Body size, inbreeding, and family interactions in the burying beetle *Nicrophorus vespilloides*

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It is impossible to convey completely the excitement of discovery, of seeing the result of an experiment and knowing that you know something new, something fundamental, and that for this moment at least, only you, in the entire world, knows it.

— Stuart Firestein

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Declarations

This dissertation is submitted in accordance with the requirements for a Doctorate of Philosophy by the School of Biological Sciences at the University of Edinburgh. The work included in this thesis has not been submitted for any other degree or professional qualification. I declare that I have written this thesis under the guidance of my supervisor. I conducted all experimental work with help as below. All other work was my own.

Chapters 2 & 3: These experiments were designed with the help of my supervisor, Dr Per Smiseth. The majority of the data were collected by Jon Richardson under my supervision. I performed all analyses presented here and wrote the subsequent manuscripts and chapters in collaboration with Dr Per Smiseth.

Chapter 4: The data were collected with assistance from Cerian Halford and Rita Rácz. I performed all the analyses presented here and wrote the subsequent manuscript and chapter in collaboration with Dr Per Smiseth.

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Lay Summary

This dissertation examines how parents' body size and their relatedness to each other affect how well their offspring perform. My study species was the burying beetle *Nicrophorus vespilloides*, which breeds on small carcasses and has elaborate parental care. Parents feed their offspring, defend them against predators and competitors, and deposit antimicrobial substances on the carcass to prevent bacterial and fungal growth. The first part of this work focuses on the parents' body size, which affects reproduction by influencing how many eggs are laid, how big the eggs are, and how much care the parents are able to provide. The second part of this work focuses on inbreeding, which occurs when relatives mate with each other and produce inbred offspring. Inbred offspring typically suffer reduced survival and/or reproductive success. These negative effects of inbreeding on the offspring are called inbreeding depression. My main findings are that (*i*) parental body size affects cooperation between parents caring for their offspring; (*ii*) parents that were previously involved in a fighting contest provide more care to their offspring, regardless of whether they won or lost that contest; (*iii*) parental care can partly compensate for the negative effects of mating with a relative, but this effect depends on the caring parent's body size; (*iv*) the number of siblings an inbred larva is competing with does not affect the magnitude of inbreeding depression in that brood; and (*v*) inbred females avoid mating with inbred males but outbred females do not show a preference between inbred and outbred males.

Abstract

There are three social dimensions within a family: parent-parent interactions, parent-offspring interactions, and offspring-offspring interactions. All of these interactions are subject to evolutionary conflict, which occurs whenever interacting individuals have divergent evolutionary interests. Family interactions and family conflict are often influenced by phenotypic and genotypic traits of the parents and the offspring. An important phenotypic trait is body size, which can affect fecundity, mating success, and fighting ability. An important genotypic trait is inbreeding status (i.e., whether an individual is outbred or inbred), which can influence its overall quality or condition. In this thesis, I investigate the independent and interactive effects of inbreeding and parental body size on family interactions in the burying beetle *Nicrophorus vespilloides.* I first show that the body size of the two parents influences the resolution of sexual conflict over the amount of parental care (Chapter 2) and over the consumption of a shared resource (Chapter 3). Here, the shared resource refers to the carcass from which both the parents and the offspring feed over the course of the breeding attempt. I then show that females that won or lost a fighting contest provide more care to their offspring compared to beetles with no fighting experience (Chapter 4). This indicates that female burying beetles make parental investment decisions based on their experience with a contest (which is independent of body size) rather than the outcome of that contest (which is dependent on body size):. In the second half of my thesis, I examine whether family interactions also influence and are influenced by inbreeding depression (Chapters

5–8). I find that a female's mating preference for an outbred versus an inbred male is conditional on her own inbreeding status: inbred females preferentially mate with outbred males, whereas outbred females are equally likely to mate with an outbred or an inbred male (Chapter 5). Even though sibling competition does not appear to have an effect on the offspring's inbreeding depression (Chapter 6), the presence of the mother during larval development can reduce the severity of inbreeding depression (Chapter 7), and this effect depends on the mother's body size (Chapter 8). In Chapter 9, I discuss the broader implications of these findings for evolutionary biology, ecology, and conservation biology.

Chapter 1: General Introduction

There are three social dimensions within a family: parent-parent interactions, parent-offspring interactions, and offspring-offspring interactions (O'Connor 1978, Mock and Parker 1998). All of these interactions are subject to evolutionary conflict, which occurs when a trait increases the fitness of one individual but not the fitness of the other individuals it is interacting with (Lessells 2012). Thus, whenever individuals with divergent evolutionary interests interact with each other, there is potential for evolutionary conflict (Mock and Parker 1998).

Parental care, for example, is an important biological process that can improve offspring growth and survival but also reduces the parent's ability to invest in future reproductive attempts (Clutton-Brock 1991, Royle et al. 2012). Offspring are under selection to demand more resources than the parent is selected to provide, leading to conflict between parents and offspring over the allocation of resources (Trivers 1974). Parents need to make decisions about how much to invest in current offspring versus any future offspring they might be produce (Parker et al. 2002). In addition, in species with biparental care, there is conflict between the two parents over their individual contributions to parental care (Godfray 1995, Houston et al. 2005, Harrison et al. 2009). This type of conflict arises because the benefit of care to each parent depends on the parents' combined effort, whereas the cost depends on each parent's personal effort (Lessells 2012). As a result, each parent is under selection to reduce its personal cost by shifting as much of the workload as possible over to its partner. Lastly, there is conflict between offspring in a brood, in the form of sibling competition: offspring try to extract as many resources from their parents as possible, potentially at the expense of their siblings (Mock and Parker 1997).

These family interactions and conflict may be influenced by various phenotypic or genotypic traits of the parents and/or the offspring. For example, an important component of a parent's phenotype is its body size, which can affect its fecundity, mating success, and fighting ability (e.g., Price 1984, Honeka 1993, Jennions and Blackwell 1996, Schuett 1997, Bonduriansky 2011). The parent's body size might therefore influence how much care it provides to its offspring and might also alter the interactions between the two parents when there is biparental care (McNamara et al. 1999, Houston et al. 2005, Steiger 2013). Furthermore, one important genotypic trait is the parents' and the offspring's inbreeding status (i.e., whether they are outbred or inbred), which influences their overall quality or condition. The reduced performance of inbred individuals may alter the reproductive decisions of inbred parents or the parental care behaviour of outbred parents caring for inbred offspring.

