of the life history of many organisms, and these interactions can have a dramatic influence on an individual's fitness. When behaviour expressed in social interactions influences fitness, a type of selection results that has been termed "social selection" to distinguish it from selection that results from abiotic factors or ecological selection (Crook 1972; West-Eberhard 1983; Wolf et al. 1999). Similarly, traits expressed during social interactions, which typically are behaviours, have been termed "interacting phenotypes" (Moore et al. 1997; Moore et al. 1998) because such phenotypes are predicted to follow a different evolutionary trajectory than other morphological or life history traits (Moore et al. 1997; Moore et al. 2002; Moore et al. 1998; Wolf et al. 1999). This altered evolutionary trajectory occurs because interacting phenotypes can be both an agent and a target of selection. Therefore, unlike abiotic factors influencing fitness, interacting phenotypes can have a heritable basis because they can themselves evolve, this leads to complex evolutionary dynamics (Moore et al. 1997; Moore et al. 2002; Moore et al. 1998). Thus information on both selection and inheritance is particularly important for traits that influence social interactions.

For many organisms, one of the most common, and also commonly studied, social interaction is that between parents and offspring. However, most studies of parent-offspring interactions have focused on the benefits and costs of parenting or offspring signalling (Clutton-Brock 1991; Rosenblatt & Snowdon 1999; Wright & Leonard 2002). Offspring growth and survival can depend on the amount of resources a parent provides, which in turn may depend on the intensity of offspring signalling. Many fewer studies have quantified patterns of genetic variation and covariation in parent and offspring behaviours expressed during interactions (Agrawal et al. 2001; Hager & Johnstone 2003;

Kolliker et al. 2000; Kolliker & Richner 2001). Even more surprising is that, to our knowledge, no studies provided measures of selection gradients associated with parental care and begging. Yet theoretical work suggests that it is critical to understand the inheritance of parent and offspring behaviours (Cheverud & Moore 1994; Moore et al. 1998) and the nature of selection arising from the effects of care or begging on offspring traits (Wolf & Brodie 1998; Wolf et al. 1999) to fully appreciate how parental care and offspring begging might evolve.

In this study I set out to empirically determine the nature of inheritance and selection associated with parent-offspring interactions involving care. My goal was twofold: first, to determine if there was coadaptation in parent-offspring communication in a species where both parental provisioning of food and begging for food can be quantified directly, and second, to quantify the strength and nature of selection arising from parenting or begging. I used a cross-fostering design to eliminate common environment effects on parenting or begging between relatives, and we examined both genetic correlations and the influence of variation in parent and offspring traits on offspring performance and fitness using the burying beetle *Nicrophorus vespilloides* as our model.

Burying beetles provide a convenient organism to study parental care and offspring begging under nearly natural environments in the laboratory (Eggert & Muller 1997; Scott 1998), and have proven useful in quantitative genetic studies of maternal effects (Rauter & Moore 2002a; Rauter & Moore 2002b). Cross-fostering is easily accomplished in burying beetles (Rauter & Moore 2002a). There are also theoretical reasons to expect variation in responses to care (Rauter & Moore 2002b), as burying beetles are not completely dependent on care. Offspring can either beg or self-feed, and *N. vespilloides* has been

described as a “partially-begging” species (Smiseth et al. 2003; Smiseth & Moore 2002; Smiseth & Moore 2004b; Smiseth & Moore 2004d).

I first tested the hypothesis that parental care and offspring begging coevolve, that is, there is a genetic correlation between the two reflecting coadapted levels of expression because the correlation is between different individuals, i.e. parents and offspring (Wade 1998; Wolf 2000; Wolf & Brodie 1998). Next, I tested the hypothesis that parental and offspring behaviour result in selection by quantifying how parental behaviour associated with care and offspring behaviour associated with begging influence offspring growth and development (i.e., we calculated performance gradients; (Arnold 1983). I also quantified selection by examining the association between growth and development with fitness (i.e., I calculated selection gradients; (Lande & Arnold 1983). Calculation of performance and associated selection (fitness) gradients allows us to measure adaptive significance directly (Arnold 1983). I therefore adopted this quantitative genetic approach to measuring both selection and inheritance to provide an analysis of parent-offspring interactions based on formal evolutionary theory involving multivariate selection (Arnold 1983; Brodie et al. 1995; Lande & Arnold 1983). To the best of my knowledge, such an integrated approach quantifying both patterns of inheritance and the strength and pattern of multivariate selection has not been attempted in a single study for this type of trait.

## **1.2 Materials and Methods**

### **1.2.1 Burying beetle basic biology**

Burying beetles have a rapid generation time and well-described behaviour and ecology (comprehensively reviewed in (Eggert & Muller 1997; Scott 1998). Furthermore, burying beetles are easily reared in the laboratory under conditions very similar to nature,

making them excellent subjects for investigations of parental care, genetics, and evolution. Burying beetles breed on vertebrate carcasses, which form the sole food resource for offspring. Carcasses can be located either by males or by females. If a male locates the carcass, he emits pheromone to attract a female. If a female locates a carcass, she lays eggs some distance from the carcass. The female, or male and female together, then remove fur or feathers, roll the carcass into a ball, keep it free of fungus and bacteria, and prepare a cavity in the top of the carcass from which the offspring forage (self-feed) from partially digested meat. The parents can also forage for themselves from the carcass from this cavity area while they are partially digesting the carcass for the offspring (Scott & Gladstein 1993).

At 20° C, larvae hatch approximately 60 hours after the eggs are laid (P.T. Smiseth and A.J. Moore, unpublished data), and they crawl to the prepared carcass. Once they reach the carcass, *Nicrophorus vespilloides* larvae require direct provisioning of food from their parents for the first 12 hours, with a decreasing reliance after that (Eggert et al. 1998). Parental care goes well beyond providing previously secured food for the larvae in burying beetles, and involves direct and detailed social interactions between parents and offspring. Larvae can either forage for themselves (self-feed) or beg for food from the parents (Smiseth et al. 2003; Smiseth & Moore 2002; Smiseth & Moore 2004d). Begging can stimulate direct parental provisioning, which occurs by regurgitation of pre-digested carrion into the mouth of the larvae and is indicated by mouth-to-mouth contact between the parent and a larva (Rauter & Moore 1999; Rauter & Moore 2004; Rauter & Moore in press; Smiseth et al. 2003; Smiseth & Moore 2004b; Smiseth & Moore 2004d). Parents never feed larvae unless they are begging. Parental care in *Nicrophorus* is consistent with most

common expectations for parental care reflecting our mammalian bias and with studies of avian parental care (Rauter & Moore 1999).

Development of *N. vespilloides* larvae is rapid, and individuals reach the adult stage approximately 30 days after they hatch. In *N. vespilloides*, larvae feed from the carcass for 5-7 days, although parental care is variable and decreasing over this time. Begging ceases after 72 hours although parents continue to visit the crater until the larvae disperse (Smiseth et al. 2003). Once the carcass has been consumed, larvae disperse by crawling from the remains of the carcass and have no more contact with the parent. At this point larvae have completed feeding and growth and enter a “wandering stage” for 7-10 days. These wandering larvae then bury themselves in the soil and pupate, emerging to adulthood after an additional 7-10 days. Adults are sexually mature 7-10 days after they emerge.

Although I conducted my study in the laboratory, I maintained conditions to resemble those in the field as closely as possible. Behaviour expressed in the laboratory and field is very similar (Beeler et al. 1999; Scott 1998). These beetles reproduce underground where temperature fluctuations should be minimised and the carcass can be relatively well hidden from predators and competitors. The lack of predators and competitors in laboratory conditions may affect larval survival after dispersal from the carcass, when the larvae burrow underground and pupate under the soil. However, mortality due to predation after dispersal would not reflect direct fitness effects of the interaction with parents, which was the focus of this study.

### **1.2.2 Experimental Design**

Smiseth and Moore (2002) provide details on the field origin (Wales) and laboratory husbandry of the beetles studied. More than 100 beetles were collected from the field and

maintained as outbred stock for several generations in the laboratory. For the current study, 34 independent breeding pairs were established from newly emerged, randomly selected, nonsibling male and female virgin beetles. When the beetles reached sexual maturity, they were set up in a clear plastic container containing 1cm depth of damp soil and placed in an observation room lit with red light. The following day the pair was provided with a 10–17 g mouse carcass, fresh frozen and thawed before the experiment (supplied by Livestock Direct, Sheffield). The mouse was provided 3 to 4 hours before “sunset” (= lights off), a time when *N. vespilloides* typically search for carrion in nature (Kocarek 2001). The carcass was checked for the presence of larvae twice a day.

There were two rearing treatments for each family: offspring reared by a related female (the biological mother as the caretaker) or by an unrelated female (a foster mother as the caretaker). Burying beetles use temporal cues in kin recognition (time of arrival of larvae to the carcass;(Muller & Eggert 1990), which facilitates cross-fostering. In these experiments each female produced and reared two broods (one as a biological mother and one as a foster mother). Larvae that arrived at the carcass within 24 hours (i.e., first instars) were removed, counted and returned to the carcass maintained by their natal mother or that of an unrelated foster mother whose larvae hatched at the same time. The order of the related or unrelated caretaker treatments were randomized for each family.

Once the family units had been established, the carcasses and females were moved to new boxes containing fresh soil to prevent the arrival of any additional larvae after the manipulation. At this time, the male was also removed so that behavioural observations were of female uniparental care only. The male was removed because uniparental and biparental care have equivalent effects on offspring fitness (Smiseth et al 2005). The manipulated broods were left for a further 24 hours, and observations of larval begging and

parental provisioning were then carried out, when the larvae were ~48 hours old and second instars.

### **1.2.2.1 Measures of Behaviour**

In this study I was interested in the covariances between natural levels of diversity in direct parental care for the offspring and offspring performance (or fitness) rather than manipulating parental care as has been done previously (e.g., (Eggert et al. 1998; Rauter & Moore 2002b). Variation in parental behaviour that did not involve direct interactions with the larvae (parental defence of the carcass, maintenance of the carcass) may be important influences on offspring fitness, but they are beyond the scope of this study, which focused specifically on interacting phenotypes of parental and offspring behaviour. Therefore, for this experiment I focused on behaviours expressed when parents and larvae are in close proximity (defined as within 1 pronotum length or less away from the larvae) and thus must involve an interaction. Close proximity is required for offspring begging; that is, offspring never beg to attract a parent, begging only occurs if parents are next to the offspring (Rauter & Moore 1999; Smiseth & Moore 2002).

The measures of both parental and offspring behaviours are identical to those we have used in previous studies (Smiseth et al. 2003; Smiseth & Moore 2002; Smiseth & Moore 2004b; Smiseth & Moore 2004d). Two parental behaviours were examined; the first was the percentage of time the parent spent in the cavity in close proximity to the offspring but without feeding the offspring. This behaviour was recorded because parents can forage for themselves when they are in the cavity (Scott & Gladstein 1993). The second behaviour was the percentage of time the parent spent directly provisioning the offspring with food when they were in close proximity. Begging is a necessary and sufficient trait to elicit

provisioning. Two larval traits indicating levels of larval begging were scored during my behavioural observations, the first being the average percent of time spent begging by each larva in the brood when the parent was present and in close proximity. Larvae start begging only when the adult is at a distance that corresponds approximately to the width of the parent's pronotum (Rauter & Moore 1999; Smiseth & Moore 2002). The percent of time spent begging is therefore a measure of the average begging effort by each larva in the brood (Smiseth & Moore 2004b). The second trait indicator was the mean number of larvae begging in scans where at least one larva was begging. This measure reflects the proportion of siblings that, at a given time, are competing for food from the parent (Smiseth & Moore 2002).

Parental and larval behaviours were recorded using instantaneous scan sampling (Martin & Bateson 1986). Observations lasted for 30 minutes and were made 24 hours after larvae arrived on the carcass. I chose this period for observation because, although there is consistent behaviour within families (A.J. Moore, unpublished data), this is the day when there is the highest level of interaction between parents and larvae (Smiseth et al. 2003).

#### **1.2.2.2 Coadaptation of Parenting and Begging**

I adapted a method similar to that of (Agrawal et al. 2001; Kolliker et al. 2000) and used a cross-fostering design to examine the potential genetic covariance between parent-offspring interactions independently of family environment. The main difference between our study and these previous studies is that we measured the amount of begging or amount of parenting in direct response to an interaction with a caretaker rather than relying on an indirect measure of begging inferred from offspring performance (Agrawal et al. 2001) or

measured from artificial recordings (Kolliker et al. 2000). This latter study also measured parental provisioning as a response to manipulated playbacks.

The cross-fostering method used eliminates the environmental influences of parents on offspring and vice versa by eliminating sources of variation caused by a shared environment. With this design, begging behaviour of offspring is measured when an unrelated caretaker rears them, and parenting of caretakers is measured when they interact with unrelated larvae. Any covariance between relatives must then reflect genetic influences because the only remaining common influence is shared inheritance. This measure may be inflated by additive components of epistasis, but using parent-offspring covariances eliminates dominance components of variation and so should be close to the additive genetic correlation. I therefore estimated this genetic correlation using a Pearson product-moment correlation between behaviours of relatives expressed in independent social contexts. The complete design, where parents also took care of their biological offspring, allowed us to compare traits expressed in family and cross-fostered groups as well. Within-social environment correlations (i.e., parents taking care of their own offspring) will differ from between-social environments (i.e., parents interacting with unrelated offspring) by an amount that reflects common environmental influences, thereby permitting a further estimate of how common environment inflates family resemblance in interacting phenotypes.

### **1.2.2.3 Measures of Offspring Fitness and Performance**

Components of offspring fitness were scored as survival from one life-history stage to another until adult emergence. Offspring performance was also measured based on traits that we know can be influenced by parental care (Rauter & Moore 2002a; Rauter & Moore

2002b; Smiseth et al. 2003) and that I predicted would be associated with fitness as they are fundamental life-history characters: mass of a larva at dispersal from the carcass (i.e., the point at which larval foraging and parental care ceased), the duration of time the larvae stayed on the carcass before dispersing as wanderers, the duration of the developmental stage from dispersal to entering the pupal stage (i.e., the “wandering” stage where larvae disperse off the resource and no longer forage), and the duration of the pupal stage (i.e., from entering the pupal stage until emergence as an adult beetle). For duration of time the larvae spent on the carcass, there was no variation within families because all larvae disperse from the carcass at the same time.

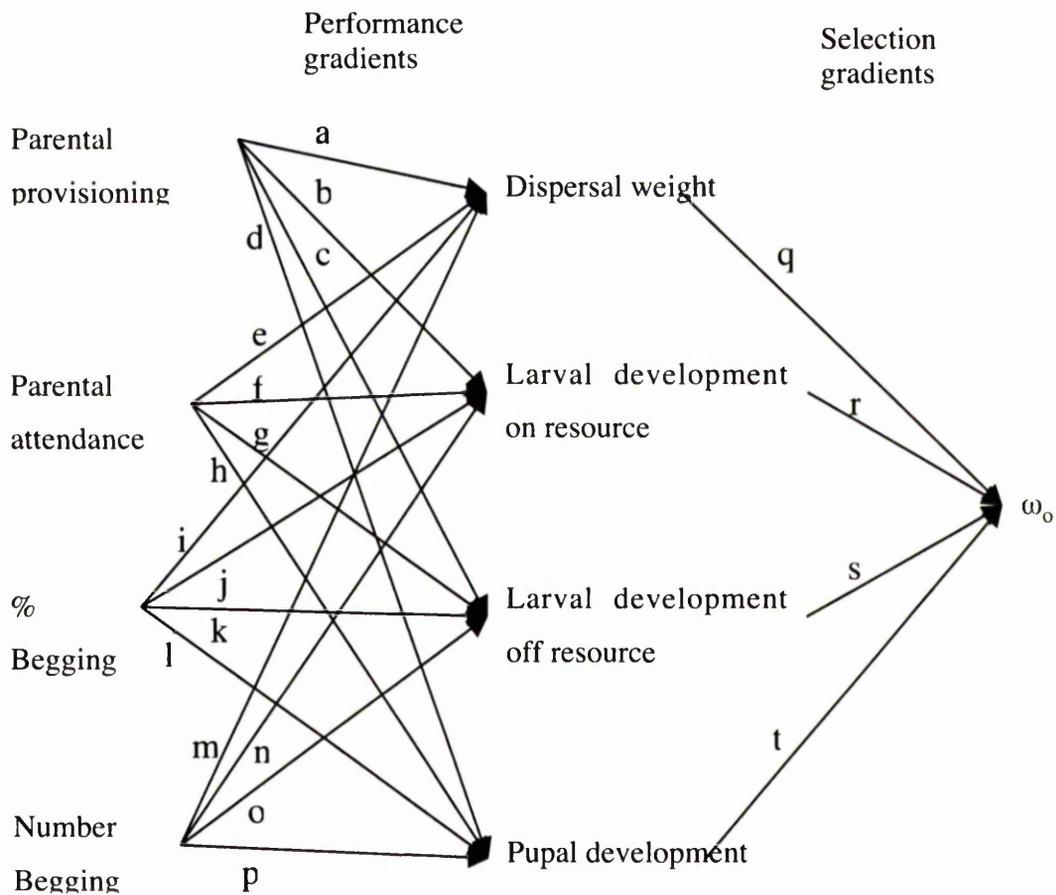
#### **1.2.2.4 Quantifying Selection Arising from Parental and Offspring Behaviour:**

##### **Selection and Performance Gradients**

Linear regression models were used to calculate standardized selection gradients (Lande and Arnold 1983) that affect life-history traits associated with larval performance. Performance gradients were also calculated (Arnold 1983) relating parental and offspring behaviour to offspring traits. This two-step process is necessary because, although the life-history characters or fitness of individuals can be measured, behaviour of parents can only be measured towards entire families and the average amount of begging for the brood because individually identifiable marks on the larvae, either natural or applied, do not persist between larval stages (moult). However, analyzing parental performance gradients, offspring performance gradients, and fitness (selection) gradients allows the integration of all measures in a formal evolutionary framework and model (Figure 1.1), put the work in terms that are used in equations for evolutionary change, and compare relative strengths of the effects (Arnold 1983; Brodie et al. 1995; Lande & Arnold 1983).

Survival from one life-history stage to the next was scored as 0 (died) or 1 (survived), and fitness was converted to relative fitness (Lande & Arnold 1983). Relative fitness was regressed on values of standardized life-history characters (mean = 0, SD = 1) using general linear models in SYSTAT to generate selection gradients (Brodie et al. 1995; Lande & Arnold 1983). Significance was determined using logistic regression. Associations between offspring life-history traits were investigated with Pearson product-moment correlation, and significance determined using Bonferroni-corrections. The association between survival and relatedness of caretaker (biological or foster mother) was tested using a contingency test.

**Figure 1.1: Model of social selection arising from parent-offspring interactions in *Nicrophorus vespilloides*. Paths a-h are parental performance gradients ( $\beta_{tz}$ ; the direct effect of parental care behaviour  $z_p$  on offspring performance,  $f$ , measured as growth and development), paths i-p are offspring performance gradients ( $\beta_{tz}$  the effects of offspring begging behaviour  $z_o$  on offspring performance  $f$ ) and paths q-t (values presented in table 1.4) are standardized linear selection gradients ( $\beta_{wf}$  the fitness gradient arising from the influence of the offspring performance trait [ $f$ ] on fitness [ $w_o$ ], measured by survival). Values for paths q-t are presented in table 1.2.**



Performance gradients were calculated by regressing parent family averages for offspring life-history characters on all measured parental and offspring behaviours. Effects of parental and offspring behaviour on each performance trait in offspring was investigated with a separate regression. Average values of the standardized offspring performance characters were used to avoid inflating degrees of freedom, as there was a single behavioural measure for each family.

### 1.2.3 Statistical Controls

My interest was restricted to the effects of social interactions on offspring traits, and we therefore experimentally minimized the potential influence of abiotic traits known to influence burying beetle behaviour (Smiseth & Moore 2002). Given the model of social selection I was testing (figure 1.1), I did not wish to include abiotic factors as covariates in our analyses because factors such as brood size and resource size are not properties of individuals but may still influence resemblances among individuals (Wolf et al. 1999). I therefore sought to experimentally minimise any covariances that might be induced by minimising the variation in these factors.

Providing a mouse of similar size to all families (mean = 13.2 SD = 2.0g) should minimise variation in the amount of resource available to affect parent and offspring behaviour. To confirm that the experiment controlled for this factor I also examined the influence of carcass size statistically. A linear regression of carcass size on the parental behaviours confirmed that the range of carcass sizes we used had no significant effect on percent of time parents spent near larvae ( $R^2 < 0.001$ ,  $F_{1, 64} = 0.005$ ,  $P = 0.942$ ) or the percent of time spent provisioning ( $R^2 < 0.001$ ,  $F_{1, 64} = 0.049$ ,  $P = 0.825$ ). A linear regression of carcass size on offspring behaviours further confirmed that carcass size had no

significant effect on either number of begging larvae ( $R^2 = 0.004$ ,  $F_{1,63} = 0.282$ ,  $P = 0.598$ ) or the percent of time spent begging by each larva ( $R^2 = 0.002$ ,  $F_{1,62} = 0.170$ ,  $P = 0.681$ ) or on brood size ( $R^2 = 0.010$ ,  $F_{1,64} = 0.640$ ,  $P = 0.427$ ). These results are consistent with previous studies that show that carcass size has little effect on parent or offspring behaviour (Smiseth & Moore 2002; Smiseth & Moore 2004d).

Rates of parental provisioning to offspring increase with very large differences in brood size (range 5 – 25 larvae; (Rauter & Moore 2004). Variation in brood size was therefore minimised as much as possible (mean = 10.6, SD = 4.7 larvae), matching brood size for fostered and natal families. The success of this approach was confirmed with linear regression. I found that the brood sizes we used in this study had no significant effect on either of the parental behaviours (percent of time near larvae  $R^2 = 0.017$ ,  $F_{1,64} = 1.129$ ,  $P = 0.292$ ; percent of time provisioning larvae  $R^2 = 0.001$ ,  $F_{1,64} = 0.056$ ,  $P = 0.813$ ).

Brood size can influence begging in *N. vespilloides* as well, but the influence is complex (Smiseth & Moore 2002). There is a curvilinear relationship between brood size and begging, with increased levels of begging in very small broods (< 5) and decreased levels of begging in large broods. Our brood sizes did not include these extremes, so any effects were expected to be minor. Supporting this, there was no significant linear relationship between brood size and total number of observations where begging was observed ( $R^2 = 0.002$ ,  $F_{1,64} = 0.105$ ,  $P = 0.747$ ) or total number of begging acts observed ( $R^2 = 0.019$ ,  $F_{1,64} = 1,257$ ,  $P = 0.266$ ). Nonetheless, to maintain consistency between studies, the same measures of begging were used as in other studies (Smiseth et al. 2003; Smiseth & Moore 2002; Smiseth & Moore 2004b; Smiseth & Moore 2004d) where brood size is in the denominator of the behavioural measures of begging.

Each female cared for two different broods, and although the order of caring for related or unrelated broods was randomized, females may behave differently when inexperienced or experienced as a parent. To check for this, I used repeated-measures analysis of variance to compare the parental care provided to the first and second clutches. Clutch order was found to have no significant effect on time spent in proximity of larvae ( $F_{1,32} = 0.023, P = 0.880$ ) or provisioning of the larvae ( $F_{1,32} = 0.220, P = 0.643$ ).

### **1.3 Results**

#### **1.3.1 Coadaptation of Parenting and Begging**

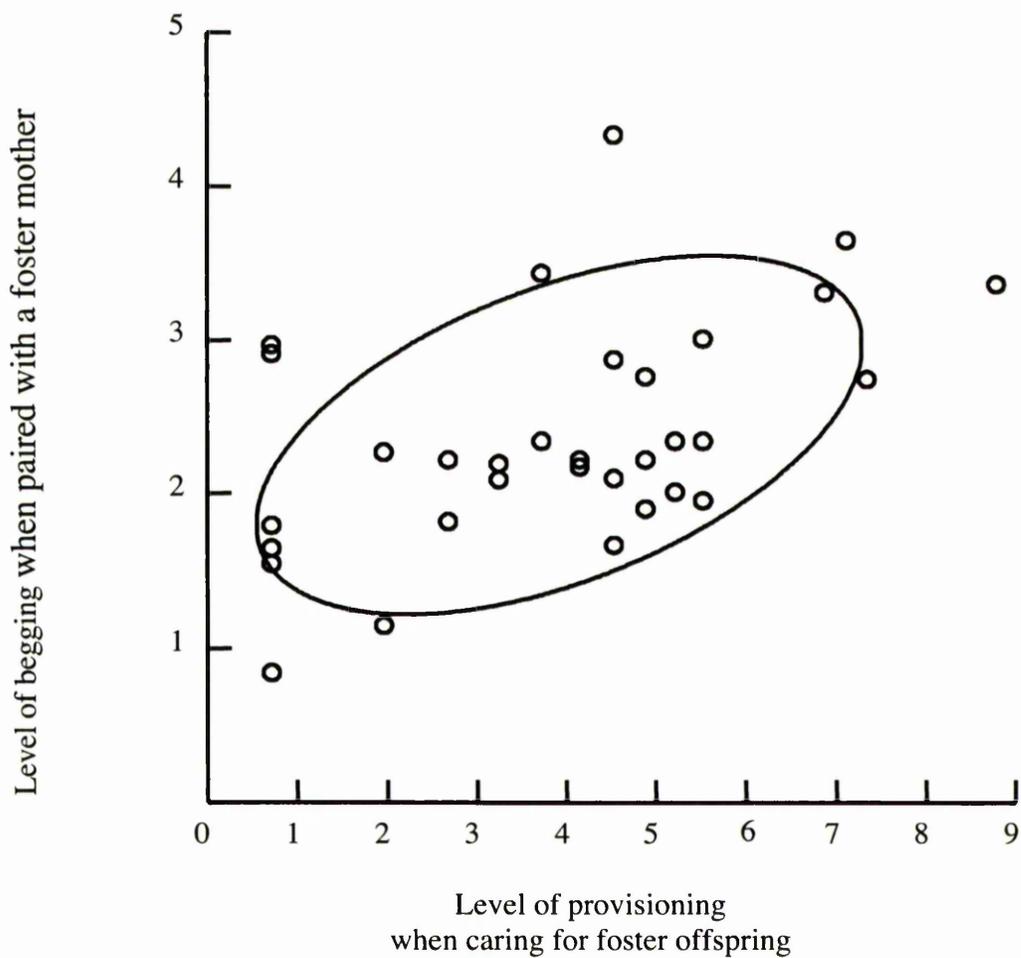
Parents and offspring performed the same behaviours regardless of whether they were interacting with relatives or non-relatives. The patterns of correlations between behavioural acts were similar (table 1.1). Begging and provisioning within biological families was more strongly correlated than between unrelated individuals ( $r = 0.63$  versus  $r = 0.53$ ), although this is not a statistically significant difference ( $t_s = 0.986, P > 0.5$ ). In general, common environmental effects did not increase resemblance among relatives much beyond the effects of inheritance (table 1.1), and in some cases the effects appear to decrease the resemblance among relatives (i.e., the correlation between average number of begging larvae and % of time parent spent in the cavity, and number of begging larvae and average % time spent begging by a larvae). Furthermore, all of the correlations between relatives, when controlling for common environment effects by using behaviour expressed towards or by a foster brood, are significantly different from zero, suggesting that all social behaviours are influenced by genetics and there is a genetic covariance between all combinations of parenting and begging traits. For example, investigating the hypothesized genetic relationship between begging and provisioning, there was a strong positive

relationship within families between begging to an unrelated caretaker and parental provisioning of food to unrelated larvae (figure 1.2). Thus, families with high levels of begging have parents that show high levels of provisioning to the larvae, indicating a strong positive genetic correlation ( $r = 0.51$ ,  $SE = 0.09$ ,  $P = 0.003$ ). This joint expression of begging and provisioning appeared to be beneficial; individuals reared by their biological mothers were more likely to survive than larvae reared by a foster mother ( $\chi^2 = 13.308$ ,  $df = 1$ ,  $P < 0.001$ ).

**Table 1.1: Family mean correlations,  $\pm$  SE, and levels of significance among parental and offspring behaviours. Below the diagonal are correlations between behaviours expressed within genetic families (i.e., by relatives in a shared social environment). Above the diagonal are correlations between behaviours of relatives expressed in an independent social environment (i.e., in a cross-fostered environment and therefore in an independent social environment). SE calculated for Pearson product moment correlation as in (Sokal & Rohlf 1995).**

<b>Behaviour</b>	<b>% total time a parent spent in the cavity</b>	<b>% time spent provisioning larvae</b>	<b>Average number of larvae begging</b>	<b>% of time spent begging by a larva</b>
<b>% time a parent spent in the cavity</b>		<b>0.551</b> $\pm 0.084$ (P < 0.001)	<b>0.511</b> $\pm 0.088$ (P = 0.003)	<b>0.559</b> $\pm 0.084$ (P < 0.001)
<b>% time spent provisioning larvae</b>	<b>0.730</b> $\pm 0.065$ (P < 0.001)		<b>0.545</b> $\pm 0.085$ (P < 0.001)	<b>0.505</b> $\pm 0.089$ (P = 0.003)
<b>Average number of larvae begging</b>	0.199 $\pm 0.113$ (P = 0.284)	<b>0.484</b> $\pm 0.091$ (P = 0.006)		<b>0.461</b> $\pm 0.092$ (P = 0.008)
<b>% of time spent begging by a larva</b>	<b>0.744</b> $\pm 0.064$ (P < 0.001)	<b>0.634</b> $\pm 0.076$ (P < 0.001)	0.124 $\pm 0.118$ (P = 0.499)	

**Figure 1.2: Relationship between provisioning by mothers, measured as % time spent provisioning unrelated offspring, and begging by offspring, measured as % time spent begging by larvae to an unrelated parent. The 95% density ellipse is indicated on the figure and the positive correlation between these traits ( $r = 0.51 \pm 0.09$ ) is highly significant ( $P = 0.003$ ).**



### **1.3.2 Quantifying Selection Arising from Parental and Offspring Behaviour: Selection and Performance Gradients**

There is strong selection associated with growth and development of larvae (table 1.2). There was selection for larger size at dispersal and faster larval development, both on and off the carcass (table 1.3). In addition to the linear selection on the duration of wandering, larval development off the carcass, there was significant non-linear selection associated with selection against extremes (table 1.3). Non-linear selection components were not significant for either mass at dispersal or duration of larval development on the resource.

**Table 1.2: Linear ( $\beta_{wfi}$ ) and non-linear ( $\gamma_{wfi}$ ) standardized selection gradients (Lande & Arnold 1983) relating the effect of offspring performance characters ( $f_i$ ) on relative fitness ( $w$ ) in *Nicrophorus vespilloides*.**

<sup>1</sup> Larval development while foraging and being fed from the carcass

<sup>2</sup> Larval development while no longer interacting with parents or siblings; also called the “wandering” stage (Eggert & Muller 1997).

Offspring performance character ( $f_i$ )	N	Linear relationship ( $\beta \pm SE$ )	P	Non-linear relationship ( $\gamma \pm SE$ )	P
Mass at dispersal	737	<b>0.055</b> (0.017)	0.002	-0.012 (0.011)	0.143
Duration of larval stage on the resource <sup>1</sup>	737	<b>-0.049</b> (0.017)	0.005	0.022 (0.010)	0.805
Duration of larval stage off the resource <sup>2</sup>	668	<b>-0.093</b> (0.014)	<0.001	<b>-0.021</b> (0.006)	<0.001

There were significant correlations among all four of our offspring performance measures except between dispersal mass and duration of larval development on the resource. Combining the two periods of larval development, on and off the carcass, there was a highly significant negative correlation between total larval development and pupal development ( $r = -0.206$ ,  $N = 611$ ,  $P < 0.001$ ). Shorter larval development is thus offset by longer pupal development – a trade-off that was seen in another burying beetle species in

which it has a genetic basis (Rauter & Moore 2002a; Rauter & Moore 2002b). Controlling for body mass at dispersal, the partial correlation between larval development and pupal development was even stronger ( $r = -0.267$ ).

**Table 1.3: Correlations among offspring performance characters.**

Correlations in bold are significant with Bonferroni-corrected levels of significance.

	Duration of larval stage on the resource	Duration of larval stage off the resource	Duration of pupal stage	Adult Size (pronotum)
<b>Dispersal weight</b>	0.046 N = 737 P = 0.211	<b>0.087</b> N = 668 P = 0.025	<b>0.315</b> N = 611 P < 0.001	<b>0.553</b> N = 601 P < 0.001
<b>Duration of larval stage on the resource</b>		<b>0.119</b> N = 668 P = 0.002	<b>-0.175</b> N = 611 P < 0.001	<b>0.216</b> N = 601 P < 0.001
<b>Duration of larval stage off the resource</b>			<b>-0.160</b> N = 611 P < 0.001	<b>0.170</b> N = 601 P < 0.001
<b>Duration of pupal stage</b>				<b>0.097</b> N = 601 P = 0.017

Maternal and offspring performance gradients suggest a consistent pattern of trade-offs in parental and offspring behaviour (table 1.4). In all cases, there was a positive relationship between levels of provisioning and offspring performance, but a negative relationship between time spent in the crater without provisioning the larvae and offspring performance (table 1.4). This same pattern of balance between offspring behaviours was seen as well, with a positive effect of time spent begging, but a negative effect of number of offspring begging on offspring performance (table 1.4). However, parental and offspring behaviour appear to influence different offspring performance characters.

Parental performance gradients were largest in relation to offspring development (table 1.4). Increased provisioning significantly reduced the duration of time larvae spent on the carcass, decreased the time spent as wandering larvae off the resource, and significantly increased time as pupae (table 1.4). In contrast, increased time spent by the parent in the cavity without provisioning had the opposite effects on development, although only the partial regression between time in the cavity and duration of time spent as wandering larvae was significant (table 1.4). There was not a significant relationship between parental behaviours and differences in larval mass at dispersal (table 1.4).

In contrast to parental behaviour, offspring performance gradients were largest in relation to larval mass (table 1.4). Increasing the amount of time a larva spent begging had a positive effect on the final mass of larvae, while increasing numbers of begging larvae had a negative effect on larval mass. The number of begging larvae also had significant negative influences on development. An increase in the number of begging larvae increased the time spent as a wandering larvae off the resource and decreased the time as a pupae, both of which are negatively related to fitness (tables 1.3 and 1.4). The amount of

time a larva begged was not significantly related to changes in development, although the pattern of association remained consistent (table 1.4).

**Table 1.4: Performance gradients ( $\beta_{fz_i}$ ) relating parental care behaviours and offspring begging behaviours to offspring performance measured as growth (mass) or rate of development. Performance gradients are calculated between offspring and their biological parent, independent of the social environment.**

Independent variables (parent and offspring behaviours) were standardized to a mean of 0, SD = 1 to allow direct comparisons of strength of selection.