In this thesis, I test the independent and interactive effects of inbreeding status and parental body size on a wide range of family interactions: parental care, biparental cooperation, sexual conflict, sibling competition, and mate choice. In addition, I investigate the consequences of these interactions for both the parents' and the offspring's fitness.

1.1 Body size

Body size is one of the most important components of an individual's phenotype. Body size is commonly described in terms of length or mass and is often used as an indicator of an individual's condition (Jakob et al. 1996). Many studies across a wide range of taxa have shown that body size can have major effects on individual fitness. For example, a larger body size is generally associated with a longer lifespan (Holm et al. 2016). Furthermore, larger females tend to have higher fecundity (e.g., birds: Saether et al. 1997, Barbraud 2000; reptiles: Clobert et al. 1998; amphibians: Gibbons and McCarthy 1986; fishes: Kraak and Bakker 1998; insects: Honeka 1993, Bonduriansky 2001; crustaceans: Kiorboe and Sabatini 1995), and larger males tend to have higher reproductive success because they are preferred by females (e.g., mammals: Charlton et al. 2007; birds: Price 1984, fishes: Bisazza and Marconato 1988; insects: Savalli and Fox 1998). Body size also affects fighting ability and thus an individual's capacity to acquire and defend resources that might be necessary for breeding, such as territories, nests, and food (e.g., Jennions and Blackwell 1996, Schuett 1997). For example, larger burying beetles (*Nicrophorus vespilloides*) are more likely to win fighting contests over a breeding resource (Otronen 1988), and larger river bullhead males (*Cottus gobio*) are more successful at acquiring nest sites and defending their brood from conspecifics (Bisazza and Marconato 1988).

An indirect consequence of the effects of body size on reproductive potential is that small and large parents may adopt different life-history strategies based on their size (e.g., Rauter et al. 2010). For example, if a small individual has limited prospects for future breeding opportunities, it might be beneficial for it to put maximum effort into the current breeding attempt. In contrast, a large individual may allocate less to current reproduction in order to take advantage of future breeding opportunities.

1.2 Inbreeding

Inbreeding is a process that occurs over two generations. It refers to the mating between close relatives in the parental generation followed by the production of inbred offspring in the subsequent generation. When parents are related to each other, it becomes more likely that they will pass on the same alleles to their offspring

(Falconer and Mackay 1996). The resulting increase in homozygosity in inbred offspring is used as a measure for the degree of inbreeding, which is called the inbreeding coefficient (Wrights 1922). The inbreeding coefficient F ranges from 0 to 1, indicating a completely outbred and heterozygous population or a completely inbred and homozygous population, respectively (Crow and Kimura 1970).

Due to the increase in homozygosity, inbreeding often leads to a reduction in the fitness of inbred offspring, known as inbreeding depression (Charlesworth and Charlesworth 1987). This is caused by a higher likelihood that recessive, deleterious alleles are expressed (Charlesworth and Charlesworth 1987) and by the loss of any benefits due to overdominance (Lynch and Walsh 1998). Inbreeding depression can affect a wide range of fitness-related traits across an individual's entire lifespan, such as development time, survival rate, body size, mating success, fecundity, and many other life-history traits (Crow and Kimura 1970, DeRose and Roff 1999, Mattey et al. 2013).

Earlier work suggests that fitness traits tend to be more strongly affected by inbreeding than non-fitness traits (Falconer and Mackay 1996, DeRose and Roff 1999). This is because fitness-related traits are under directional selection, and inbreeding reduces the mean value of such traits; this occurs when the dominant allele for a particular trait increases the value of that trait, whereas the recessive allele reduces its value (Falconer and Mackay 1996). This is referred to as directional dominance (Falconer and Mackay 1996). In contrast, morphological traits are expected to be less affected by inbreeding because (*i*) they are additive with no dominance variation or (*ii*) their dominance is either not directional or less directional than fitness-related traits (DeRose and Roff 1999).

Inbreeding depression studies originally focused on early-life fitness traits (Fernandez et al. 1995, Kempenaers et al. 1996), but it is now widely accepted that in order to accurately assess the fitness costs of inbreeding, it is necessary to measure a range of fitness traits across an individual's entire lifespan (Charlesworth and Charlesworth 1987). In fact, in some species, inbreeding depression is as severe or more severe in late life than early life (Wolfe 1993, Keller 1998, Keller et al. 2008). It is well documented that different species suffer inbreeding depression in different traits, but there is also substantial variation in the overall magnitude of inbreeding depression between and within species (Crnokrak and Roff 1999, Keller and Waller 2002, Moorad and Wade 2005). One factor contributing to between-species variation is that deleterious alleles are usually purged in species with a history of inbreeding, making inbreeding depression less severe than in species with no prior history of inbreeding (Pemberton 2008). Environmental stress is another factor underlying variation in the severity of inbreeding depression between and within species (Marr et al. 2006, Fox and Reed 2011, Reed et al. 2012). This topic has recently received a lot of attention (Avilés and Bukowski 2006, Marr et al. 2006, Fox and Reed 2011, Reed et al. 2012, Meunier and Kölliker 2013), and there is now good evidence that inbreeding depression may be influenced both by the biotic environment (e.g., parasitism or competition) and the abiotic environment (e.g., temperature). Stressful environments are predicted to exacerbate inbreeding depression (Marr et al. 2006, Fox and Reed 2011, Reed et al. 2012), whereas benign environments may buffer against inbreeding depression (Avilés and Bukowski 2006, Meunier and Kölliker 2013).

Inbreeding and inbreeding depression can have important evolutionary consequences (Szulkin et al. 2013). In species where inbreeding depression is severe, various mechanisms may evolve to avoid the costs of inbreeding; these include sex-biased dispersal, mate choice, and extra-pair copulations (Pusey and Wolf 1996, Foerster et al. 2003). Although there is evidence for inbreeding avoidance in many species, there is remarkable variation in animal breeding strategies, with some species showing inbreeding tolerance and others even showing inbreeding preference (Szulkin et al. 2013). The costs of inbreeding relative to the costs of avoiding inbreeding should determine whether animals should avoid mating with their relatives (Kokko and Ots 2006).