Offspring performance character (f)	Social behaviour of parents and offspring ( $z_i$ )			
	% time spent provisioning larvae $\beta_{fz1}$ (SE)	% time spent in cavity $\beta_{fz2}$ (SE)	% time spent begging by a larva $\beta_{fz3}$ (SE)	Number of larvae begging $\beta_{fz4}$ (SE)
<b>Dispersal weight</b>	-0.002 (0.005) P = 0.729	0.005 (0.004) P = 0.3654	<b>0.018</b> (0.003) P < 0.001	<b>-0.009</b> (0.004) P = 0.021
<b>Duration of larval stage on the resource</b>	<b>-0.394</b> (0.171) P = 0.025	0.275 (0.156) P = 0.083	-0.008 (0.113) P = 0.941	<b>0.280</b> (0.139) P = 0.049
<b>Duration of larval stage off the resource</b>	-0.572 (0.308) P = 0.069	<b>0.782</b> (0.282) P = 0.007	-0.163 (0.205) P = 0.430	0.192 (0.250) P = 0.447
<b>Duration of pupal stage</b>	<b>0.449</b> (0.162) P = 0.008	-0.169 (0.150) P = 0.264	0.127 (0.108) P = 0.244	<b>-0.279</b> (0.132) P = 0.039

## 1.4 Discussion

This study is unique in simultaneously investigating the role of both selection and inheritance on parent-offspring interactions. I am therefore able to provide a more complete picture of how such interactions can evolve. I show that differences in the expression of parental and offspring behaviour in *Nicrophorus vespilloides* persisted regardless of the social environment in which they were expressed (table 1.1). There were significant positive correlations between parent and offspring behaviour related to the biological origins of the individuals assayed but independent of the social environment within which the behaviour was performed, demonstrating that there was significant genetic covariation underlying pairs of parent and offspring behavioural traits. I found significant natural selection on offspring performance (table 1.2), and corresponding parental and offspring performance gradients arising from parental and offspring behaviour, respectively, affecting offspring performance (table 1.4). Thus, this study places parent and offspring interactions within the framework of formal quantitative genetic theory, and allows us to interpret patterns of selection and potential inheritance (Arnold 1983). Specifically, the environment-independent correlations support quantitative genetic theories of parent-offspring coadaptation (Wolf 2000; Wolf & Brodie 1998), and the selection and performance gradients suggest hypotheses for how joint expression levels of provisioning and begging may be adaptive in this system.

### 1.4.1 Coadaptation of Parenting and Begging

Similar to (Agrawal et al. 2001) work on burrower bugs, and (Hager & Johnstone 2003) work on inbred strains of laboratory mice, I found that offspring fitness was highest when reared by parents that share genes in common with the offspring. This is because of a

better match between genetically-influenced parent and offspring behaviour, causing more dependent larvae to have more attentive mothers. This is evidence for a coadaptation between parent and offspring behaviour. In addition, there was the expected genetic correlation between parent and offspring traits in *N. vespilloides* given a coadaptation (table 1.1). This correlation was positive – genetic influences led to increased levels of provisioning in parents and increased levels of begging in larvae. The positive genetic correlation is in contrast to (Agrawal et al. 2001), who found a negative correlation, but consistent with the positive correlation reported by (Kolliker et al. 2000) in great tits and suggested by the results of (Hager & Johnstone 2003). However, there are positive correlations for all pairs of parent and offspring behaviours, suggesting that it is not possible for individuals to maximise interactions with potentially positive fitness consequences while minimising interactions that have potentially negative fitness consequences (see below) because negative genetic correlations would be expected if there were an evolved balance of positive and negative interactions. Evidence for why parent-offspring behaviour reflects a coadaptation requires the information from analyses of selection and performance gradients.

#### **1.4.2 Quantifying Selection Arising from Parental and Offspring Behaviour: Selection and Performance Gradients**

The offspring performance traits measured were strongly related to fitness in *N. vespilloides* (table 1.2). There was selection for larger offspring and those that progressed faster through the larval stage. In addition, there was a negative correlation between the duration of the larval and pupal stages, suggesting that it is advantageous to spend as much time as possible in the pupal rather than the larval stage (table 1.3). These results are

consistent with a previous study of *Nicrophorus pustulatus* (Rauter & Moore 2002b), which found evidence for a similar trade-off between components of offspring development maintained by negative genetic correlations between developmental stages in *N. pustulatus*. Overall, heavier larvae (and ultimately larger adults) took longer to develop (table 1.3), suggesting there may be an upper limit to selection for reduced development time because of trade-offs between components of development. Shifting development to different stages may ameliorate this constraint. Finally, selection on offspring performance traits was not strictly directional (table 1.2). Although the relationship between larval size and fitness was linear, there was a significant non-linear relationship between fitness and the duration of development. This further supports the idea of an upper limit to the rate of offspring development.

Given the strength and pattern of selection on offspring performance, the offspring performance gradients for the two begging behaviours we measured appeared to have conflicting function or influence (table 1.4). The average time spent begging to a parent by each larva in the brood had a positive impact on offspring performance, increasing larval body mass at dispersal and thus adult size at eclosion. This supports a signalling function for begging that is positively related to fitness because only begging offspring are fed by the parents (Smiseth & Moore 2004d). In contrast, there was a negative performance gradient for the mean number of larvae begging during each begging bout for both dispersal weight and the duration of pupal development. These negative fitness effects are likely to arise from intrabrood competition for access to food provisioned by the parent (e.g., (Stockley & Parker 2002; Wright & Leonard 2002) because the number of begging offspring is a measure of the number of siblings that, at a given time, are competing for food from the parents (Smiseth & Moore 2002). This may also reflect an influence of

brood size because larger broods may exhibit more competition but I found no effect of clutch size on offspring begging behaviours. This suggests that competition varies between families, depending on the offspring's reliance on their mother for food. The overall effects is that more competition results in a negative effect on offspring performance.

Parental performance gradients show a similar pattern to offspring performance gradients, but the effects are on different offspring characters. In general, provisioning is beneficial because there were significant performance gradients for rate of larval development both on and off the resource. Parental provisioning appears to be a superior way for offspring to obtain food, given that offspring can forage for themselves as well (Smiseth & Moore 2004d). Experiments with other burying beetles, where offspring development was compared under conditions where care was provided or eliminated, have also shown that care positively influences larval growth and development (Anduaga & Huerta 2001; Eggert et al. 1998; Rauter & Moore 2002b). Thus, while a positive effect of parental provisioning is unsurprising, this is the first study to quantify the effects of natural levels of variation in care. This positive effect is directly related to feeding and not a function of other social interactions between parents and offspring. Increased time that a parent spent near larvae without feeding them was negatively related to rate of larval development. This is probably because parents may forage for themselves when near the opening to the cavity where food is readily available, as well as regurgitating food to the larvae (Scott & Gladstein 1993). Personal foraging increases the potential future fitness of parents even though it decreases offspring fitness (Scott & Gladstein 1993). Thus, *N. vespilloides* parents are competitors for the food resource as well as caretakers to their offspring, resulting in potential parent-offspring conflict.

### 1.4.3 Integration of Selection and Inheritance: The Adaptive Nature of Parent and Offspring Behaviour Expressed in Interactions

If offspring benefit by both begging more and receiving more provisioning, why don't all offspring and parents show high levels of begging and provisioning? Why should the genetic correlation be positive? Agrawal et al. (2001) suggest that the negative correlation in the burrower bugs they study there is a coadaptation because there will be combinations of high begging/low provisioning or low begging/high provisioning that result in equal offspring fitness on average. A negative correlation equalizes the benefits and costs of both. Under this reasoning, a positive correlation would seem to be not adaptive given that high/high combinations would be superior to low/low combinations.

Agrawal et al.'s (2001) argument is based on the results of a model of stabilising selection on the interacting phenotypes (Wolf & Brodie 1998). Selection is often more complex than pure stabilising selection when there are parent-offspring interactions (Kolliker 2003). The results support a pattern of complex selection in *N. vespilloides* that is often directional and sometimes includes non-linear components. Further, the sign of the selection or performance gradient depended on which traits were examined in our study. This suggests that the complex pattern of selection found arising from parent-offspring interactions in *N. vespilloides*, combined with genetic covariances among traits with contrasting effects, provides insights into why parent-offspring covariances may be difficult to predict. It also suggests that the parent and offspring traits in *N. vespilloides* are coadapted, regardless of the sign of the genetic correlations due to the complex pattern of selection.

This study suggests that in *N. vespilloides*, parents can have negative effects on their offspring by spending time in the cavity without feeding them. However, parents cannot feed their offspring unless they enter the cavity. Likewise, a parent feeds only begging larvae, but there can be competition for food that has negative effects on offspring performance. The pattern of genetic covariances suggest that parents that are likely to spend more time feeding themselves while in the cavity also spend more time feeding their offspring, and vice-versa. Families that are likely to have high levels of larval begging also have higher numbers of begging larvae and increased competition. This suggests that there are multiple stable combinations of behaviour in *N. vespilloides*.

The reasons for the positive genetic correlations between all traits is not known, but positive correlation can arise if there are trade-offs elsewhere in the system (Arnold 1992; Houle 1991). My results suggest that in *N. vespilloides*, one such trade-off may arise because a short larval period is most strongly influenced by parental provisioning while growth (mass) is most strongly influenced by offspring begging. The presence of family members may, however, constrain the evolution towards offspring optima, because the different parental and offspring behaviours are not independent of each other. Balancing the positive and negative effects of interactions with relatives therefore requires a trade-off because larger individuals require a longer development time because individuals cannot easily grow quickly and grow to be large. A positive coadaptation overcomes some of the costs associated with larval competition and the presence of parents because the parents will provision the offspring more often; that is, positive directional selection will result in increased provisioning even more if larvae beg and create more competition. If, in contrast, the parents do not associate with the offspring, the offspring will not beg, in which case both the costs and the benefits of begging and the presence of parents will be minimised.

Parent-offspring interactions are complex interacting phenotypes and are both targets and agents of selection (Moore et al. 1998). Most models (Wolf 2000; Wolf & Brodie 1998) have focused on levels of offspring begging and parental provisioning, but we suggest that this may be too simplistic a view. My results suggest that all behaviours that are potentially functionally related should be taken into account when studying social selection and evolution in parent-offspring interactions.

## **2 Adaptive maternal effects in ageing mothers**

## 2.1 Introduction

The environment experienced by offspring can be as important as the genes they receive from their parents. The environment may range from the prenatal constituents of the egg produced by their mother, to extended postnatal parental care provided to altricial offspring (Mousseau & Fox 1998). A growing literature demonstrates that such “maternal effects” on offspring phenotype are of considerable evolutionary importance and can themselves be adaptive (Mousseau & Dingle 1991).

One important maternal effect is maternal age (Cheverud & Moore 1994). Age-related maternal effects on offspring phenotype can profoundly influence offspring fitness (Cheverud & Moore 1994; Mousseau & Dingle 1991). Female age has fitness effects on offspring survival in many insects, including *Drosophila*. Increasing female age reduces larva to adult viability in *Drosophila melanogaster* (Kern et al. 2001) and egg to adult viability in *D. serrata* (Hercus & Hoffmann 2000). However, a negative impact on offspring fitness through maternal age is not universal. In the cockroach, *Nauphoeta cinerea*, maternal age at first reproduction has no effect on larval viability (Moore & Harris 2003).

Maternal effects may be manifested at two postzygotic developmental stages, prenatal (Qvarnstrom & Price 2001) and postnatal (Cheverud & Moore 1994). These can both be influenced by maternal age. Prenatal effects are due to material resources of the egg and the oviposition site. Changes in egg size as a mother ages lead to prenatal maternal age effects on offspring fitness (Mousseau & Dingle 1991). For example, in the bruchid beetle, *Callosobruchus maculatus*, eggs laid later in life may be smaller than those laid earlier, due to a gradual depletion of a female’s resources, leading to differences in offspring size, development time and survival (Fox 1993).

Maternal age has been shown to have postnatal maternal effects by influencing parental care behaviours. In mammals, a mother may become more successful at raising her offspring as she ages (Clutton-Brock 1984). This may be because as a mother gets older her reproductive investment in each offspring will increase due to a decrease in the number of potential future offspring she can produce; this is the Residual Reproductive Value, RRV, Hypothesis (Clutton-Brock 1984; Pianka & Parker 1975; Trivers 1972).

Previous experience of providing parental care is an important factor in offspring survival, which may mask any effect due to maternal age (Cameron et al. 2000). An experiment looking at the effect of female age on parental care, independent of experience, was performed on Mongolian gerbils. It was found that females that are older at the time of their first reproduction display higher levels of parental care towards their offspring than younger females (Clark et al. 2002).

In this study I examine age-related changes on prenatal and postnatal maternal effects, and ask if mothers make adaptive changes in response to ageing. I investigated the effects of maternal age on offspring fitness, parental care behaviours and offspring begging behaviours using female burying beetles, *Nicrophorus vespilloides*, 2 and 6 weeks old adults. This is a large age difference in burying beetles because the breeding season is only three months long. Burying beetles are a commonly studied example of direct parental care as they behave naturally under laboratory conditions and are easily manipulated (Eggert & Muller 1997; Scott 1998). Maternal effects on offspring development, survival and size have also previously been shown in both *N. vespilloides* (Lock et al. 2004) and *N. pustulatus* (Rauter & Moore 2002a). Successful reproduction by *Nicrophorus* species is dependent upon locating a mate and the carcass of a small vertebrate, which is required for food and as a nest for developing larvae (Scott 1998). However, a vertebrate carcass is

unpredictable and adults may have to wait before encountering one (Scott 1998). Therefore, upon finding a carcass parents exhibit parental care behaviours to maximise the fitness of their offspring as they may not survive long enough to find another carcass and breed again (Scott 1998; Tallamy & Brown 1999).

It is not clear whether a mother increases the amount of care she provides (postnatal maternal effect) because her RRV is decreasing, or because the quality of her offspring is declining (prenatal maternal effect). To separate these two maternal age effects on parental care and offspring fitness a cross-fostering design was used, where offspring were cared for either by a female of the same age as their mother, or in the other age group. Given that a female burying beetle may only live long enough to find one carcass, due to their unpredictability, I would expect her RRV to decrease as she gets older, and therefore expect females in the older age group, 6 weeks post-eclosion to display more parental care. I also expect a prenatal maternal effect, leading to a decrease in offspring quality as their mother ages, based on the evidence from reduction of offspring viability in *Drosophila* (Hercus & Hoffmann 2000; Kern et al. 2001) and decreased egg quality in the bruchid beetle, *Callosobruchus maculatus* (Fox 1993).

## **2.2 Materials and methods**

### **2.2.1 General Procedures**

The beetles used in this experiment were the first generation offspring of a laboratory population that originated from 110 females and 57 males. The wild-caught individuals were trapped in Japanese insect traps, baited with rotting meat, in Sunbank Wood, Mersey Valley, UK in August 2003. Laboratory bred individuals were housed

individually in clear plastic containers (17 x 12 x 6 cm) at 20°C under a 15:9h light:dark cycle. The beetles were fed decapitated mealworms (*Tenebrio* spp.) twice a week.

### **2.2.2 Experimental Design**

For this study 74 independent breeding pairs were selected. In 36 pairs females had eclosed 2 weeks earlier, these are “young” females. In 39 pairs females had eclosed 6 weeks earlier, these were “old” females. These ages were chosen because a female becomes sexually mature at 2 weeks old and 6 weeks old is half way through the three- month breeding season of burying beetles (Scott 1998). It is also likely that a female may not breed until she is 6 weeks old due to the unpredictability of a vertebrate carcass. All females were mated to males that had eclosed 2 weeks earlier. Each pair was provided with a mouse carcass between 10 and 14g in mass (Livefoods Direct, Sheffield, UK) at 5pm, which is two hours before the subjective night in the laboratory, mimicking dusk when beetles in the wild typically search for carrion. Sixty hours later, when eggs were observed in the soil, the female and carcass were moved to another box. Eggs were collected and placed in a Petri dish on damp filter paper. The male was removed at this point because uniparental and biparental care have equivalent effects on offspring fitness (Smiseth & Moore 2002).

Larvae hatched within 24 hours after eggs were collected. Offspring were fostered to an unrelated female who was either the same age as their mother, or a female of the other age. Burying beetles use temporal cues for kin recognition (Eggert & Muller 2000) and as a result cross-fostering is easily performed. All offspring were fostered because a previous experiment has shown that offspring cared for by a natal mother have greater survival than those cared for by a foster mother (Lock et al. 2004). This produced four treatments: 6

weeks post-eclosion caretaker with offspring from a 6 weeks post-eclosion mother (21 broods); 6 weeks post-eclosion caretaker with offspring from a 2 weeks post-eclosion mother (18 broods); 2 weeks post-eclosion caretaker with offspring from a 6 weeks post-eclosion mother (18 broods); 2 weeks post-eclosion caretaker with offspring from a 2 weeks post-eclosion mother (18 broods).

To investigate brood size effects on parental care unmanipulated broods were set up at the same time as the manipulated broods. Ten females of each of the two age groups, 6 weeks and 2 weeks post eclosion were mated with a 2-week-old male and provided with a mouse of a standardised size. Males were removed the next day. The female and her brood were left until the larvae dispersed from the carcass, when the number of surviving offspring was counted.

#### **2.2.2.1 Measures of behaviour**

Burying beetles are a valuable species for the study of parental care. In many insect species the parents ensure a food supply for hatching larvae. Burying beetles use a vertebrate carcass as a food source for offspring, removing hair or feathers, keeping it free of fungus and preparing a cavity for offspring to forage for themselves (Scott & Gladstein 1993). In *Nicrophorus* there is also a direct interaction between parents and offspring, with offspring begging for food and parents regurgitating pre-digested carrion into the mouths of the larvae. So that larvae are able to either self feed or beg to be fed by their parents. Care occurs for 5-7 days but is most important during the first 24 hours (Eggert et al. 1998; Smiseth & Moore 2002).

For this experiment, behavioural observations of parents and offspring were carried out 24 hours after each caretaker was given her brood. Parental and larval behaviours were

recorded using the instantaneous scan sampling (Martin & Bateson 1986). Observations lasted 30 minutes, during which all behaviours performed by parents and offspring were scored, as in previous studies (Smiseth & Moore 2002; Smiseth & Moore 2004b; Smiseth & Moore 2004c; Smiseth & Moore 2004d). The 2 parental care behaviours examined were: 1) percentage of time the caretaker spent in the cavity in close proximity to the offspring, that is within one pronotum length of the offspring, without being fed; 2) percentage of time the caretaker spent directly provisioning the offspring, indicated by mouth-to-mouth contact between parent and larva (Eggert & Muller 1997; Scott 1998).

For offspring behaviours 2 parameters indicating offspring begging were obtained: 1) the percent of time spent begging by each larva in the brood when the parent was present and in close proximity, a measure of begging effort by each larva; 2) the mean number of larvae begging in scans where at least one larva was begging, a measure of sibling competition for food from the parent (Smiseth & Moore 2002).

#### **2.2.2.2 Fitness and life history**

Life-history characters associated with fitness were measured. These were: 1) mass of the entire brood before being given to the caretaker, to give an average hatch mass for each larva; 2) mass of each larva at dispersal from the carcass, when parental care and larval foraging cease; 3) mass gain during the parental care period; 4) the duration of development from dispersal to pupation, this is a non-feeding wandering phase; 5) the duration of the pupal stage, from pupation until emergence as an adult beetle.

### 2.2.3 Statistical analyses

To investigate a brood size effect on offspring survival to dispersal in the unmanipulated broods a one-way analysis of variance was used. Carcass size was included as a covariate. Prenatal maternal effects were investigated using a one-way analysis of variance of the mean hatching mass of larva in each family.

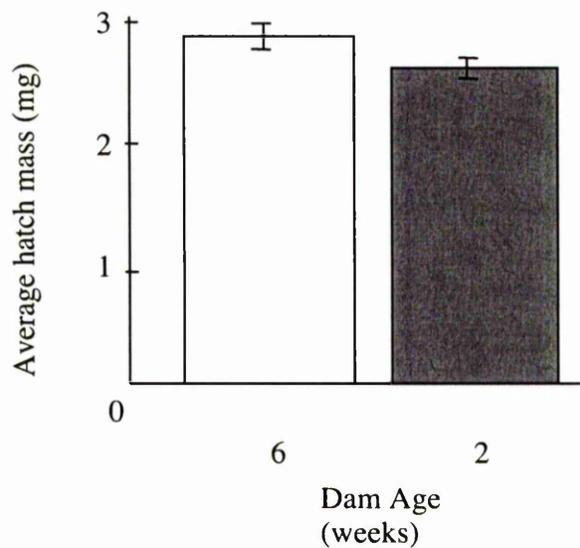
The postnatal effects of female age were tested using two-way analysis of variance. This allowed us to test for the effects of dam age, caretaker age and the interaction between dam age and caretaker age. For life history and developmental traits mean values were used for each family. This was not necessary for behavioural traits as there was only one behavioural measure for each family.

## 2.3 Results

### 2.3.1 Prenatal dam age effects

The age of a female did not affect the number of offspring that survived until dispersal from the carcass, when the brood was not manipulated ( $F_{1,18}=0.456$ ,  $p=0.504$ ). Dam age had a significant effect on the average mass of a larva at hatching (Figure 2.1). Offspring from dams that were aged 6 weeks post-eclosion were heavier than those from dams aged 2-weeks post eclosion ( $F_{1,69}=1.236$ ,  $p=0.270$ ).

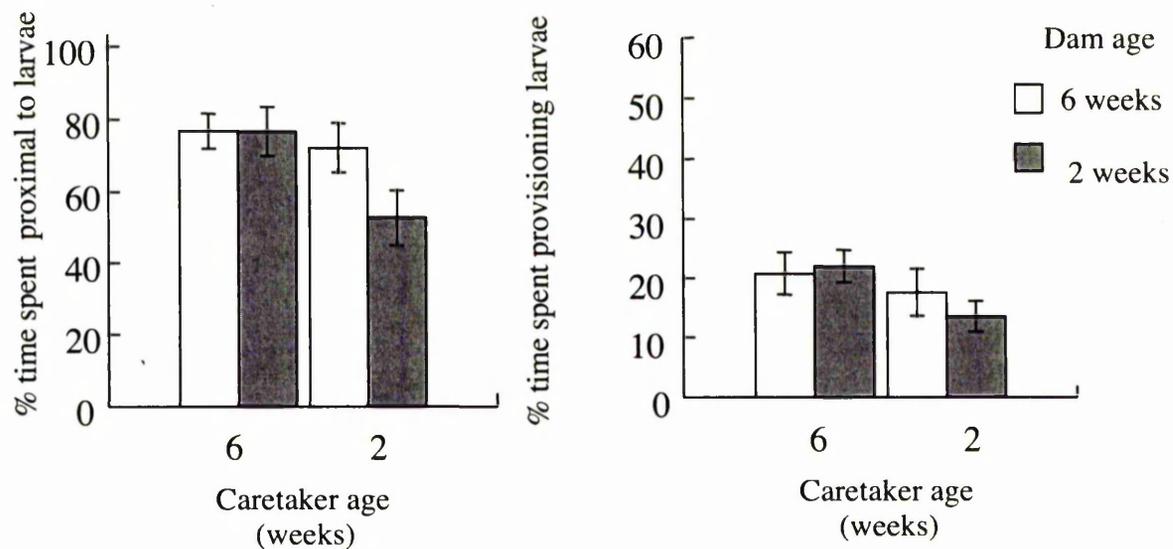
**Figure 2.1: The effect of dam age (6 weeks or 2 weeks old) on the average weight of larvae at hatching**



### 2.3.2 Postnatal dam age effects

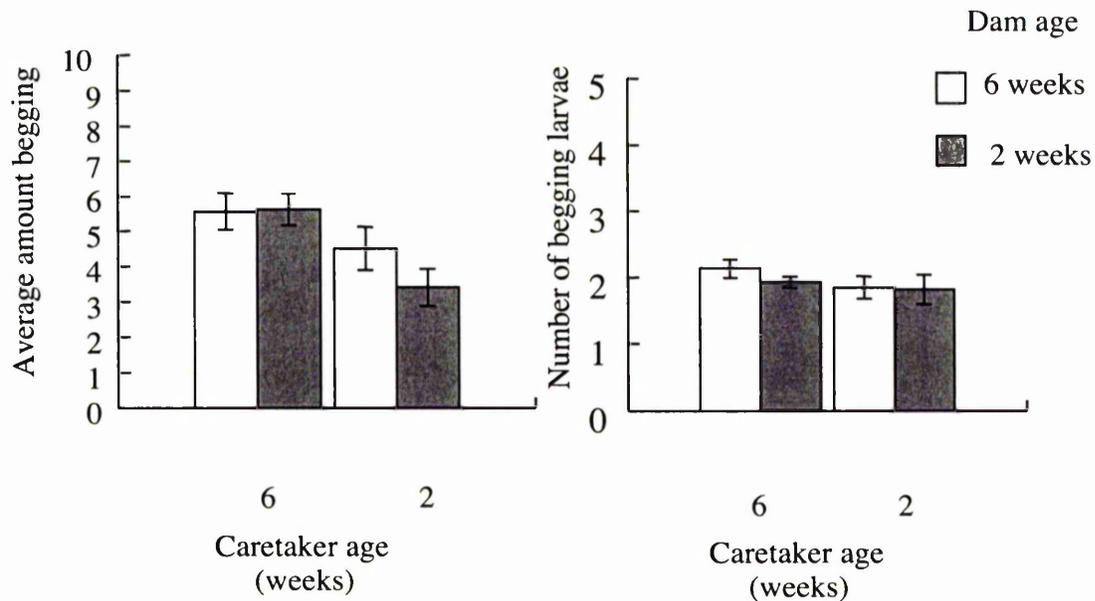
The age of the female was also found to affect parental care behaviours. Older caretakers spent more time performing parental care behaviours than younger caretakers (Figure 2.2). The amount of time the female was observed proximal to the larvae was influenced by the age of the caretaker ( $F_{1,71}=5.065$ ,  $p=0.028$ ), with older caretaker females spending more time in close proximity to the offspring. There was no effect of the age of the dam ( $F_{1,71}=2.398$ ,  $p=0.126$ ) or the interaction between dam age and caretaker age ( $F_{1,71}=2.321$ ,  $p=0.132$ ). The amount of time the caretaker female spent provisioning was also influenced by her age, with older females spending longer provisioning larvae ( $F_{1,71}=3.350$ ,  $p=0.071$ ) but there was no influence of dam age ( $F_{1,71}=0.195$ ,  $p=0.660$ ) or the interaction between dam age and caretaker age ( $F_{1,71}=0.690$ ,  $p=0.409$ ).

**Figure 2.2: The effect of caretaker age and age of the dam of the offspring she is caring for on two parental care behaviours, time spent near to larvae and time spent provisioning larvae**



The number of begging offspring (Figure 2.3) was not affected by caretaker age ( $F_{1,62}=0.189$   $p=0.666$ ), dam age ( $F_{1,62}=0.462$ ,  $p=0.499$ ) or the interaction between caretaker age and dam age ( $F_{1,62}=0.961$ ,  $p=0.331$ ). The amount of time spent begging by the average larva was affected by the age of the caretaker ( $F_{1,71}=9.854$ ,  $p=0.002$ ) but not the age of the dam ( $F_{1,71}=1.012$   $p=0.318$ ) or the interaction between caretaker age and dam age ( $F_{1,71}=1.258$   $p=0.266$ ). Offspring spent longer begging to the older caretaker females.

**Figure 2.3: Effect of dam age and the caretaker providing parental care, on two measures of offspring begging, amount of begging and number of larvae begging**

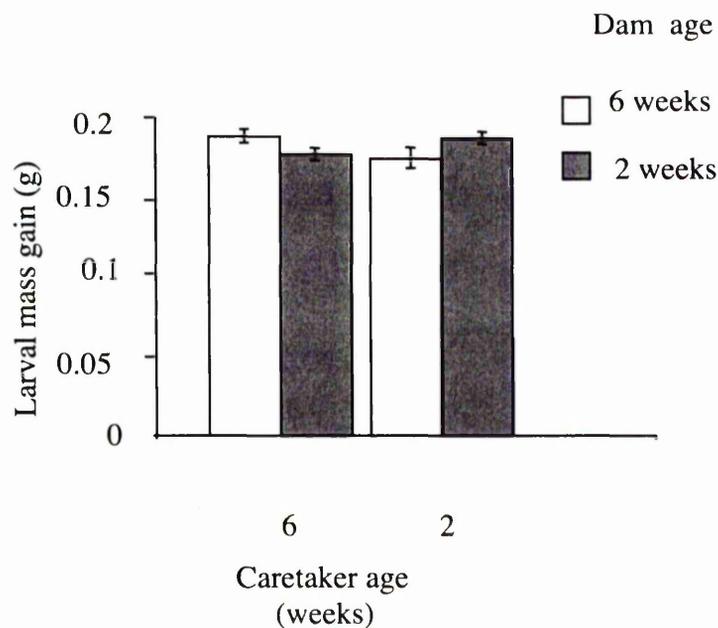


### 2.3.3 Overall impact of dam age and caretaker age on offspring fitness

#### 2.3.3.1 Mass gain during the parental care period

The interaction between prenatal and postnatal maternal effects was found to have a significant effect on mass gain (Figure 2.4). The increase in larval mass during the parental care period, from hatching until dispersal from the carcass, was influenced by an interaction between dam age, a prenatal maternal effect, and caretaker age, a postnatal maternal effect ( $F_{1,67}=6.015$ ,  $p=0.017$ ). Offspring gained the most mass when raised by a caretaker female the same age as their dam. There was no effect of dam age ( $F_{1,67}=0.122$ ,  $p=0.728$ ) or caretaker age ( $F_{1,67}=0.014$ ,  $p=0.905$ ) independently.

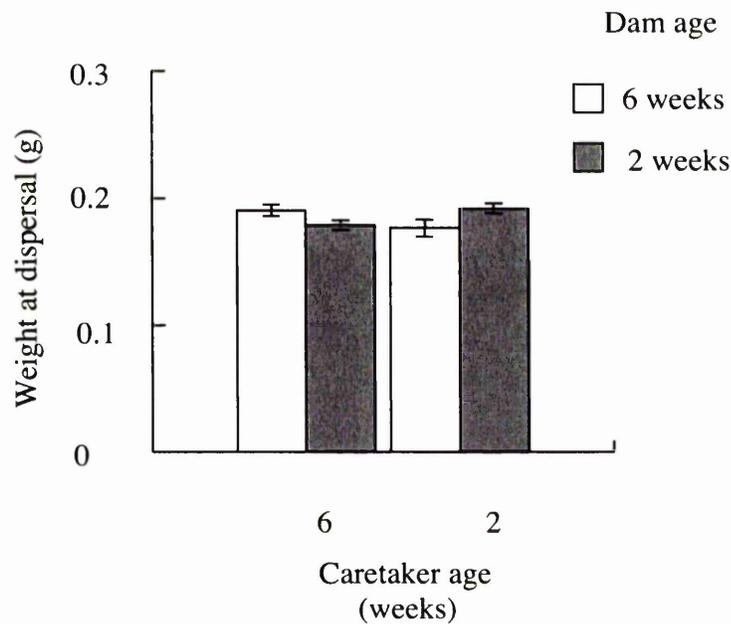
**Figure 2.4: Effect of dam age and caretaker age on larval mass gain during the parental care period**



### 2.3.3.2 Mass at dispersal

An interaction effect between dam age and caretaker age was found in the mass of larvae at dispersal from the carcass (Figure 2.5). Mass at dispersal was not affected by caretaker age ( $F_{1,71}=0.160$ ,  $p=0.690$ ) or dam age ( $F_{1,71}=0.004$ ,  $p=0.949$ ) but is influenced by the interaction between dam age and caretaker age ( $F_{1,71}=8.455$ ,  $p=0.005$ ). When the age of the dam and caretaker are the same offspring are the heaviest with no difference between females aged 2 weeks and 6 weeks post-eclosion. If dam age and caretaker age are not the same offspring are lighter.

**Figure 2.5: Effect of dam age and caretaker age on larval weight upon dispersal from the carcass at the end of the parental care period**

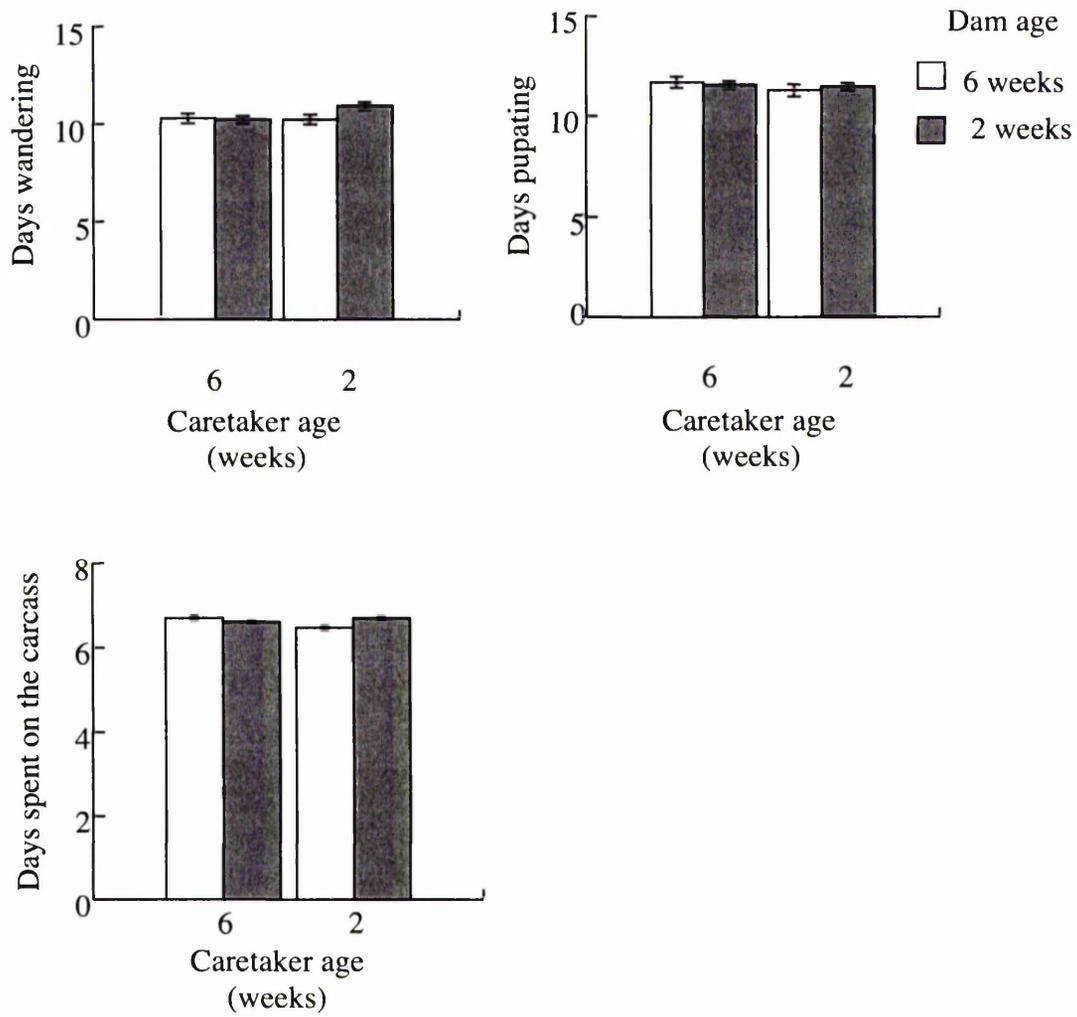


### 2.3.3.3 Development time

The interaction between dam age and caretaker age did not have an effect on the amount of time larvae spent on the carcass ( $F_{1,71}=0.188$ ,  $p=0.661$ ), which is the duration of the parental care period (Figure 2.6). There was also no effect of the age of offspring's dam ( $F_{1,71}=0.608$ ,  $p=0.438$ ) or caretaker ( $F_{1,71}=1.403$ ,  $p=0.240$ ).

The duration of other development periods were not affected by female age either (figure 2.6). The number of days a larva spent wandering was not affected by the age of its dam ( $F_{1,71}=0.002$ ,  $p=0.966$ ), its caretaker ( $F_{1,71}=1.031$ ,  $p=0.313$ ) or the interaction between the age of its dam and the age of its caretaker ( $F_{1,71}=0.427$ ,  $p=0.517$ ). The duration of an offspring's pupation was also unaffected by the age of its dam ( $F_{1,71}=1.863$ ,  $p=0.177$ ), the age of its caretaker ( $F_{1,71}=1.747$ ,  $p=0.190$ ) or the interaction between the age of its dam and the age of its caretaker ( $F_{1,71}=2.865$ ,  $p=0.095$ ).