1.3 Study System: Burying Beetle *Nicrophorus vespilloides*

The *Nicrophorus* genus comprises of 75 species of burying beetles that are mainly found in North America and Northern Europe (Peck 1982). There are several attributes that make burying beetles a highly tractable study system. Firstly, they survive and breed well under laboratory conditions, so it is easy to maintain a population in the laboratory. Secondly, they have a relatively short life cycle with a generation time of approximately 6 weeks, making it feasible to conduct multigenerational studies. This short generation time also makes it possible to measure fitness traits across an individual's entire life cycle to gain a more accurate measure of fitness (e.g., Mattey et al. 2013). Lastly, parents and offspring perform all of the same behaviours in the laboratory as in the field, allowing us to study parental care under controlled, experimental conditions (Scott 1998).

1.3.1 Family interactions in *N. vespilloides*

The burying beetle *Nicrophorus vespilloides* is an excellent study system for investigating family dynamics and has been used in many studies of family conflict and cooperation both in the field and the laboratory (Eggert and Sakaluk 1995, Scott 1998, Smiseth and Moore 2004, Smiseth et al. 2005, Trumbo 2006, Smiseth et al. 2007a, Smiseth et al. 2007b, Trumbo 2007, Suzuki and Nagano 2009, Boncoraglio and Kilner 2012, Mattey and Smiseth 2015). *Nicrophorus vespilloides* has biparental care, and larvae compete with each other for food from their parents. These interactions have important fitness consequences for both the parents and the offspring. Parents provide elaborate care that improves offspring growth and survival (Eggert 1992, Scott 1998) but is costly to the parents, as it reduces their immunity, fecundity, and future reproductive success (Rozen et al. 2008, Ward et al. 2009, Arce et al. 2012). Parental care is facultative in this species, which means that the offspring are semi-independent, and some of them can still survive to the juvenile stage even in the complete absence of care. This allowed me to conduct experiments where one or both of the parents were removed (Chapters 4, 6–8). Although offspring suffer reduced survival in the absence of both parents, the removal of one parent has no fitness consequences for the offspring under laboratory conditions (Smiseth et al. 2005).

Like all *Nicrophorus* beetles, *N. vespilloides* breeds on carcasses of small mammals and birds (Scott 1998). These carcasses are a rare but extremely valuable resource, so there is fierce intraspecific and interspecific competition for them (Scott 1998). Once a pair of adult beetles acquires a carcass they can use for breeding, they remove its fur or feathers, roll it into a ball, bury it into the ground, and lay eggs around it (Scott 1998). Over the course of the breeding bout, the parents maintain the carcass by depositing oral and anal antimicrobial secretions that prevent bacterial and fungal growth (Rozen et al. 2008, Arce et al. 2012). The parents also create a crater on the carcass by breaking the skin, making it easier for larvae to start feeding once they arrive on the carcass (Eggert and Müller 1997).

Approximately 60 hours after the eggs are laid, the larvae start hatching, they crawl to the carcass, and start feeding on the crater created by the parents. Even though larvae can feed on their own, they also beg for food from their parents. Larval begging is tactile, with larvae raising their head towards the parent while waving their legs or touching the parent with their legs (Smiseth and Moore 2002). In response to this tactile begging, parents regurgitate predigested carrion to the larvae through mouth-to-mouth contact. Parents can feed multiple larvae at the same time, but when too many larvae are begging simultaneously, the parent cannot feed all of them. Larval begging for food thus gives rise to sibling competition, which becomes more intense in larger broods (Smiseth et al. 2007). Although there is no evidence that larval begging is energetically costly in this species (Smiseth and Parker 2008), begging may be costly because of a higher risk of infanticide (Andrews and Smiseth 2013). Females are typically more involved in care and tend to stay on the carcass longer than males (Fetherston et al. 1994, Eggert et al. 1998, Smiseth and Moore 2002, Rauter and Moore 2004, Smiseth et al. 2005). Males generally abandon the brood to find a new mate soon after the larvae hatch (Scott 1998). Abandonment by both parents usually occurs when the carcass is small (Ward et al. 2009).

As the larvae grow bigger, they feed on their own more and beg less to the parents (Smiseth et al. 2003). Larvae disperse from the carcass into the surrounding soil 4–5 days after hatching, which corresponds to the end of the parental care period. The larvae wander in the soil for about 10 days after dispersal, at which point they pupate. Approximately 10 days after pupation, they eclose as adult beetles, and approximately 10 days after eclosion, they become sexually mature (Eggert and Müller 1997).

1.3.2 Body size in *N. vespilloides*

Body size is an important determinant of survival, fighting ability, and reproductive behaviour in *N. vespilloides* (Otronen 1988, Rauter et al. 2010, Steiger 2013). Firstly, larval body size affects survival to eclosion, with small larvae suffering much higher mortality than large larvae (Figure 1.1). Secondly, body size influences post-eclosion lifespan, with large beetles living longer than small beetles (J Moorad, unpublished data). Thirdly, adult body size is the strongest determinant of whether a burying beetle will be successful at acquiring a carcass and defending it against conspecific competitors (Otronen 1988). As mentioned above, burying beetles breed on carcasses of small vertebrates, for which there is fierce competition (Scott 1998). Otronen (1988) found that *N. vespilloides* males with a greater body mass were more likely to win a contest over a carcass. Safryn and Scott (2000) then showed that in *Nicrophorus orbicollis*, body size (pronotum width) was more important than body mass in predicting the outcome of a contest. Lastly, female body size has been shown to influence egg size, the number and size of the offspring produced, and the amount of care the mother provides during larval development (Rauter et al. 2010, Steiger 2013). Larger *N. vespilloides* females lay larger eggs, spend more time providing care, and as a result, they have larger offspring at the end of the parental care period (Steiger 2013). In the related *Nicrophorus pustulatus*, larger females produce smaller broods with larger offspring, whereas smaller females produce larger broods with offspring that are generally smaller and more variable in size (Rauter et al. 2010).

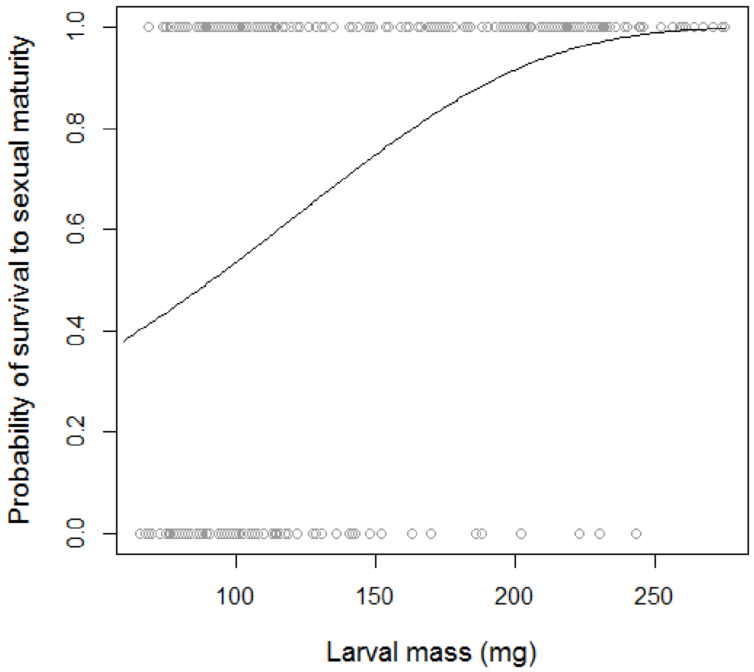

Figure 1.1: Probability of burying beetle (*N. vespilloides*) larvae surviving to sexual maturity plotted against their larval mass. These data were collected by removing larvae (n = 332) from the carcass at different stages of development, recording their individual mass (range = 60–275 mg), and tracking their mortality until sexual maturity. The amount of time from removal from the carcass to sexual maturity ranged between 27 and 33 days.

Larvae do not feed after dispersal and before eclosion, so adult body size is determined by larval mass at the dispersal stage (i.e., the end of the parental care period). This means that the amount of food provisioning by the parents can have long-term fitness consequences for the offspring, mediated through larval mass. It also means that it is possible to experimentally generate small and large adult beetles by removing larvae from the carcass at different times, when they are within a specified size range (Steiger 2013). I used this methodology for several experiments included in this thesis (Chapters 2–4 and 8).

1.3.3 Inbreeding in *N. vespilloides*

There is evidence for severe inbreeding depression in *N. vespilloides* (Mattey et al. 2013). Inbred offspring generated through brother-sister matings suffer reduced survival at the larval and pupal stages, a shorter lifespan after eclosion, as well as lower reproductive success (Mattey et al. 2013). Despite these significant fitness costs of inbreeding, Mattey and Smiseth (2015) found that *N. vespilloides* females were equally likely to mate with related and unrelated males. One potential explanation for the absence of inbreeding avoidance in *N. vespilloides* is that the risk of inbreeding is low in natural populations of this species (Mattey and Smiseth 2015). When inbreeding is rare, we would expect only weak selection on inbreeding avoidance mechanisms, such as mate choice based on relatedness cues (Kokko and Ots 2006). Although little is currently known about the risk of inbreeding for burying beetles in the wild, inbreeding is likely to be rare given the generally large population sizes and the long dispersal distances of beetles searching for suitable carcasses for breeding (Petruška 1975, Mattey and Smiseth 2015). The assumption that *N. vespilloides* does not regularly inbreed is consistent with the observation of severe inbreeding depression (Mattey et al. 2013), which suggests that there is no history of purging of recessive, deleterious alleles.

Nicrophorus vespilloides can therefore be used as a model for understanding how the fitness costs of inbreeding are shaped by family interactions in species that do not regularly inbreed. There are two important reasons it is useful to focus on species where inbreeding is relatively rare. Firstly, inbreeding depression is a greater concern for species with no prior history of inbreeding because deleterious recessive alleles have not yet been purged. As a result, inbreeding can have detrimental fitness consequences in these species. Secondly, once a species has a significant history of inbreeding, parental behaviours and other family interactions may be modified by selection due to inbreeding. Hence, species with a history of inbreeding might not be appropriate as models for endangered species that have only recently become subject to inbreeding.

In principle, all populations are potentially at risk of inbreeding in the future, especially given increasing habitat loss and other human-induced disturbances that increase the chances of inbreeding (Andersen et al. 2004). It is thus important to better understand how species that have recently become subject to inbreeding may cope with inbreeding depression. Whenever inbreeding occurs in a population with no prior history of inbreeding, the associated fitness costs may be mediated through pre-existing mechanisms that evolved to serve an adaptive function in a different context (Mattey and Smiseth 2015).

1.4 Aims

In this thesis, I investigate the effects of inbreeding and parental body size on a number of family interactions and social behaviours associated with reproduction: parental care, biparental cooperation, sibling competition, mate choice, and sexual conflict. The general aim of this work is to improve our understanding of how the fitness consequences of body size (phenotype) and inbreeding (genotype) are mediated through interactions between family members (Figure 1.2).