**Figure 2.6: Effect of dam age and caretaker age on three offspring life history traits, number of days spent in the non-feeding wandering phase, number of days spent in the pupal chamber and number of days spent on the carcass**



## 2.4 Discussion

This study is the first to consider prenatal and postnatal maternal effects on offspring, whilst controlling for maternal experience. I focussed on the effect of maternal age and similar studies should now be carried out investigating the effects of other maternal traits. Maternal age has been shown to be an important factor in both offspring fitness and parental care behaviours in a number of species but the effect on both has not been investigated in the same species before. Using *N. vespilloides* we were able to look at the effects of female age on offspring fitness, parental care behaviours and offspring begging behaviours using a cross fostering experiment between females of different ages.

The finding that larvae from older dams have a heavier average hatch mass than younger dams contradicts the more common finding, egg size decreases as females age and their resources for egg production decline (Fox 1993). However, a reduction in egg size due to maternal age is not universal in insects, and an increase is commonly observed in orthopterans and heteropterans (Fox & Czesak 2000). In *N. vespilloides* the assumption that progeny fitness increases with progeny size may be untrue because parental care increases the complexity of the interaction between parents and offspring (Fox & Czesak 2000).

An important difference in my study to previous studies is that I measured the first reproductive event of females, so that female age effects are independent of parenting experience. Therefore, egg size may simply reflect greater time for acquisition of resources. On the other hand, variation in egg size in the seed beetle *Stator limbatus* is an adaptive response by the female due to her environment, to increase offspring survival (Fox et al. 1999). The increase in offspring size in *N. vespilloides* in this experiment may also be an adaptive response by mothers to increase offspring survival.

Parental care behaviours were also influenced by maternal age. Older caretaker females were found to provide more parental care, as predicted. An increase in parental care as females age has also been found in the Mongolian gerbil, independent of experience (Clark et al. 2002). This result is consistent with the Residual Reproductive Value, RRV, hypothesis that a mother will increase her investment in individual offspring as she ages because her number of potential future offspring will decrease (Clutton-Brock 1984; Pianka & Parker 1975; Trivers 1972). It is also possible that females increase the amount of time they spend performing parental care behaviours as they get older due to the decreasing quality of her offspring. The increase in average hatch mass of offspring suggests a pre-natal adaptive response by females to declining fitness of their offspring as females' age increases, in order to maximise offspring fitness.

This study is unique in investigating the effect of female age on offspring begging behaviour. Offspring spent more time begging to older caretakers but this is probably because those females spent more time near the larvae and larvae only beg when the female is present (Smiseth & Moore 2002). I also found that dam age has no effect on offspring begging behaviours. This suggests that a female alters her parental care behaviours based on her age, not the age of the offspring for which she is caring because offspring behaviour is not affected by the age of their dam.

Despite an increase in larval hatch mass as dams age, a lack of difference in the begging behaviours of offspring and an increase in the parental care behaviours of caretaker females as they age, there is an interaction between dam age and caretaker age when offspring are cross fostered. Massgains by larvae from dams of the two age groups during the parental care period were highest when dam age and caretaker age were the same. This may be due to an integration of prenatal and postnatal maternal effects, to optimise

offspring fitness. Evidence for variation in care strategies has previously been found in the burying beetle *N. pustulatus* (Rauter & Moore 2002b). Variation in prenatal and postnatal parental care may be maintained to allow parental care behaviours to be adaptive to optimise offspring fitness.

Offspring from females of both age groups had the heaviest mass at dispersal when their dam and caretaker are the same age. This may provide further evidence for an adaptive response to increasing maternal age, due to the decreasing fitness of their offspring. Offspring from older dams are heavier at hatching and older caretakers provide more care, which would suggest the offspring with an old dam and an old caretaker should have the heaviest dispersal mass at the end of the parental care period. However, offspring from both age groups are equally fit when their dam and caretaker female are the same age.

There was also an interaction effect of dam age and caretaker age on the amount of time larvae spent on the carcass, with larvae spending less time when the dam age and caretaker age are the same. This may be because offspring were receiving inappropriate care and stayed on the carcass longer to self-feed, in an attempt to increase their mass at dispersal. Contrary to many studies, there were no dam age effects on development times, indicating that maternal age has no effect on development times. Offspring of older mothers of seed beetles have a longer lifespan (Fox et al. 2003). However, the long-term effect of maternal age on offspring lifespan was not investigated in this study.

This experiment suggests that offspring from younger mothers are less fit when cared for by an older caretaker, despite receiving more care. This may be because when an offspring's dam and caretaker are the same age the offspring is receiving appropriate care, the care it needs. Offspring need is based on physiological changes in the female (prenatal maternal effects), which results in a change in the fitness of her offspring and also in the

size of offspring she produces and the parental care she provides (postnatal maternal effects). When offspring receive the care they need they are equally fit due to a complex balance of negative and positive effects on offspring. Negative effects are that parents also feed themselves from the carcass (Smiseth & Moore 2004c). Positive effects are that parental care behaviours have positive effects on offspring performance and fitness (Lock et al. 2004). Thus, the fitness of offspring is unaffected by maternal age due to the adaptive prenatal and postnatal maternal effects of contributions to eggs and parental care behaviours.

### **3 Parental effects due to variation in maternal and paternal care**

### 3.1 Introduction

Parents influence offspring both through the genes they pass on and through parental effects, i.e. the environment they provide to their offspring (Mousseau & Fox 1998). In species with parental care, the environment provided by the parents can be a major source of the environmental influences during every life history stage of offspring. From a quantitative genetic perspective, the parental care environment is often investigated as a maternal effect, especially prenatal effects such as egg constituents (Roubertoux et al. 1990). However, in species with parental care, parental effects can be postnatal as well as prenatal, although quantitative geneticists have investigated this less often (Cheverud & Moore 1994).

One of the difficulties in estimating how parental care results in parental effects is quantifying care behaviour (Agrawal et al. 2001; Kolliker & Richner 2001; MacColl & Hatchwell 2003). Often parental care behaviours are not limited to those aimed directly at the offspring, and therefore difficult to measure, or behaviours may be hard to observe. For example, (Hager & Johnstone 2003) measured maternal provisioning as the decrease in maternal mass during two hours of offspring feeding. Therefore, the problems associated with the direct measurement of parental care have led to few studies investigating the effects of variation in parental care.

One species where parental care is direct and variation has been documented is burying beetles, in the genus *Nicrophorus* (Eggert & Muller 1997; Scott 1998). Burying beetles provide extended postzygotic parental care to maximise the fitness of their offspring (Scott 1998; Tallamy & Brown 1999) and to increase the development rate of the larvae (Rauter & Moore 2002b).

Successful reproduction by *Nicrophorus* is dependent upon locating a mate and the carcass of a small vertebrate, which is required for food and as nests for developing larvae (Eggert & Muller 1997; Scott 1998). Parents prepare the carcass for their larvae by removing its hair or feathers, covering it with oral or anal secretions to delay decay, and chew a hole in the top from which larvae feed (Milne & Milne 1976). The carcass is then buried and the female lays eggs in the soil nearby. After hatching the larvae crawl into the hole on the top of the carcass, where they are cared for by their parents (Beeler et al. 1999; Eggert & Muller 2000; Eggert et al. 1998; Jenkins et al. 2000; Scott 1998). During the parental care period parents are observed performing carcass maintenance as they walk around the carcass, cleaning it and depositing antibacterial and proteolytic secretions to delay decomposition (Eggert & Muller 1997; Scott 1998). Larvae are able to self-feed from the carcass but also beg by stimulating their parents' mouthparts, when the parent is in close proximity to the larvae (Rauter & Moore 1999). The parents provision the larvae by regurgitating pre-digested carrion directly, through mouth-to-mouth contact (Milne & Milne 1976).

In burying beetles parental care can be uniparental by either females or males (Trumbo 1991). When parents cooperate to provide biparental care there are sex-specific differences in the time parents spend performing different parental care behaviours (Smiseth et al. 2005; Smiseth & Moore 2004a). In contrast, under laboratory conditions both females and males are equally competent single parents (Bartlett 1988; Smiseth et al. 2005). There is no difference in the amount of care provided by females and males under uniparental conditions, on average (Smiseth et al. 2005; Smiseth & Moore 2004a).

Previous studies investigating sex-differences in the parental care behaviours of *Nicrophorus* species have compared time allocation to parental care behaviours (Smiseth et

al. 2005; Smiseth & Moore 2004a), and the effects on offspring quality (Muller et al. 1998). These studies do not investigate the influence of individual parental variation on offspring phenotype.

In this study I adopt a quantitative genetic approach to investigate environmental influences associated with variation in parental care in the burying beetle, *Nicrophorus vespilloides*. I capitalise on the fact that on average both sexes are equally competent parents (Bartlett 1988; Fetherston et al. 1994; Smiseth et al. 2005; Trumbo 1991) and split offspring between their parents. This allowed me to control for prenatal maternal effects and specifically investigate postnatal parental care effects.

Typically, paternal effects are thought to be rare (Lande & Price 1989). The partial regression coefficient of the strength of maternal effects ( $m$ ) is calculated as the difference between the mother-offspring correlation and the father-offspring correlation (Lande & Price 1989). However in many species, such as *N. vespilloides*, fathers provide postnatal parental care, indicating the existence of paternal effects (Lacey 1998). Following on from the Lande and Price (1989) method for estimating the strength of the maternal effect ( $m$ ), the strength of the paternal effect ( $f$ ) is estimated as the difference between the offspring-father regression and the offspring-mother regression when there is paternal care (Arnold 1994). Due to the split-family design used in this experiment we were able to calculate the partial regression coefficients,  $m$  and  $f$ , as the difference between the parent-offspring correlation in shared and unshared social environments, because in unshared environments a parent of the other sex is providing parental care.

Due to the quantitative nature of behaviour there is huge variation in parental care behaviour between individuals. I measured the influence of variation on offspring using five offspring phenotypic characters, three parental care behaviours, one larval morphology

character, and one larval life history character. The characters used all have reliable experimental measures and have been shown to be heritable and have parental effects in other species. I used different types of offspring phenotypic traits to see if they are influenced in a similar way by the effects of parental variation. Life history traits are under strong directional selection and I therefore expect parental care variation to have a smaller effect than on behavioural traits, which are more plastic.

The aim of this experiment was to measure the genetic and environmental contributions of parents to variation in offspring traits in *N. vespilloides*. To do this I measured the resemblance between parents and offspring (heritability) and the causal coefficients describing parental effects on five offspring phenotypic traits. This allowed me to compare the genetic variation in the parental care behaviours of females and males, and their influences on offspring phenotype.

## **3.2 Materials and Methods**

### **3.2.1 General Procedures**

The burying beetles used in this experiment were derived from a laboratory population bred to minimize inbreeding. The laboratory population originated from 110 females and 57 males trapped in Japanese insect traps. Twenty insect traps were baited with rotting beefsteak and hung from the trees in Sunbank Wood in the Mersey Valley in August 2003. The traps were checked after one week and two weeks. Wild-caught individuals were housed individually in plastic containers (17 x 12 x 6 cm) in the laboratory. Females were provided with a defrosted mouse carcass (Livefoods Direct, Sheffield, UK) and left to breed. Any females that did not breed were provided with a male and a new carcass a week later. This produced the first generation of the laboratory populations. Prior to experimental

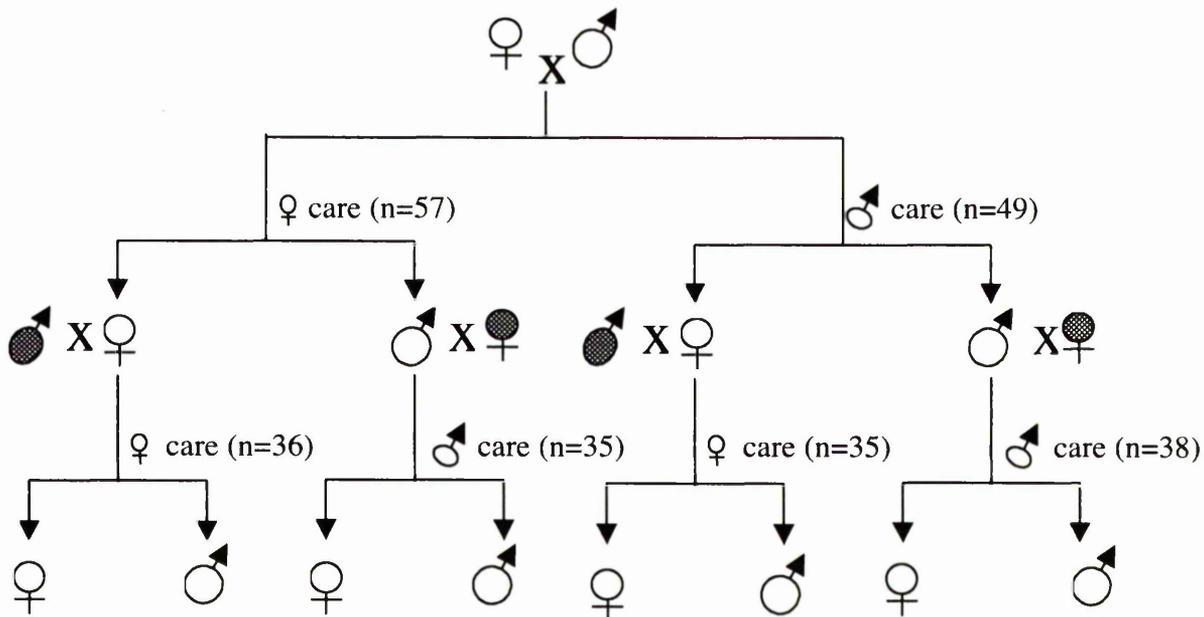
use the beetles were housed individually in clear plastic containers (17 x 12 x 6 cm) at 20°C under a 15:9h light:dark cycle and fed decapitated mealworms (*Tenebrio*) twice a week.

### **3.2.2 Experimental procedure**

#### **3.2.2.1 Parental generation**

All beetles used in this experiment were aged 2-3 weeks post-emergence as an adult, to control for age effects on parental care and offspring fitness. The breeding design is shown in figure 3.1. Virgin parental generation pairs were set up in a clear plastic box (17 x 12 x 6 cm) containing 2 cm depth of soil. Each pair was provided with a mouse carcass between 15 and 20g (Livefoods Direct, Sheffield, UK) at 5pm, which is two hours before the dark cycle begins in the laboratory and when beetles in the wild typically search for carrion.

**Figure 3.1: Breeding design used to investigate parental and grandparental sex effects on offspring life history traits. The hatched symbols represent stock beetles, which did not provide any parental care.**



Forty-eight hours after the parents were provided with a carcass the eggs were collected and placed in a Petri dish on damp filter paper. One parent was placed with the carcass in a new plastic box. The Petri dishes were placed on top of the box containing the carcass to keep account of which larvae belonged to which female. The parent who was not placed in the box with the carcass was put in a box by itself, with a label to identify the pair to which the individual belonged. Whether the female or male was kept with the carcass was decided at random.

Petri dishes were checked in the morning and afternoon for newly hatched larvae. Broods of 8 to 12 larvae were weighed and given to the parent in the box containing the carcass. The time at which they were given to the parent was noted, so that behavioural observations could be carried out exactly 24 hours later.

During this time the parent who had been removed from the carcass was fed a small amount of organic beef mincemeat, alternated with decapitated mealworms (*Tenebrio*), every three days, so that it experienced a change in diet quality similar to that of its partner, who was able to feed from the carcass. Upon dispersing from its brood the parent who had provided care was fed the same diet as its partner who had not provided care for a week. The male and female were then paired again.

At the second pairing, the male and female were again provided with a carcass, and eggs were collected 48 hours later. This time the parent who had been removed before was put in the box with the carcass and given the same number of offspring as the first parent. Parental care observations were carried out exactly 24 hours after the parent was given its offspring. This allowed separate male and female care of full-sib offspring, independent of brood order, because each parent is providing parental care for the first time. Therefore, each family was comprised a brood that received maternal care and a brood that received paternal care.

Larvae were weighed at dispersal from the carcass, at the end of the parental care period, and placed into individual plastic boxes (11x11x3.2cm). Dates of dispersal and pupation were recorded for all offspring to quantify the time spent in the wandering phase (see Traits measured).

### **3.2.2.2 Offspring generation**

Two weeks after emergence as an adult a son and daughter from each brood were each paired with an unrelated stock beetle. Again the pair was provided with a mouse of between 15 and 20g 2 hours before the dark cycle. Eggs were collected into Petri dishes 48 hours later. The stock beetle was removed when eggs were collected. The experimental beetle stayed with the carcass. When the larvae hatched, the son or daughter was provided with a larval brood the same size as the brood cared for by its parent. Exactly 24 hours later behavioural observations were carried out. This provided us with behavioural data for sons and daughters that were cared for by either their father or mother.

Larvae were weighed individually at dispersal from the carcass, and placed into their own small plastic boxes. Dates of dispersal and pupation and were recorded to give a measure of time spent wandering.

### **3.2.2.3 Traits measured**

The three parental care behaviours examined were: 1) percentage of time the parent spent maintaining the carcass to prevent decay; 2) percentage of time the parent spent in the cavity in close proximity to the offspring, that is within one pronotum length of the offspring, without being fed; 3) percentage of time the parent spent directly provisioning the offspring, indicated by mouth-to-mouth contact between parent and larva (Eggert & Muller 1997; Scott 1998).

The development trait was the duration of the developmental stage from dispersal to entering the pupal stage (i.e., the “wandering” stage where larvae disperse from the resource and no longer forage). The morphological trait I measured was larval weight at dispersal. Wandering duration and larval weight at dispersal are both known to be

influenced by parental care (Rauter & Moore 2002a; Rauter & Moore 2002b; Smiseth et al. 2003).

In this experiment three parental care behaviours, one development trait and one morphological trait were examined. In *Nicrophorus* care occurs for 5-7 days but is most important during the first 24 hours (Eggert et al. 1998; Smiseth & Moore 2002). Behavioural observations of the parental and offspring generations were carried out exactly 24 hours after each parent was given their brood. Parental care behaviours were recorded using the instantaneous scan sampling (Martin & Bateson 1986). Observations lasted 30 minutes, during which all behaviours performed by parents and offspring were scored, as in previous studies (Smiseth & Moore 2002; Smiseth & Moore 2004d), however only 3 measures of parental care behaviours are considered here because they are the most easy to distinguish: parent within near proximity to the larvae, carcass maintenance and larval provisioning.

### **3.2.3 Statistical methods**

#### **3.2.3.1 Differences between Mothers and Fathers**

One of the assumptions of my analyses is that there is no selection for caretaker sex bias i.e., the traits are the same in males and females (Falconer & Mackay 1996). Also, the traits are expected to be the same across generations. Previous work on burying beetles has found no difference in the average levels of females and males when providing uniparental care (Bartlett 1988; Smiseth et al. 2005). I used analysis of variance to test for differences between parental care behaviours of mothers and fathers and life history traits of their larvae in the parental generation. ANOVA was also used to test for differences in parental care behaviours and larval life history traits between sons and daughters in the offspring

generation. These analyses tested for sex differences in parental care. Differences between parental care behaviours and larval life history traits of the parental and offspring generations were also compared using ANOVA.

### **3.2.3.2 Resemblance between parents and offspring**

The resemblance between parents and offspring were calculated from parent-offspring regressions between the same behaviours in both generations. Mean values for all offspring generation (mid-offspring) parental care traits were regressed on the traits measured on the parent who provided care, to give a shared environment estimate of heritability. Mid-offspring traits were also regressed on the traits measured on their parent who did not provide care, giving an estimate of heritability between parents and offspring that did not share an environment. This eliminated any shared environment effects. I calculated estimates of the degree of resemblance between parents and offspring (heritability) as twice the regression coefficients obtained from parent-offspring regression (Falconer & Mackay 1996). The heritability estimate of a trait was regarded as significant when it was more than two standard errors from zero (Lynch & Walsh 1998).

### **3.2.3.3 Estimates of Maternal (m) and Paternal (f) effects**

Estimates of maternal (m) and paternal (f) effects were calculated following (Lande & Price 1989). The coefficient of the strength of the maternal effect is usually calculated as the difference between the offspring-mother regression and the offspring-father regression (Arnold 1992). The coefficient of the strength of the paternal effect is usually calculated as the difference between the offspring-father regression and the offspring-mother regression

(Arnold 1992). Due to the split family experimental design used in this experiment  $m$  is therefore calculated as the difference between the shared environment mother-offspring regression and the unshared environment mother-offspring regression. The coefficient of the strength of a paternal effect ( $f$ ) is calculated as the difference between a shared environment father-offspring regression and an unshared environment father-offspring regression. The parental effect coefficients,  $m$  and  $f$ , are bound by  $\pm 1$  (Price 1998). In this experiment the coefficients measure the strength of postnatal maternal and paternal effects because prenatal effects were the same for each family.

### **3.3 Results**

#### **3.3.1 Descriptive statistics**

There was no significant difference between the sexes in any of the parental care behaviours in the parental generation (table 3.1). The sex of the caretaker did not influence offspring traits. There were not significant differences in the effects of care by the two sexes on offspring (table 3.1). The same pattern held for the next generation as well (table 3.2). I therefore was able to consider male and female parental care equivalent.

**Table 3.1: The influence of the sex of the caretaker in the parental generation on three parental care behaviours, one offspring development traits and one offspring life history trait**

	<b>Mother mean</b> <b>(SE)</b>	<b>Father mean</b> <b>(SE)</b>	<b>ANOVA</b>
<b>Parental care behaviours</b>			
<b>Carcass maintenance (%)</b>	16.1 (1.7)	17.1 (1.8)	$F_{1,86}=0.167, p=0.683$
<b>Proximal to larvae (%)</b>	59.8 (5.4)	60.4 (4.9)	$F_{1,83}=0.008 p=0.937$
<b>Provisioning larvae (%)</b>	12.1 (1.4)	17.4 (2.5)	$F_{1,57}=3.829, p=0.055$
<b>Offspring traits</b>			
<b>Wandering phase (days)</b>	11.8 (0.3)	11.5 (0.2)	$F_{1,97}=1.053, p=0.367$
<b>Mass at dispersal (g)</b>	184.7 (3.1)	183.0 (2.8)	$F_{1,160}=0.162, p=0.689$

**Table 3.2: The influence of the sex of the caretaker in the offspring generation on three parental care behaviours, one offspring development traits and one offspring life history trait**

	<b>Female mean (SE)</b>	<b>Male mean (SE)</b>	<b>ANOVA</b>
<b>Parental care behaviours</b>			
<b>Carcass maintenance (%)</b>	14.8 (1.7)	14.9 (1.7)	$F_{1,114}=0.001, p=0.980$
<b>Proximal to larvae (%)</b>	57.9 (4.9)	57.3 (4.7)	$F_{1,104}=0.007 p=0.933$
<b>Provisioning larvae (%)</b>	10.0 (1.2)	13.2 (1.3)	$F_{1,63}=1.031, p=0.312$
<b>Offspring traits</b>			
<b>Wandering phase (days)</b>	11.8 (0.3)	11.5 (0.2)	$F_{1,138}=1.031, p=0.312$
<b>Mass at dispersal (g)</b>	184.7 (3.1)	183.0 (2.8)	$F_{1,142}=1.005, p=0.318$

### **3.3.2 Resemblance between parents and offspring**

#### **3.3.2.1 Mother and mid-offspring resemblance**

The amount of resemblance between mothers and offspring when offspring were reared by their mothers was calculated as the heritability in a shared environment ( $h^2_{\text{shared}}$ ). All heritability estimates were low (table 3.3), with only wandering having a heritability more than two standard errors away from zero.

The resemblance between mothers and offspring when offspring were reared by their fathers was estimated as the heritability in an unshared environment ( $h^2_{\text{unshared}}$ ). The heritability estimates were somewhat larger than those calculated in the shared environment (table 3.3). However, again, only one trait (dispersal mass) had a heritability estimate significantly larger than zero.

#### **3.3.2.2 Father and mid-offspring resemblance**

Resemblance between fathers and offspring when that male provided the rearing environment were estimated by heritability in a shared environment ( $h^2_{\text{shared}}$ ). All the estimates were low (table 3.4), with no traits having a heritability two standard errors from zeros.

The resemblance between fathers and offspring when, the offspring were reared by their mother, was estimated by calculating heritability in an unshared environment ( $h^2_{\text{unshared}}$ ). Heritability estimates were again low (table 3.4). Two traits had heritability estimates that were more than two standard errors from zero. These were the duration of the larval wandering phase and dispersal mass.

**Table 3.3: Estimates of resemblance between mothers and offspring (maternal heritability) of three parental care behaviours, one development stage duration, and one morphological character between mother and mid-offspring in *Nicrophorus vespilloides*. Heritability estimates were calculated between parents and the offspring they reared (shared environment) and offspring that were reared by their mate (unshared environment).**

<b>Mother and mid-offspring</b>						
Shared Environment						
<b>Trait</b>	<b>Mean</b>	<b>V<sub>P</sub></b>	<b>CV<sub>P</sub></b>	<b>V<sub>A</sub></b>	<b>CV<sub>A</sub></b>	<b>h<sup>2</sup> (SE)</b>
Maintenance	19.202	226.179	78.321	34.288	30.495	0.22 (0.29)
Proximity	57.513	952.702	53.668	117.226	18.825	0.12 (0.42)
Provisioning	13.504	51.261	53.019	-0.794	-6.599	-0.12 (0.45)
Wandering	11.843	3.357	15.471	2.04	12.060	0.63 (0.31)
Dispersal Mass	0.183	3.8E-4	10.652	2.7E-05	2.845	0.06 (0.25)
Unshared Environment						
<b>Trait</b>	<b>Mean</b>	<b>V<sub>P</sub></b>	<b>CV<sub>P</sub></b>	<b>V<sub>A</sub></b>	<b>CV<sub>A</sub></b>	<b>h<sup>2</sup> (SE)</b>
Maintenance	16.017	136.4	72.917	80.844	56.136	0.47 (0.31)
Proximity	55.706	1056.82	58.358	122.436	19.863	0.12 (0.41)
Provisioning	14.918	62.332	52.923	41.32	43.089	0.71 (0.57)
Wandering	11.637	4.459	18.146	1.76	11.400	0.26 (0.18)
Dispersal Mass	0.183	3.64E-4	10.420	1.4E-4	6.417	0.27 (0.13)

**Table 3.4: Estimates of resemblance between fathers and offspring (paternal heritability) of three parental care behaviours, one development stage duration, and one morphological character between fathers and mid-offspring a shared and an unshared environment in *Nicrophorus vespilloides*.**

<b>Father and mid-offspring</b>						
Shared Environment						
Trait	Mean	V <sub>P</sub>	CV <sub>P</sub>	V <sub>A</sub>	CV <sub>A</sub>	h <sup>2</sup> (SE)
Maintenance	16.176	124.051	68.854	19.482	27.286	0.16 (0.42)
Proximity	56.905	1043.662	56.771	453.012	37.403	0.37 (0.33)
Provisioning	17.591	96.824	55.937	-53.53	-41.592	-0.64 (0.51)
Wandering	11.848	2.276	12.733	0.924	8.113	0.36 (0.27)
Dispersal Mass	0.184	2.27E-04	8.181	4.4E-05	3.605	0.27 (0.39)
Unshared Environment						
Trait	Mean	V <sub>P</sub>	CV <sub>P</sub>	V <sub>A</sub>	CV <sub>A</sub>	h <sup>2</sup> (SE)
Maintenance	15.44	137.622	75.980	-8.062	-18.390	-0.06 (0.34)
Proximity	56.55	1084.212	58.227	565.844	42.065	0.52 (0.37)
Provisioning	16.053	97.619	61.548	-78.704	-55.264	-0.50 (0.31)
Wandering	11.544	2.655	14.115	2.148	12.696	0.94 (0.37)
Dispersal Mass	0.184	3.38E-4	9.993	2.2E-4	8.145	0.60 (0.29)

### 3.3.3 Estimates of Maternal (m) and Paternal (f) effect coefficients

Estimates of maternal (m) and paternal (f) postnatal effects are shown in table 3.5. All effects were small except for the maternal effect on larval provisioning, which was quite large and negative.

For three traits there was a difference in the direction of the parental effects depending on the sex of the parent. There was a negative maternal effect on carcass maintenance but a positive paternal effect. There was a positive maternal effect on parental proximity to offspring and on larval wandering phase. These two traits were influenced by a negative paternal effect. For the other two traits the maternal and paternal effects were in the same direction. Mothers and fathers negatively affected larval provisioning behaviour. Maternal and paternal effects positively influenced larval mass at dispersal. However, due to the small strength of nearly all parental effects these differences may be due to the small sample size of the data set.

**Table 3.5: Estimates of maternal (m) and paternal (f) effects on three parental care behaviours, one larval development trait and one larval life history trait. Values of m and f were obtained using the method of Arnold (1994).**

<b>Trait</b>	<b>M</b>	<b>f</b>
<b>Carcass maintenance (%)</b>	-0.13	0.11
<b>Near Proximity (%)</b>	0	-0.07
<b>Larval Provisioning (%)</b>	-0.37	-0.07
<b>Wandering duration (days)</b>	0.02	0.11
<b>Mass at Dispersal (g)</b>	0.01	-0.1

### **3.4 Discussion**

Phenotypic differences in parental care behaviours and their phenotypic effects on offspring life history traits have been investigated previously in burying beetles (Bartlett 1988; Eggert & Muller 1997; Fetherston et al. 1994; Smiseth et al. 2005; Smiseth & Moore 2004a). In this study I measured the effects of parent sex on genetic and phenotypic variation, and on the heritability of parental care behaviours and life history traits. I also investigated the genetic correlation between parental care behaviours and between life history traits under female and male care environments. Finally, I calculated matrices of the causal coefficients describing maternal (m-matrix) and paternal (f-matrix) effects. My goal was to investigate the differences in maternal and paternal effects on offspring characters.

#### **3.4.1 Resemblance between parents and offspring**

The degree of phenotypic resemblance between related individuals is proportionate to the heritability of traits between parents and offspring (Falconer & Mackay 1996). The total heritability of a trait is determined by the contributions of direct and indirect (environmental) genetic effects on the phenotype of the trait (Rauter & Moore 2002a). In this experiment the contributions of indirect and direct genetic effects were separated by estimating heritability values from regression between parents and offspring they shared a social environment with, and those from an unshared environment. Estimates of heritability from shared environment regressions will also include environmental effects of parental care. Therefore, any differences in the heritability estimates in shared and unshared environments will be due to maternal and paternal effects of parental care.

My estimates of resemblance were either low or zero. There was a sex difference in the resemblance, despite finding no difference in the parental care provided by females and

males. This is consistent with previous studies that have found that single parents provide a similar level of care on average (Bartlett 1988; Bartlett & Ashworth 1988; Fetherston et al. 1994; Smiseth et al. 2005; Smiseth & Moore 2004a; Smiseth & Moore 2004c; Trumbo 1991). When parents and offspring shared an environment the resemblance between the trait larval wandering was larger between mothers and offspring than between fathers and offspring. However, this trend was reversed in an unshared environment, with resemblance being greatest between fathers and offspring. This suggests that mothers have a larger environmental influence on this trait, through their parental care behaviours, but fathers had a more direct genetic effect. This means that both parents have an influence on this trait.

There was moderate resemblance for larval dispersal mass between mothers and offspring in an unshared environment. The resemblance between fathers and offspring for this trait is also high, in an unshared environment. The moderate and high values of inheritance from mothers and fathers suggest that again the trait is influenced by both mothers and fathers. Larvae with a larger dispersal mass have a higher survivorship to adulthood (Chapter 1, published as Lock et al 2004). Hence, there is strong selection for large larvae. Larval dispersal mass is positively correlated with parental performance (Chapter 1, published as Lock et al 2004). The resemblance between parents and offspring may occur because dispersal mass has such a large effect on offspring survivorship. Therefore, larval mass at dispersal is similar to that of both their mother and father.

In burying beetles uniparental care is rare in nature, especially for fathers, suggesting that sex differences have evolved under biparental due to conflict between the parents over parental care (Muller et al 1998, Smiseth et al 2004, Smiseth & Moore 2004a, Rauter & Moore 2004). Under biparental care the value of the male's participation is unclear because uniparental and biparental care have equivalent effects on offspring fitness

(Smiseth & Moore 2002). It has been suggested the male stays to copulate with the female, to ensure paternity of the brood and a possible second brood, if the female finds another carcass before mating with a different male (Müller et al 1998).

The estimates of resemblance between parents and offspring were influenced by whether offspring and parents shared an environment. In general estimates of resemblance between parents and offspring were slightly larger when parents and offspring did not share an environment. This indicates that parents may have a negative effect on the phenotype of their offspring when in a shared environment. However, estimates of maternal and paternal effect coefficients were very small, providing little evidence of parental effects.

#### **3.4.2 Maternal and Paternal effects**

The finding that maternal and paternal effects were either very small or negative is consistent with the findings of other experiments investigating maternal effects in insects (reviewed in Reinhold 2002), despite the elaborate parental care behaviours displayed by male and female burying beetles. There was only one trait with a relatively large maternal effect, larval provisioning. The coefficient of the strength of the maternal effect ( $m$ ) on this trait was larger than that of the other traits and negative,  $-0.37$ . This indicates that maternal provisioning of larvae has a negative maternal effect on larval provisioning by the next generation. Thus, if in behavioural observations a mother provisions her offspring a lot, behavioural observations of those offspring when they are parents will show small levels of provisioning.

Negative maternal effects are predicted when selection for an increased value of an offspring character may result in a decrease in the character, and selection for an increase in an offspring character may result in a decrease in maternal performance. This causes

evolution to proceed in opposition to adaptation due to inheritance (Cheverud & Moore 1994).

One study to report a negative maternal effect was carried out on Springtails (Janssen et al. 1988). The regression slope for age at maturity between mothers and daughters was negative, indicating a negative maternal effect on age at maturity (Janssen et al. 1988). The negative effect was interpreted as a result of springtails producing two broods a year, so that successive generations encounter different environments, Summer or Winter, but grandparents and their grandchildren will encounter the same environment. Under these conditions a negative maternal effect is adaptive because it will result in differences in the maturity age of mother and daughters, which will correspond with changes in the environment (Janssen et al. 1988). However, the negative maternal effect was not found when the experiment was repeated to test whether it allowed an adaptation to the environment (Stam et al. 1998).

In *N. vespilloides* there is a positive relationship between maternal provisioning and offspring begging; this means that offspring that beg a lot have parents that provision them a lot (Chapter 1, published as Lock et al 2004). However, the negative maternal effect implies that when these offspring are parents they will spend less time provisioning their larvae. A reduction in parental care has been found to have harmful effects on offspring fitness in another burying beetle species, *N. pustulatus* (Eggert et al 1998, Rauter & Moore 2002b).

An experiment on *N. vespilloides* also found that larval fitness is significantly lower when parental care is reduced (Lock et al in prep). Parental care behaviours of offspring, as parents, that received either full or reduced parental care were also observed. No significant difference was found in parental care but the morphological and life history result data

suggest there was a difference in parental care behaviours, which may not have been due to disturbance of the beetles during the observational period. Offspring cared for by a mother whose had received reduced parental care as a larva had a larger dispersal mass and shorter wandering phase than those whose mother had received full parental care (Lock et al in prep).

The negative maternal effect may occur due to the interaction between prenatal and postnatal effects, so that mothers invest either in eggs or in parental care. Hence, offspring that received a high level of maternal provisioning will produce fitter, high quality eggs, which hatch into larvae that require less provisioning. The decreased fitness of these individuals, due to reduced parental care, causes them to produce lower quality eggs, and therefore offspring that require a high level of maternal provisioning.

### 3.4.3 Conclusions

In the burying beetle *N. vespilloides*, parental effects, due to individual variation in parental care, were found to have a variable effect on the resemblance between parents and offspring of, depending on the sex of the parent. The split-family experimental design allowed estimation of the partial regression coefficients measuring the effect on the mother (m) and father's (f) phenotypes on offspring. These were found to be very small on all traits, apart from the maternal effect on provisioning behaviour. This had a large, negative maternal effect.