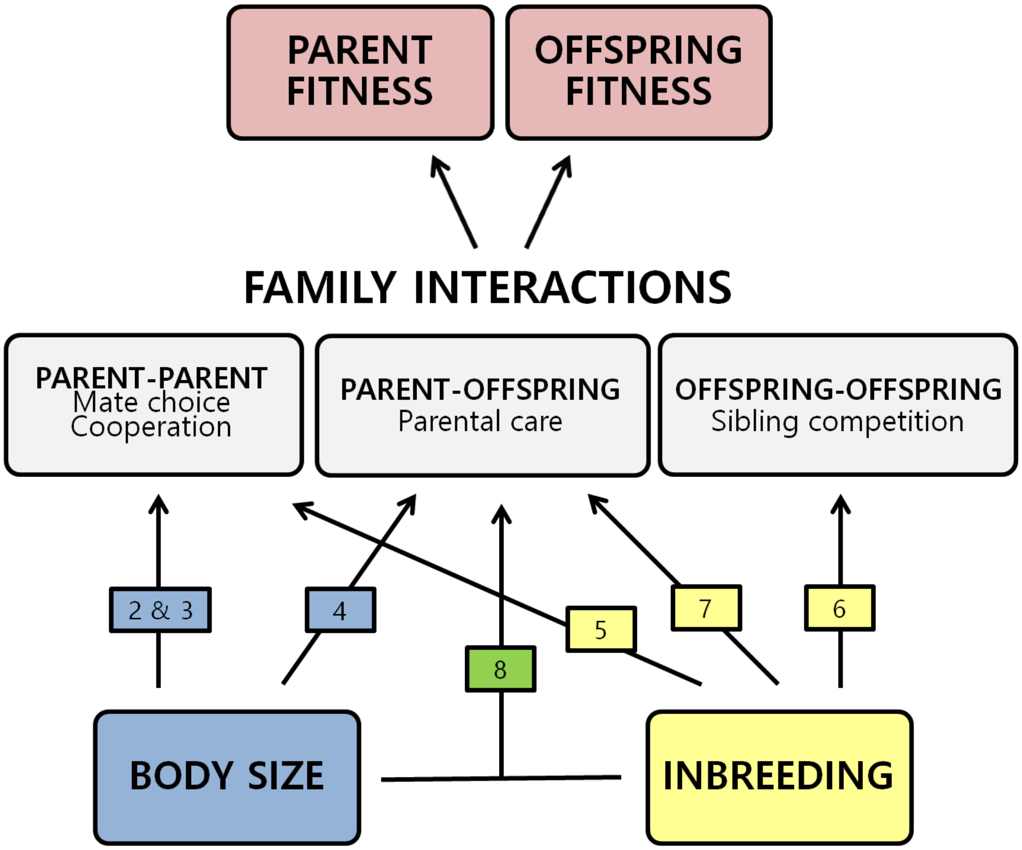

Figure 1.2: Diagram illustrating the topics and interactions discussed in this thesis. The numbers on the arrows indicate the chapters that focus on each interaction.

Chapters 2, 3, and 4 focus on body size, whereas chapters 5, 6, and 7 focus on inbreeding. In Chapter 8, I investigate how the interaction between body size and inbreeding influences the parent's reproductive success and the offspring's fitness. In Chapter 9, I discuss the main findings of this thesis and its broader implications for ecology and evolutionary biology. Below, I provide an overview of Chapters 2–8:

Chapter 2: Parental body size and sexual conflict over parental care
Whenever parents cooperate to raise their brood, sexual conflict arises because each parent is under selection to reduce its personal cost by shifting as much of the workload as possible over to its partner. Given that the costs of parental care depend on the parent's condition, I test whether parents

change the amount of care they provide in response to their own body size (i.e., their condition), their partner's body size, or their partner's behaviour. In addition, I investigate how such adjustments in parental care influence offspring fitness.

Chapter 3: Parental body size and sexual conflict over consumption of a shared resource

Here, I investigate how the parents' body size influences the resolution of conflict over the consumption of a shared breeding resource. As mentioned above, burying beetles breed on carcasses of small vertebrates, which serve as a source of food for the two parents and their offspring. Sexual conflict arises because the more each parent consumes from the carcass, the less is left for its partner and the developing offspring. In this chapter, I test whether parents change the amount of carrion they consume in response to their own body size, their partner's body size, or their partner's consumption. I then assess whether changes in the parents' consumption rate have consequences for the offspring's fitness.

Chapter 4: Size-dependent fighting success and parental care

In this chapter, I test whether the parent's body size indirectly affects its parental effort by influencing whether it wins or loses a fighting contest with a conspecific. Given the rarity of small carcasses suitable for breeding, burying beetles have to compete fiercely with same-sex conspecifics to acquire and defend a carcass. During these fights, beetles gain information about their size and condition relative to other individuals in the population. For example, losers might perceive themselves as being in relatively bad condition, indicating that they have limited prospects for future breeding. In this chapter, I test whether loser females invest more in current reproduction than winner females (of the same size) and whether this has consequences for their own and their offspring's fitness.

Chapter 5: Inbreeding and mate choice

In this chapter, I test whether outbred and inbred females show different mating preferences when choosing between outbred and inbred males. Earlier work in *N. vespilloides* has shown that partners of inbred parents spend more time providing care to their larvae (Mattey and Smiseth 2015), potentially due to a lower quality of care provided by inbred individuals. Female choice based on the male's inbreeding status may thus have consequences for both the female's and the offspring's fitness.

Chapter 6: Inbreeding and sibling competition

Here, I test whether sibling competition exacerbates inbreeding depression. Recent studies have shown that environmental stresses can increase the severity of inbreeding depression (Fox and Reed 2011, Reed et al. 2012). Sibling competition is an important determinant of the offspring's growth and survival in many species and may therefore be a key source of environmental stress to the offspring (Mock and Parker 1997, Roulin and Dreiss 2012).

Chapter 7: Inbreeding and parental care

Environmental stresses are expected to exacerbate inbreeding depression (Fox and Reed 2011, Reed et al. 2012), but benign conditions, such as parental care, may buffer against inbreeding depression (Meunier and Kolliker 2009). In this chapter, I test whether the presence of the mother during larval development reduces the severity of inbreeding depression in the offspring.

Chapter 8: Inbreeding and maternal effects due to body size

After showing that inbreeding depression is less severe in the presence of maternal care (Chapter 7), I investigate whether this buffering effect depends on the mother's phenotype. Maternal condition affects the quantity or quality of care provided to the offspring (Andersen et al. 2000, Bales et al. 2002, Steiger 2013), so maternal traits such as body size, age, and nutritional condition, might influence inbreeding depression in the offspring. This type of a causal influence of the maternal phenotype on the offspring phenotype would represent a maternal effect (Wolf and Wade 2009). In this chapter, I test whether the mother's body size alters the severity of inbreeding depression in the offspring through its effect on parental care ability.

Chapter 2: Parental body size and sexual conflict over parental care

This chapter has been published as referenced below, and this publication appears as Appendix A in this thesis:

> Pilakouta N, Richardson J, Smiseth PT (2015) State-dependent cooperation in burying beetles: parents adjust their contribution towards care based on both their own and their partner's size. *Journal of Evolutionary Biology* 28:1965-1974.

Abstract

Handicapping experiments on species with biparental care show that a focal parent increases its contribution to care when its partner is handicapped. Such results are interpreted as evidence for negotiation, whereby each parent adjusts its amount of care based on that of its partner. However, it is currently unclear whether the focal parent responds to a change in its handicapped partner's behaviour or state. To address this gap, I conducted an experiment on the burying beetle *Nicrophorus vespilloides* where I first experimentally generated different-sized male and female parents by varying the duration of larval development. I then used a 2 × 2 factorial design in which a small or large male was paired with a small or large female. I found that small females provided less direct care (food provisioning and interactions with larvae) than large females, and males and females provided less direct care when paired with a small partner. Thus, the focal parent adjusted its

contribution towards care based on both its own state and that of its partner. There was evidence for negotiation between the two parents as the focal parent adjusted its contribution based on the amount of care by its partner. However, there was no evidence that negotiation accounted for how the focal parent responded to its partner's size. These results have important implications for our understanding of biparental cooperation, as they show that each parent not only adjusts its contribution based on the amount of care provided by its partner but also based on its own state and its partner's state.