# **4 Parental and grandparental effects on offspring development**

## 4.1 Introduction

Maternal effects occur when the phenotype of an individual is due not only to its genotype and the environment it experienced during development, but also by the phenotype of its mother (Wade 1998). Maternal effects are ubiquitous and can occur at any stage of development. Therefore, there are many different types of maternal effects (Cowley & Atchley 1992). For example, in species that provide parental care, offspring experience two distinct parental environments, prenatal and postnatal (Cowley & Atchley 1992). Prenatal maternal effects typically reflect cytoplasmic factors in the mother's egg, for example, the amount of yolk, hormones and mRNA's (Mousseau & Fox 1998). Prenatal maternal effects can also reflect the uterine or internal environment in species that are viviparous (Roubertoux et al. 1990). Postnatal maternal effects occur due to differences in resource provisioning of mothers, natural variation in the treatment of offspring by mothers of different genotypes, and previous experience of parental care (Crusio & Schmitt 1996).

The nature of prenatal and postnatal maternal interactions on offspring postnatal development is not well characterised (Wolf et al. 1998) because the role of interactions between progeny genotype and parental environment has been largely ignored (Rhees et al. 1999). However, embryo manipulation studies have proven invaluable in separating prenatal and postnatal maternal effects (Cowley 1991). Cross-fostering studies have also allowed further investigation of postnatal maternal effects (Rhees et al. 1999).

The environmental effects of the father on offspring phenotype are usually thought to be minimal or nonexistent because most males provide little in the way of prenatal parental environments (Bernardo 1996). However, postnatal paternal effects can be important (Fox et al. 1995), especially in species that display biparental or paternal care. For biparental systems the term "parental effect" is more appropriate because both mothers

and fathers provide parental care. A parental effect can be defined as an effect on offspring phenotype that is not caused by the genotype of the offspring, non-parental environmental components or a combination of the two, it is therefore due to the parental environment (Lacey 1998). A parental effect occurs when the parent responds to a change in its environment or condition by adapting the parental care it provides to its offspring. The resultant parental effect is a phenotypic modification of an offspring trait (Lacey 1998).

Parental effects are clearly important modifiers of offspring phenotypes but parental effects can persist into subsequent generations as a grandparental effect (Mazer & Damuth 2001). Grandparental effects occur when a grandparent's parental performance influences the parental care environment provided by its offspring, therefore affecting the phenotype of its grandchildren (Rossiter 1996). Such effects may be subtle and are often overlooked. For example, (Magiafoglou & Hoffmann 2003) investigated grandparental effects of cold exposure in *Drosophila serrata*. In this species the cross-generational effect on offspring viability depends on the sex and generation exposed to cold shock (Magiafoglou & Hoffmann 2003).

Burying beetles are one of only a handful of insect species that exhibit extended biparental care (Zeh & Smith 1985). Male and female beetles will fly in search of small vertebrate carcasses in the afternoon and evening (Muller & Eggert 1987). Upon finding a carcass the adults compete, until a single dominant pair remains (Trumbo 1990). The pair then cooperates to bury the carcass, removing hair or feathers and shaping the carcass into a ball (Milne & Milne 1976; Scott 1998; Trumbo 1990). Eggs are laid in the soil and hatch about sixty hours later as altricial larvae that crawl into a hole the parents have chewed in the carcass, where they are provisioned regurgitated carcass by both parents (Scott 1998). Burying beetles are not completely dependent on care and can either beg or self-feed;

leading to *N. vespilloides* being described as a “partially-begging” species (Smiseth et al. 2003; Smiseth & Moore 2002; Smiseth & Moore 2004a; Smiseth & Moore 2004b; Smiseth & Moore 2004d).

Providing elaborate parental care allows burying beetles to maximise the lifetime fitness of their current brood, because due to the rarity of and competition for small carcasses, it may be a pair’s only opportunity to reproduce (Scott & Gladstein 1993). The details of burying beetle parental care behaviours are summarised in two recent reviews (Eggert & Muller 1997; Scott 1998).

In *N. vespilloides*, both parents can cooperate to raise the brood, providing biparental care (Bartlett 1988). Both sexes may also rear broods as single parents (Bartlett 1988; Smiseth et al. 2005). Under biparental conditions there are sex-specific differences in the time parents allocate to different parental care behaviours, with females spending more time provisioning larvae and males spending more time maintaining the carcass (Smiseth et al. 2005; Smiseth & Moore 2004a). However, under laboratory conditions both females and males are equally competent single parents (Bartlett 1988; Smiseth et al. 2005). There is no difference in the amount of care provided by females and males under uniparental conditions (Smiseth & Moore 2004a; Smiseth et al. 2005).

My goal was to examine prenatal and postnatal parental effects and grandparental effects on life history traits in the burying beetle *Nicrophorus vespilloides*. I carried out a split-family experiment to separate postnatal variation in parental care from prenatal maternal effects. I was able to capitalise on the fact that in burying beetles the average level of care provided by females and males as single parents is the same (Bartlett 1988; Fetherston et al. 1994; Rauter & Moore 2004; Smiseth et al. 2005; Trumbo 1991) and use the natural variation between individuals in parental care behaviours to investigate the

effects of environmental variation on offspring. To investigate grandparental effects we continued the experiment to a third generation to investigate whether parental effects persist as grandparental effects.

## **4.2 Materials and Methods**

### **4.2.1 General Procedures**

The burying beetles used in this experiment were from a laboratory population bred to minimize inbreeding. The laboratory population originated from 110 females and 57 males trapped in Japanese insect traps. Twenty insect traps were baited with rotting beefsteak and hung from the trees in Sunbank Wood in the Mersey Valley in August 2003. The traps were checked after one week and two weeks. Wild-caught individuals were housed individually in clear plastic containers (17 x 12 x 6 cm) in the laboratory. Females were provided with a defrosted mouse carcass (Livefoods Direct, Sheffield, UK) and left to breed. Any females that did not breed were provided with a male and a new carcass a week later. This produced the first generation of the laboratory populations.

Prior to experimental use the beetles were housed individually in clear plastic containers (17 x 12 x 6 cm) at 20°C under a 15:9h light:dark cycle and fed decapitated mealworms (*Tenebrio*) twice a week.

### **4.2.2 Experimental procedure**

#### **4.2.2.1 Parental effects**

All beetles used in this experiment were aged 2-3 weeks post-emergence as an adult, to control for age effects on parental care and offspring fitness. The breeding design is shown in figure 3.1. Virgin parental generation pairs were set up in a clear plastic boxes

(17 x 12 x 6 cm) containing 2 cm depth of soil. Each pair was provided with a mouse carcass between 15 and 20g (Livefoods Direct, Sheffield, UK) at 5pm, which is two hours before the dark cycle begins in the laboratory and when beetles in the wild typically search for carrion.

Forty-eight hours after the first generation parents were provided with a carcass the eggs were collected and placed in a Petri dish on damp filter paper. One parent was placed with the carcass in a new plastic box. Whether the female or male was kept with the carcass was decided at random.

The next day the Petri dishes were checked in the morning and afternoon for newly hatched larvae. Broods of 8 to 12 larvae were weighed and given to the parent in the box containing the carcass. The larvae and parent were left until they dispersed from the carcass at the end of the parental care period.

During this time the parent who had been removed from the carcass was fed a small amount of organic beef mincemeat, alternated with decapitated mealworms (*Tenebrio*), every three days, so that it experienced a change in diet quality similar to that of its partner, who was able to feed from the carcass. Upon dispersing from its brood the parent who had provided care was fed the same diet as its partner who had not provided care for a week. The male and female were then paired again.

At the second pairing, the male and female were again provided with a carcass, and eggs were collected 48 hours later. This time the parent who had been removed before was put in the box with the carcass and given the same number of offspring as the first parent. This allowed separate male and female care of full-sib offspring, independent of brood order, because each parent is providing parental care for the first time. Therefore, each

family was comprised a brood that received maternal care and a brood that received paternal care.

Previous experiments on *N. vespilloides* have found that single parents provide a similar level of care to their first and second broods (Lock et al. 2004). An increase in offspring mass at hatching is only found in the second brood of single mothers when the father assisted with biparental care in her first brood (Jenkins et al. 2000). Thus, brood order should have no effect on offspring life history traits. Regardless of this, we randomized which parent provided care first by tossing a coin.

All second generation broods had been weighed prior to being placed with their parent. Larvae were weighed again at dispersal from the carcass, at the end of the parental care period, and placed into individual plastic boxes (11x11x3.2cm). This gave the massweight gain value the parental care period. Dates of hatching, dispersal, pupation and eclosion were recorded for all offspring, in order to gain values for the time spent in the wandering phase, between dispersal and pupation, and the time spent as pupae, before emergence as an adult.

#### **4.2.2.2 Grandparental effects**

Two weeks after emergence as an adult a son and daughter from each second generation brood was paired with an unrelated stock beetle. Again the pair was provided with a mouse of between 15 and 20g two hours before the dark cycle. Eggs were collected into Petri dishes 48 hours later. The stock beetle was removed when eggs were collected. The experimental second generation beetle stayed with the carcass. When the third generation larvae hatched the second generation son or daughter was provided with a larval brood the same size as cared for by its parent. The broods were weighed at hatching, and

larvae were weighed individually at dispersal from the carcass, and placed into their own small plastic boxes. Dates of hatching, dispersal, pupation and eclosion were recorded. This gave measurements of mass gain, time wandering and time pupating.

#### **4.2.2.3 Traits measured**

The offspring life history traits we measured are known to be influenced by parental care (Rauter & Moore 2002a; Rauter & Moore 2002b; Smiseth et al. 2003). The life history traits measured were: the duration of the developmental stage from dispersal to entering the pupal stage (i.e., the “wandering” stage where larvae disperse off the resource and no longer forage), the duration of the pupal stage (i.e., from entering the pupal stage until emergence as an adult beetle) and mass gain during the parental care period (the change is larval mass from when they were placed on the carcass until dispersal from the carcass when parental care ceased).

#### **4.2.3 Statistical methods**

Statistical analyses were carried out using JMP 10 for the Macintosh. The magnitudes of G x E interactions were investigated using analysis of variance (Via & Conner 1995). A 2-way mixed model analysis of variance was used due to the presence of both fixed and random effects as sources of variation (Ayres & Thomas 1990). Family was a random factor while the sex of the parent or grandparent was a fixed factor. The SAS mixed-model method was used because it has a natural application for estimating differences in the expression of a character in different environments (Fry 1992).

In the 2-way mixed model ANOVA the main effects tested were the family and the sex of the parent. I also tested for an interaction between family and parental care

environment. I viewed the interaction between family and parental care environment graphically by producing reaction norms for each offspring life history trait. The family mean of each life history character expressed by offspring whose mother (or grandmother) had provided care to third (or second) generation offspring was plotted on the left axis, and family mean of each life history character expressed by offspring whose father (or grandfather) had provided care to the third (or second) generation offspring was plotted on the right axis. The genotypic means are connected by a line, the slope of which represents the phenotypic response of each family to a change in its parental care environment.

### 4.3 Results

#### 4.3.1 Parental effects on offspring life history traits

As expected, offspring did equally well when cared for by a female or a male parent. The overall mean values of life history traits, shown in table 4.1 were very similar in second generation offspring cared for by their mother or father. Further analysis investigated parental care environment effects separately on each family.

**Table 4.1: Mean values of life history traits for second generation offspring reared by either their mother or father. Standard errors are shown in brackets**

<b>Life history trait</b>	<b>Mean 2<sup>nd</sup> generation values with maternal care</b>	<b>Mean 2<sup>nd</sup> generation values with paternal care</b>
<b>Time spent wandering (days)</b>	11.5 (0.1)	11.4 (0.1)
<b>Time spent pupating (days)</b>	10.4 (0.1)	10.5 (0.1)
<b>Mass gain (g)</b>	0.2 (0)	0.2 (0)

The two-way mixed model ANOVA investigated parental effects (Table 4.2). There was a main effect of family on the number of days larvae spent in the non-feeding wandering phase. Parent sex did not have a main effect on the wandering phase. There was a significant interaction effect between family and parent sex on the duration of the wandering phase.

The length of time offspring spent as pupae was influenced by a main effect of family but there was no main effect of parent sex. As found for the wandering phase, there was a significant interaction effect between family and parent sex on the duration offspring spent pupating.

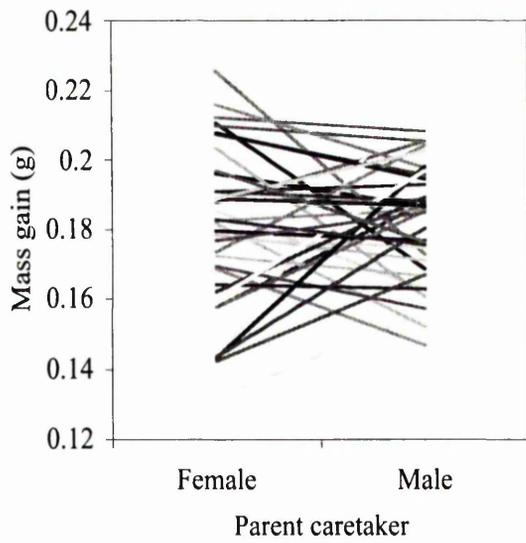
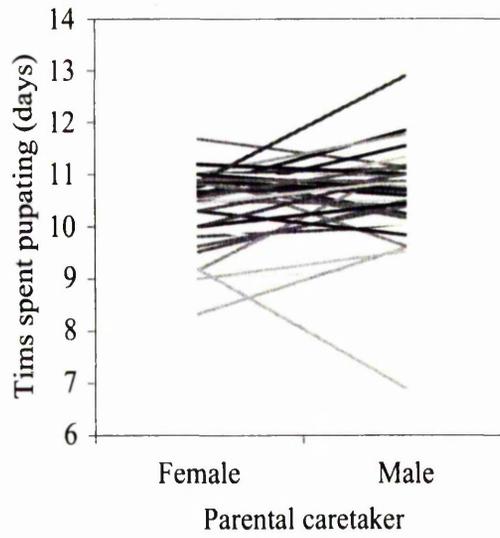
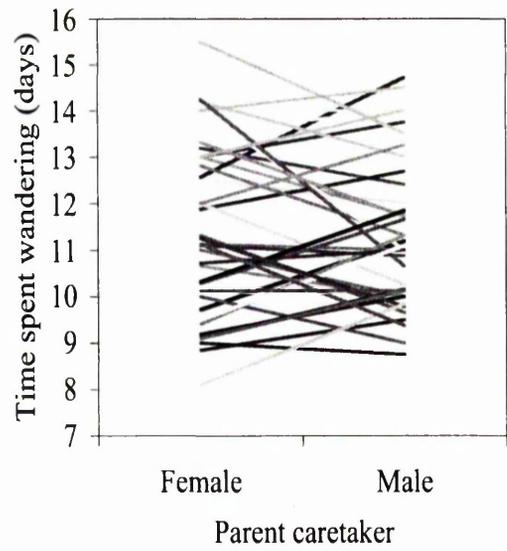
There was no main effect of family or parent sex on offspring weight gain during the parental care period. There was a significant interaction effect between the main effects, family and the sex of the parent providing care, on offspring mass gain.

**Table 4.2: Mixed model Analysis of Variance looking at the effects of family group and parent sex on three offspring life history traits**

<b>Character</b>	<b>Source of Variation</b>	<b>df</b>	<b>MS</b>	<b>F-ratio</b>	<b>F</b>	<b>P</b>
<b>Days Wandering</b>	Family	38	29.874	MSf/MSfp	5.6632	<0.001
	Parent	1	1.708	Msp/MSe	1.7122	NS
	Family x Parent	38	5.275	MSfp/MSe	5.2872	<0.001
	Error	404	403.062			
<b>Days Pupating</b>	Family	38	5.763	MSf/MSfp	2.8242	<0.001
	Parent	1	3.070	Msp/MSe	2.8428	NS
	Family x Parent	38	2.0407	MSfp/MSe	1.8896	<0.01
	Error	363	392.027			
<b>Mass gain</b>	Family	38	0.003	MSf/MSfp	1.3983	NS
	Parent	1	0.0002	Msp/MSe	0.3360	NS
	Family x Parent	38	0.0022	MSfp/MSe	4.7634	<0.01
	Error	477	0.0005			

The interaction effects for all three offspring life history traits are shown graphically in figure 4.1. Each line represents the phenotype exhibited by members of each family across an environment gradient, in this experiment female or male parental care. For all three traits there is a change in the rank and scale of the reaction norms, seen as the chaotic crossing of lines.

**Figure 4.1: Reaction norms of three offspring life history traits when either their mother or father provides parental care.**



### 4.3.2 Grandparental effects on offspring life history traits

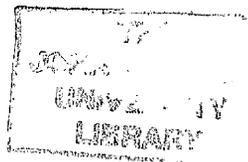
The second generation parents had similar effects on their third generation offspring, irrespective of their sex (Table 4.3). This corresponds with the similar parental sex effects of first generation parents on second generation offspring (Table 4.1).

**Table 4.3: Mean values of life history traits for third generation offspring reared by either their mother or father. Standard errors are shown in brackets**

Life history trait	Mean 3 <sup>rd</sup> generation values with maternal care	Mean 3 <sup>rd</sup> generation values with paternal care
Time spent wandering (days)	10.1 (0.1)	10.7 (0.1)
Time spent pupating (days)	10.5 (0.1)	10.4 (0.1)
Mass gain (g)	0.2 (0)	0.2 (0)

The persistence of first generation parental effects into a third generation, termed grandparental effects, was explored by investigating the main effects of grandparent sex and family on offspring life history traits (Table 4.4). Family did not significantly affect the wandering phase. There was a main effect of grandparent sex. When offspring's parents received maternal care the wandering phase was shorter than when offspring's parents received paternal care. The time offspring spent wandering was also influenced by the interaction between family and grandparent sex.

There was no main effect of family on the length of time offspring spend in the pupal chamber. There was a main effect of grandparent sex. The duration of the pupation phase was longer when offspring's parents received maternal care, than when offspring's



parents received paternal care. There was an interaction effect between family and grandparent sex on the number of days offspring spent pupating.

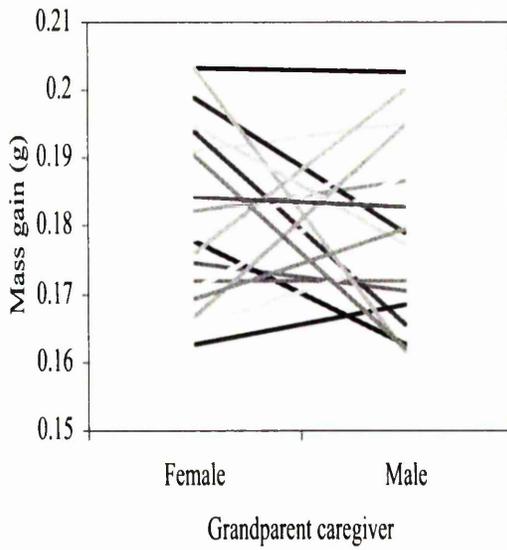
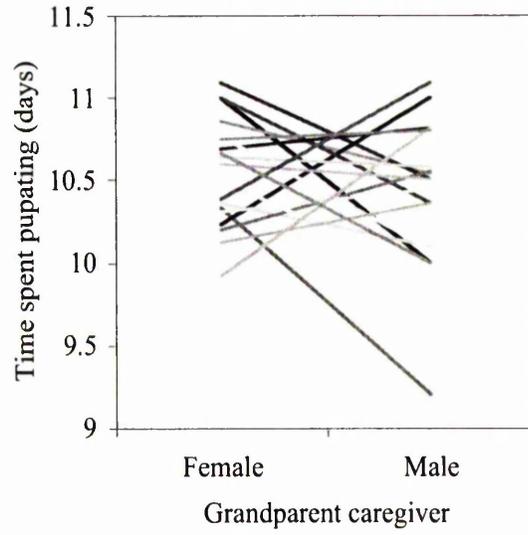
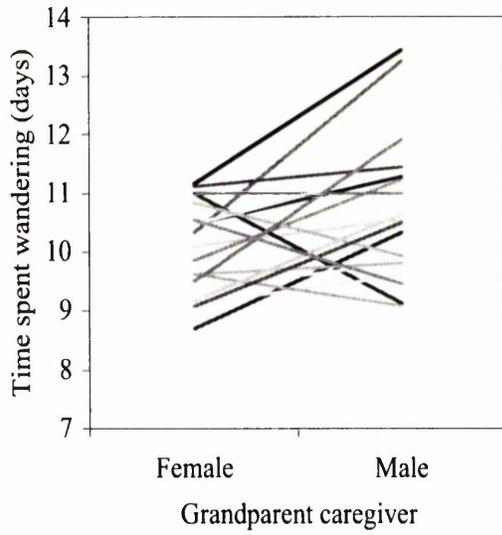
The main effects of family or grandparent sex did not influence the mass offspring gained during the parental care period. However, there was a significant interaction effect.

**Table 4.4: Mixed model Analysis of Variance looking at the effects of dam (family group) and grandparent sex (sex of parent’s caretaker) on three offspring life history traits**

<b>Character</b>	<b>Source of Variation</b>	<b>Df</b>	<b>MS</b>	<b>F-ratio</b>	<b>F</b>	<b>p</b>
<b>Days Wandering</b>	Family	18	16.023	MSf/MSfg	1.763	NS
	Grandparent	1	50.321	MSg/MSe	39.789	<0.01
	Family x Grandparent	18	9.089	MSfg/MSe	7.187	<0.01
	Error	429	1.265			
<b>Days Pupating</b>	Family	18	1.250	MSf/MSfg	0.674	NS
	Grandparent	1	2.200	MSg/MSe	4.676	<0.05
	Family x Grandparent	18	1.855	MSfg/MSe	3.942	<0.01
	Error	398	0.470			
<b>Mass gain</b>	Family	18	0.002	MSf/MSfg	1.001	NS
	Grandparent	1	0.001	MSg/MSe	0.805	NS
	Family x Grandparent	18	0.002	MSfg/MSe	3.307	<0.01
	Error	467	0.001			

The significant interaction effects between family and grandparent sex on all three offspring life history traits are shown visually as reaction norms in figure 4.2. Each line represents the phenotypic response of each family to the sex of the grandparent that provided care to their parents to a change in its parental care environment.

**Figure 4.2: Reaction norms of three offspring life history traits when either their grandmother or grandfather cared for their parents.**



## 4.4 Discussion

Recently parental effects have received considerable attention from evolutionary biologists, as they have been found to be important sources of variation in many life history traits (Mousseau & Fox 1998). Less well studied are the effects of specific maternal effects. One exception is testosterone levels in bird eggs (Gil et al. 1999). However, few studies investigate the importance of parental care as a parental effect, despite the acknowledged importance of care.

I examined the importance of parental effects through manipulation of the postnatal parental care environment. I employed a split family design to explore the separate effects of maternal and paternal care on offspring life history traits in *N. vespilloides*. This allowed me to investigate postnatal parental effects separately from prenatal maternal effects because siblings received identical prenatal care but different postnatal care. I also examined the grandparental effects of separate maternal and paternal care by investigating the life history traits of the grandchildren of the parental generation.

### 4.4.1 Parental effects

Using a split family experimental design allowed me to control for genetic variation among offspring because full-siblings were cared for either their mother or father. I was also able to control for prenatal effects because the offspring received the same pre-hatching influence from their mother, in cytoplasmic contributions to the eggs, and also from the father, because both parents buried and prepared the carcass prior to postnatal parental care. Thus I was able to compare postnatal parental effects of maternal and paternal care on offspring phenotype.

There were two main effects tested in the 2-way ANOVA: 1. which family group the offspring belonged to; 2. the sex of the parental providing the parental care environment. A significant family effect was found for the two measures of development time, number of days spent as a non-feeding “wandering” larva and number of days spent as a pupa. The result was due to resemblance between siblings, indicating that genotype or prenatal maternal effects may have more of an effect of development time. Family did not have a significant effect of larval weight gain during the parental care period. This difference may be due to the differences in the resource environment (i.e. the mouse carcass). A positive relationship between carrion size and offspring weight gain has been found previously in *N. vespilloides* (Bartlett & Ashworth 1988; Eggert & Muller 1997). However, in this experiment we controlled all carcass sizes to between 15 and 20g. Both parents also prepared and buried the carcass prior to the arrival of the larval. Therefore, the differences in weight gain may be due to individual differences in the maintenance of the carcass.

The sex of the parent providing care had no significant effect on the time offspring spent in the non-feeding “wandering” phase, the time offspring spent as pupae or offspring mass gain during the parental care period. This is consistent with previous experiments on several species of *Nicrophorus* that, under laboratory conditions, females and males are equally competent single parents (Bartlett 1988; Fetherston et al. 1994; Rauter & Moore 2004; Smiseth et al. 2005; Trumbo 1991).

Variation in the postnatal parental care environment results from differences in the parental performance phenotype. The genes controlling parental performance interact with the parent’s environment, causing variation in the parental care environment experienced by the offspring (Rossiter 1998). For all three offspring life history traits there was a

significant interaction effect between family and parent sex. Reaction norms allowed us to view these interactions graphically and examine differences in the rank and scale of the three offspring phenotypes for each family, depending on which parent was providing postnatal parental care (Via 1987).

The reaction norms for all life history traits show a change in the relative phenotypic ranks of the families across parental care environments, indicating that families vary in their response to their parental care environment (Lynch & Walsh 1997). There is also a change in the scale of the response, so the phenotype of families may react more or less strongly, depending on which parent is providing care (Lynch & Walsh 1997).

A change in phenotypic rank has been found previously in a related species, *N. pustulatus* when comparing life history traits of offspring in the presence and absence of parental care (Rauter & Moore 2002b). Not all mothers have a positive influence on their offspring, and in some families development and growth were faster and larger when their mother was absent. This surprising result was explained by the facultative nature of parental care in *N. pustulatus*, with parents abandoning their offspring in undesirable circumstances (Rauter & Moore 2002b). The same facultative parental care is also observed in *N. vespilloides*. It is therefore advantageous for there to be variation in offspring need, as well as in parental care, so that phenotypic matching between parents and offspring occurs at different levels in different families, allowing some burying beetle offspring to survive in even the worst parental care conditions.

The norm of reaction of the offspring phenotypes for each family in each parental care environment is unpredictable, due to the change in rank and scale. This may be because postnatal parental effects provide a mechanism for adaptive transgenerational phenotypic plasticity (Mousseau & Fox 1998). Plasticity is measured as the difference in

the mean phenotypes expressed in several environments by siblings (Via 1987), therefore, the slopes of the lines in Figures 4.1 and 4.2 represent the phenotypic response of each family to a change in the parental care environment. In some families there is an increase in development times or mass gain when care is provided by one parent when compared to development times and mass gain with the other parent. The parental care environment is unpredictable due to individual genetic variation, causing environment-specific parental effects (Shaw & Byers 1998). Parental effects therefore exhibit adaptive plasticity due to the evolution of environment-specific parental effects (Bernardo 1996) (Shaw & Byers 1998).

#### **4.4.2 Grandparental effects**

The grandparental care effects carry on from the previous generation and must therefore manifest as prenatal (egg) effects and postnatal (parental care behaviour) effects (Roff 1998). When exploring the persistence of parental effects into the next generation, as grandparental effects there were two main effects in the two-way ANOVA: 1. the family the offspring belonged to, defined as all the grandchildren of each grandparent pair; 2. the sex of the grandparent who had provided care to the previous generation. Family had a non-significant effect on the number of days spent wandering, the number of days spent pupating and weight gain during the parental care period. This lack of family effect may be because grandchildren were not as genetically similar, due to the breeding of their parents with laboratory stock beetles.

The sex of the grandparent that provided care to the parental generation had a significant effect on both measured of offspring development, the duration of the larval wandering phase and pupation. When parental care had been provided to their parent by

their grandmother offspring spent less time wandering and more time pupating. A negative correlation between wandering and pupation durations has been found previously in *N. pustulatus* (Rauter & Moore 2002b) and *N. vespilloides* (Lock et al. 2004). In this experiment the negative correlation was maintained when offspring had a female grandparent. However, the negative correlation was reversed when a male provided grandparental care, with offspring displaying a longer wandering phase and a shorter pupation phase. Hence, the direction of the response to selection depends on the sex of the grandparent.

A similar result has been shown in *D. serrata*, where grandparents were given a cold shock (Magiafoglou & Hoffmann 2003). When the grandmother was given a cold shock the development time of female offspring decreased. However, when it was the grandfather who was given the cold shock, the development time of female offspring increased. Therefore, the direction of a cross-generational response to environmental stimuli may be dependent on the grandparent sex (Magiafoglou & Hoffmann 2003).

In this experiment the sex difference in grandparental effects occurs because of differences in the manifestation of prenatal and postnatal parental effects, and the interaction between them. Sons and daughters of the second generation received care from either their father or mother. They were then mated with a non-experimental stock beetle. Daughters are able to respond to their parental care environment, uniparental care from either their mother or father, in two ways, prenatal and postnatal maternal effects. Sons are only able to respond through postnatal effects, because they have no influence on egg constituents, only on the postnatal parental care that they provide. This means that a paternal prenatal response is delayed by one generation, and manifested when his daughters reproduce (Roff 1998).

A delay by one generation of the paternal response was originally found in the mosquito fish, *Gambusia affinis*, when investigating embryo weight (Reznick 1981). It was found because the weight of the embryo is determined by the genotype of the mother, not the offspring, and therefore a paternal response is only seen when his daughters reproduce, in the next generation (Reznick 1981).

The sex difference caused by grandparental effects occurs as a result of a delay in the manifestation of the prenatal paternal effect, which causes a different interaction between prenatal and postnatal effects. This is seen as the maintenance of the negative correlation between wandering and pupation duration in offspring with a female grandparent, and its loss in offspring whose male grandparent provided parental care. This is evidence of the interaction between prenatal and postnatal parental effects allowing adaptation in response to environmental variation.

In a previous experiment on *N. vespilloides* I also found that the interaction between prenatal and postnatal effects causes an adaptive response in the phenotype of the offspring (Chapter 2). Offspring of younger (aged two weeks post-eclosion) and older (aged six weeks post-eclosion) dams were cross fostered to a caretaker female of either the same or different age group. Offspring were found to be heavier and hatching when their dam was in the old age group, older caretaker females also provided more parental care behaviours, however, when dam age and caretaker age were the same, offspring were gained an equal amount of weight during the parental care period. This was due to an interaction between prenatal and postnatal maternal effects, which meant that females were able to adjust their prenatal and postnatal effects, depending on their age. As a result of the interaction, offspring were most fit, i.e. gained the most mass, when prenatal and postnatal effects were from females of the same age (same condition). When there was a mismatch

between dam age and female caretaker age offspring were not as fit because there was not an interaction between prenatal and postnatal maternal effects (Chapter 2).

It is not possible to graphically show the difference in pupation and wandering durations as a result of sex differences in prenatal and postnatal parental effects. This indicates that although the interaction between prenatal and postnatal grandparental effects is important in some families, it is not as important in other families.

The 2-way ANOVA also allowed investigation of the interaction effect between the family the offspring belonged to and grandparent sex. There was a significant interaction effect on time spent wandering, time spent pupating and larval mass gain during the parental care period. The interaction effects between family and grandparent sex were illustrated using reaction norms (Figure 3). As found for the parental effects reaction norms, there is an unpredictable change in the rank and the scale of the phenotype expressed by each family when their parents received care from either their grandmother or their grandfather. This indicates that the persistence of parental effects across generations as grandparental effects causes an adaptive response in the phenotype of grandchildren to variation in the care provided to their parents by their grandparents, due to phenotypic plasticity.

Natural variation in the postnatal parental care environment provided by individuals has parental effects on offspring life history traits. These parental effects persist across generations as grandparental effects. Both parental and grandparental effects allow the adaptation of offspring as a result of phenotypic plasticity. For grandparental effects the interactions between prenatal and postnatal parental effects also allow the adaptation of offspring traits. Therefore, variation in the parental care behaviours of parents and grandparents, due to parental performance genes and the environment experienced by the

parents and offspring, cause adaptive plasticity of offspring life history traits.

## General Discussion

In species without a mapped genotype behavioural studies provide the best information on parent-offspring interactions. By coupling a quantitative genetic approach with behavioural studies it is possible to investigate the variation in parental behaviour, not just maternal effects on offspring phenotype. This provides integrated research into the genetics and evolution parent-offspring interactions.

In this thesis I studied parental care behaviours and their maternal effects on offspring phenotypes in the burying beetle, *Nicrophorus vespilloides*. Variation in parental care behaviours were produced through manipulation of the genetic relationship between parents and offspring, the physical condition of the parent, due its age, and the sex of the parent.

Chapter 1 investigated the genetic correlation and social selection arising from two parental and two offspring behaviours. This was done by examining the behaviours of parents and offspring in an unshared environment, therefore eliminating common social influences. The patterns of selection were investigated using performance and selection gradients. Evidence for the genetic coadaptation was obtained from performance gradients. Offspring and parental performance traits were found to be strongly related to offspring fitness. Selection gradients found a complex pattern of selection from parents to offspring, which results in a positive correlation between parent and offspring traits. There is a genetic coadaptation between parental care behaviours and offspring begging behaviours that is maintained by genetic correlations. Therefore, integrating information from genetics and selection provides an explanation for why variation in parental care persists.

In Chapter 2 I investigated the effect of maternal age on parental care behaviours, offspring begging behaviours and offspring life history traits. Prenatal (offspring weight at

hatching) and postnatal (parental care behaviours) effects were considered separately, whilst controlling for maternal experience, because each female was providing care for the first time. Older females were found to produce larvae with a higher hatching weight, provide more parental care and induce a higher level of larval begging. However, offspring from females of the two age groups were equally fit when their caretaker and dam were the same age. This is evidence of an interaction between prenatal and postnatal maternal effects. This allows changes of egg constituents, causing an increase larval hatching weight and an increase in parental care behaviours, as females age. Therefore, adaptation of prenatal and postnatal investment allows a female to maximise offspring fitness as she ages. This also explains why variation in parental care exists.

In chapter 3 prenatal maternal effects were controlled and the effects of postnatal female or male care were investigated using a split-family experiment. No significant difference between female and male parental care was found, however, there is individual variation in parental care behaviours. The influence of this variation was investigated by calculating estimates of resemblance between parents and offspring of behavioural, life history and morphological traits. I found that estimates of resemblance varied, depending on the sex of the parent. Comparing resemblance estimates from shared and unshared environment regressions also indicated negative parental effects on resemblance. However, most matrices of the causal components of maternal (m) and paternal (f) effects were very small. Only one trait, larval provisioning behaviour, had a quite large and negative m value, indicating a negative maternal effect. This may occur because mothers that are fed a lot as larvae could produce large offspring require less provisioning. However, this may cause them to produce lower quality eggs, and offspring that require more feeding, causing a

negative maternal effect. This is another example of an interaction between prenatal and postnatal effects

The influence of variation in parental care was also investigated in chapter 4. Due to the split-family design used offspring of each family experienced two parental care environments, maternal and paternal. The sex of the parent was found to have no effect on offspring; however, grandparent sex did have an effect. This was again due to an interaction between prenatal and postnatal parental effects and a delay by one generation in the expression of prenatal grandpaternal effects, compared to grandmaternal effects. As in chapter 2, adaptive nature of the interaction between prenatal and postnatal parental effects allows parents to maximise offspring fitness in a variable parental care environment.

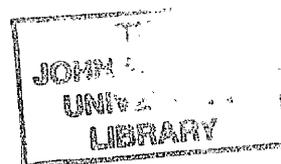
In this thesis I have used a quantitative genetics approach to investigate the interaction between parents and offspring and the effects of variation in parental care on offspring begging behaviours and life history. I found that variation in the parental care behaviours provided by adult *Nicrophorus vespilloides* allows offspring to become better adapted to their environment, through the action of maternal effects and selection.

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