2.1 Introduction

Parents of many animals, including the majority of birds (Cockburn 2006) as well as some mammals, fishes, and insects (Balshine 2012, Trumbo 2012), cooperate to provide care for their joint offspring. Until now, most work on biparental cooperation has focused on how a focal parent adjusts its contribution based on the amount of care provided by its partner (Lessells 2012). This focus is motivated by theoretical models for the evolutionary resolution of sexual conflict between caring parents (Houston et al. 2005, Lessells 2012). Sexual conflict arises because the benefit of care to each parent depends on the parents' combined effort while the cost depends on each parent's personal effort (Lessells 2012). Thus, each parent will be under selection to reduce its personal cost by shifting as much of the workload as possible over to its partner. Theoretical models suggest that this conflict can be resolved through three behavioural mechanisms: negotiation, matching, and sealed-bid decisions. Negotiation and matching occur when each parent adjusts its level of care in direct response to its partner's contribution. When there is negotiation, the focal parent responds to a reduction in the amount of care provided by its partner by increasing its contribution such that it compensates incompletely for the partner's reduction (McNamara et al. 1999). In contrast, when there is matching, the focal parent responds by matching any increase or reduction in its partner's contribution

(Johnstone and Hinde 2006). Finally, sealed-bid decisions occur when each parent makes an initial fixed decision about how much care to provide and that decision is independent of that of its partner (Houston and Davies 1985). Experimental studies on birds and other taxa have provided evidence in support of all three mechanisms (e.g., negotiation: Wright and Cuthill 1989; matching: Hinde 2006; sealed bids: Schwagmeyer et al. 2002), although a meta-analysis of studies on birds found overall support for negotiation (Harrison et al. 2009).

Much of the evidence showing that the focal parent adjusts its contribution based on the amount of care provided by its partner derives from handicapping experiments (Wright and Cuthill 1989, Harrison et al. 2009, Lessells 2012). The rationale of such experiments is to reduce the contribution of one parent, typically by adding weights to the back of the handicapped parent (birds and insects: e.g., Wright and Cuthill 1989, Suzuki and Nagano 2009) or clipping some of its flight feathers (birds only: e.g., Sanz et al. 2000) and then monitor any subsequent adjustments in the amount of care provided by the two parents. In general, such experiments show that the handicapped parent provides less care, presumably as a consequence of the increased costs of providing care, while the other parent provides more care (Wright and Cuthill 1989, Harrison et al. 2009). Traditionally, the increased amount of care by the other parent is interpreted as a response to the change in the handicapped parent's behaviour. However, an alternative interpretation is that this increase is a direct response to the change in the handicapped parent's state. Currently, there is insufficient evidence to determine whether the increase in care by the focal parent is a response to the change in the handicapped parent's behaviour or state. Here, I extend previous work by investigating whether cooperating parents adjust their contribution based on variation in their own state as well as the state of their partner, and by investigating whether any adjustments in the amount of care are in direct response to the partner's state or whether they are mediated through the partner's behaviour. I also extend the specific focus on handicapping to the wider issue of how the dynamics of biparental coopertation are influenced by variation in components of the parents' state, such as their body size, age, nutritional condition, and health.

Nicrophorus burying beetles are well suited as a system for investigating these issues because parental care is very flexible for both parents (Eggert et al. 1998, Smiseth and Moore 2004). Burying beetles breed on carcasses of small vertebrates, which provide the sole source of food for the developing larvae (Scott 1998). Both parents help prepare the carcass, protect the brood from predators and conspecifics, apply antimicrobials to the carcass, and provision the larvae with predigested carrion (Eggert et al. 1998, Rozen et al. 2008, Walling et al. 2008, Arce et al. 2012). Females often spend more time provisioning food for the larvae, and they stay on the carcass for longer than males, whilst males spend more time maintaining the carcass (Fetherston et al. 1994, Eggert et al. 1998, Smiseth and Moore 2002, Rauter and Moore 2004, Smiseth et al. 2005, Walling et al. 2008). Previous studies on this species have provided mixed evidence with some support for both negotiation (Fetherston et al. 1994, Rauter and Moore 2004, Smiseth et al. 2005, Suzuki and Nagano 2009, Smiseth and Moore 2004, Creighton et al. 2015) and sealed-bid models (Jenkins et al. 2000, Rauter and Moore 2004, Smiseth et al. 2005, Suzuki and Nagano 2009). A recent study on the effects of inbreeding on biparental care found evidence for both negotiation and sealed bid models, suggesting that these two mechanisms are not mutually exclusive (Mattey and Smiseth 2015).

The state of an individual can refer to a number of different traits, including its body size, age, nutritional condition, health, and whether it is subjected to handicapping or not. We might expect a focal parent to adjust its level of care to variation in its own state. The reason for this is that parental care incurs costs in terms of energy and time expenditure (Alonso-Alvarez and Velando 2012), and it reflects the trade-off between investment in current and future reproduction (Trivers 1972), both of which are likely to be conditional on the parent's own state. Furthermore, a focal parent may adjust its contribution based on the state of its partner if the amount of care provided by the partner is determined by the partner's state. Here, I focus specifically on body size as the state component of interest because a previous study on the same species found that large females have higher reproductive success than small ones (Steiger 2013). Thus, small females might be less capable of providing care, potentially as a consequence of physiological or anatomical differences between different-sized females. To address whether male and female parents adjust their parental care behaviour based on their own body size and that of their partner, I used a 2 × 2 factorial design where a large or small male was paired with a large or small female. To this end, I experimentally generated different-sized male and female parents by varying the duration of their larval development (Steiger 2013). I predicted that small parents would provide less care than large ones and that a focal parent would provide more care when mated to small than to large partners. I then tested whether any adjustments in the level of care by a focal parent to its partner's size were mediated through negotiation, matching, or sealed-bid decisions. If such adjustments were mediated through negotiation or matching, I predicted that they would be dependent on the amount of care provided by the partner. In contrast, if such adjustments were mediated through sealed-bid decisions, I predicted they would occur in direct response to the partner's state and thus be independent of the amount of care provided by the partner.

2.2 Methods

I used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. I maintained a large population and only mated unrelated or distantly related individuals (no common ancestors for at least two generations) to avoid inbreeding in the stock population. The beetles used in this study comprised of sixth-, seventh-, and eighth-generation beetles from lines originally collected in Edinburgh, UK and Warmond, The Netherlands. They were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20 °C and constant light. Non-breeding adults were fed raw organic beef twice a week.

2.2.1 Experimental design

In the first part of this experiment, I generated beetles of different sizes using a full-sib design based on previously established methodology for this species (Steiger 2013). This design allowed me to exclude potential confounding effects due to genetic differences between individuals of different sizes (Steiger 2013). To this end, I paired up unrelated virgin males and females, provided them with a previously frozen mouse carcass, and allowed them to produce a brood. For each of these 90 broods, I removed half of the brood from the carcass once the larvae had reached the third instar and achieved a mass of 80–120 mg (approximately two days after hatching). I recorded the mass of each of these larvae and kept them in individual containers until they reached adulthood, at which point they were used as the small parents in my experiment. I left the remaining larvae on the carcass until almost the entire carcass was consumed, removing them right before dispersal (four to five days after hatching). I again measured their individual mass and put each larva in a separate container until they reached adulthood, at which point they were used as the large parents in my experiment. The larvae do not feed after dispersal and before eclosion, and the size of a larva at dispersal therefore determines its adult body size (Lock et al. 2004).

When these small and large individuals reached adulthood, they were bred to collect data on their own and their partner's parental care behaviour. All beetles were virgins, and they were bred within two weeks after sexual maturity to avoid behavioural variation due to differences in age. To investigate the effects of male and female state on the dynamics of biparental care, I used a 2 × 2 factorial design with the following treatment groups: a large male paired to a large female ($n = 25$), a large male paired to a small female (*n* = 25), a small male paired to a large female (*n* = 25), and a small male paired to a small female (*n* = 25). The larval mass of my experimental beetles ranged from approximately 80 to 230 mg. The beetles that weighed less than 150 mg when removed from the carcass were classified as small (mean ± SD = 111 ± 14 mg), while beetles that weighed more than 150 mg were classified as large (mean ± SD = 203 ± 24 mg).

The experimental pairs (*n* = 100) were transferred to transparent plastic containers (17 × 12 × 6 cm) with 1 cm of moist soil and a previously frozen mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (22–25 g). Immediately after the eggs were laid, I moved the parents and the carcass to a new container with fresh, moist soil. When the eggs started hatching, I used the newly hatched larvae to generate experimental broods of 15 larvae by pooling larvae from eggs laid by different females across all treatments (Mattey and Smiseth 2015). This cross-fostering design ensures that any adjustments in the focal parent’s behaviour due to its own or its partner's body size can be attributed to interactions between the two parents rather than parental effects or the number of larvae in the brood (Mattey and Smiseth 2015). Due to temporal kin discrimination in this species, parents cannot distinguish between manipulated foster broods and their own broods, as long as the larvae are at the same developmental stage (Oldekop et al. 2007). Since parents kill any larvae that arrive on the carcass before their eggs are expected to hatch (Müller and Eggert 1990), I only provided experimental pairs with a brood once their own eggs had hatched. Before placing the larvae on the carcass, I weighed the brood so that I would be able to calculate offspring growth from hatching to later stages of larval development.

I conducted behavioural observations 24 hours after the parents were provided with a brood, because this stage in larval development corresponds to a peak in parental food provisioning in this species (Smiseth et al. 2003, 2007). I used instantaneous sampling every 1 min for 30 min in accordance with established protocols (Smiseth and Moore 2002, Mattey and Smiseth 2015). I recorded the number of scans each parent spent providing (*i*) direct care, defined as food provisioning to the larvae (i.e., mouth-to-mouth contact with at least one larva) or interacting with the larvae (i.e., being inside or around the crater and allowing larvae to beg), and (*ii*) indirect care, defined as carcass maintenance (i.e., deposition of secretions to the surface of the carcass or excavation of the crypt) or guarding (i.e., standing still in a position where it could defend the brood from predators or interspecific competitors).

At the end of the 30-min observation, I counted the number of larvae still alive and weighed the whole brood. The larvae were then returned to the carcass, and the parents were allowed to care for the brood undisturbed until the larvae dispersed from the carcass about three to four days later. At dispersal from the carcass, I recorded the date, number of larvae, and total brood mass.

2.2.2 Data analysis

All data were analysed using R version 3.1.1. I used general linear models for traits that had a normal error structure (number of larvae at dispersal, average larval mass at dispersal, and early larval growth rate from hatching until the observation) and generalized linear models for traits that had a negative binomial error distribution (time to dispersal) or a Poisson error distribution (female direct care, female indirect care, total direct care, and total indirect care). Because of the high proportion of zeros in the data on male care, I ran a zero-adjusted negative binomial (ZANB) regression (male direct care) and a zero-adjusted Poisson (ZAP) regression (male indirect care), using the 'hurdle' function in the 'pscl' package (Jackman 2014). A binomial structure was assumed for the zero-hurdle model, and a negative binomial and a Poisson structure for the count model on male direct and indirect care, respectively. Significant values on the count model indicate that a given variable has an effect on the amount of care provided, whereas significant values on the zero-hurdle model indicate that a given variable has an effect on the probability of providing no care versus some care. For all of these models, decisions on whether to include the interaction term and any additional effects were based on the lowest AIC score. When the difference in the AIC score was less than two, I used the simpler model.

I conducted separate analyses for the amount of direct and indirect care provided by small and large parents of each sex. All such models included the main effects of male and female size (small or large) and the interaction between male and female body size. Note that for male behaviours, male size represents the focal parent's size and female size represents the partner's size, while for female behaviours, female size represents the focal parent's size and male size represents the partner's size. I also tested for an effect of the partner's behaviour on the amount of care provided by the focal parent.

Carcass size was added as a covariate to all models on parental care because resource availability can influence parental behaviour (Mattey and Smiseth 2015). Indeed, males provided more direct care on larger carcasses ($z = 2.0$, $P = 0.047$), whereas female provided more direct care on smaller carcasses ($z = -2.4$, $P = 0.014$). Carcass size had no effect on indirect care provided by males ($z = 0.24$, $P = 0.81$) or females ($z = -1.6$, $P = 0.11$). I also added brood size at the time of the observation to all parental care models, because although I provided all parents with a brood of 15 larvae, there was some variation in the number of larvae that were alive at the time of the observation. Both males ($z = 1.99$, $P = 0.047$) and females ($z = 4.86$, $P < 0.0001$) spent more time providing direct care to larger broods, but brood size had no significant effect on the amount of male indirect care ($z = 1.66$, $P = 0.098$) or female indirect care ($z = 1.9$, $P = 0.054$).

To assess whether partner responses were mediated through a negotiation or matching process, I compared models in which the amount of time that the partner spent providing care was either added or removed as an additional effect. If such responses were mediated through a negotiation or matching process, I predicted that including the partner's behaviour would remove or reduce the effect of the partner's body size on the amount of care by the focal parent. To examine the level of compensation, I conducted separate analyses for the total amount of direct and indirect care by the two parents. These models included male and female body size, the interaction between these two factors, as well as carcass size and brood size.

Lastly, I tested whether the two parents' size had an effect on early larval growth rate, time to dispersal, number of larvae surviving to dispersal, and average larval mass at dispersal. Total direct care was added as a factor in these models, because the amount of care provided by the two parents is expected to have an effect on offspring fitness. Furthermore, I included the number of larvae dispersing as a covariate in the model for average larval mass at dispersal, since previous studies have shown a negative correlation between number and size of larvae at dispersal (Smiseth et al. 2014).

2.3 Results

As expected, I found that small females spent less time providing direct care to their offspring than large females (Table 2.1, Figure 2.1) and that small males were less likely to provide direct care than large males (zero-hurdle model: Estimate = –0.60, SE = 0.31, $z = -2.0$, $P = 0.048$). However, there was no difference in the amount of direct care provided by small and large males that provided at least some direct care (count model; Table 1). There were also no differences in the amount of indirect care provided by small and large females or by small and large males (Table 2.1, Figure 2.2).

Table 2.1: Effects of parental body size on biparental cooperation. For simplicity, I present the results for the count model for the ZANB and ZAP regressions used to analyse male direct and indirect care, respectively (see text for zero-hurdle model results). Data on female care were analysed using a GLM fitted with a Poisson error structure.

	Focal parent's size		Partner's size		Interaction	
Type of care	*z*	*P*	*Z*	*P*	*z*	*P*
M direct care	-1.7	0.093	-2.2	0.026	1.4	0.066
F direct care	-3.5	<0.001	-2.3	0.022	3.8	<0.001
M indirect care	-0.7	0.48	1.9	0.059	1.6	0.10
F indirect care	-1.9	0.059	-0.09	0.93	-0.2	0.85

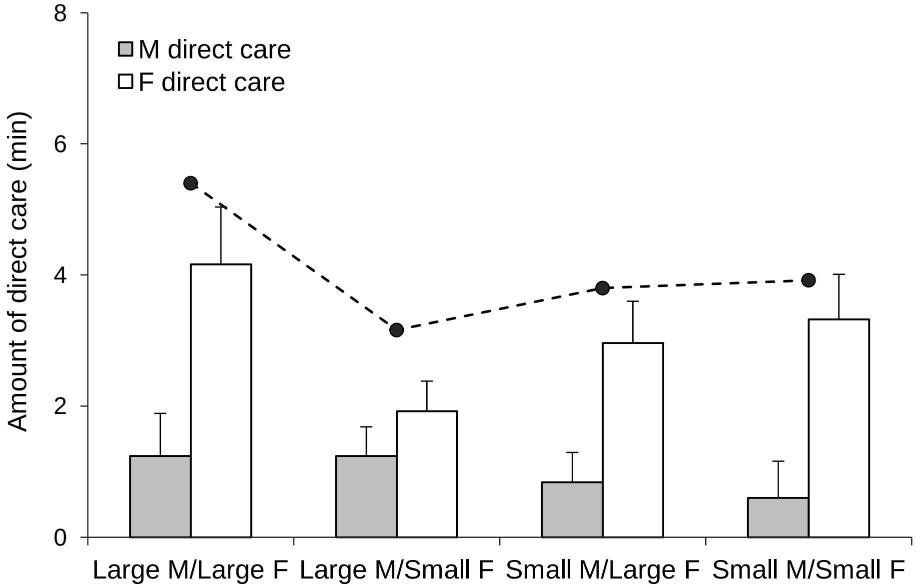

Figure 2.1: Amount of time spent providing direct care (mean ± SE) by small or large males (grey bars) and small or large females (white bars) during a 30-min observation conducted 24 hours after providing the parents with an experimental mixed brood. Direct care behaviours comprise food provisioning and interactions with larvae. The filled circles indicate the mean total direct care provided by the two parents in each treatment group. The line connecting the filled circles illustrates the level of compensation. In this case, the line declines from the treatment where both parents are large to the other three treatments, indicating that the total amount of care is reduced when at least one of the parents are small.

Table 2.2: Effects of male and female body size on total care provided by the two parents. Each row represents the total amount of time spent providing direct and indirect care during a 30-min observation period. These data were analysed using a GLM fitted with a Poisson error structure. I provide information on the parameter estimates (Est), standard errors (SE), test statistics (*z*-values), and *P*-values for the effects of the male's size, the female's size, and the interaction between the two.

	Focal parent's size		Partner's size		Interaction	
Type of care	*z*	*P*	*z*	*P*	*z*	*P*
Total direct care	-3.0	0.003	-3.7	<0.001	3.2	0.001
Total indirect care	-0.04	0.97	0.69	0.49	0.35	0.73

In addition, there was a significant effect of the partner's size on the amount of direct care provided by both sexes (Table 2.1, Figure 2.1). However, in contrast to what I expected, both males and females spent significantly less time providing direct care when they were mated to a small partner than when they were mated to a large one. As a result, the total amount of direct care provided by the two parents was significantly lower when at least one of the parents was small (Table 2.2, Figure 2.1). There was no difference in the amount of indirect care provided by males or females paired to a small or large partner (Table 2.1, Figure 2.2), and the total amount of indirect care provided by the two parents was not affected by the parents' size (Table 2.2, Figure 2.2).

To determine whether the adjustment in the amount of direct care by the focal parent based on its partner's body size was mediated through a response to the partner's behaviour, I compared models in which I included or excluded the amount of direct care provided by the partner as an additional effect in our models. I first tested for evidence for negotiation by testing whether the focal parent adjusted its contribution based on the amount of care provided by its partner. As expected if the two parents negotiate how much care each should provide, I found that females spent more time providing direct care when the male provided less direct care

(Estimate = –0.084, SE = 0.020, $z = –4.3$, $P < 0.0001$). Furthermore, males were more likely to provide direct care when their partner was providing less direct care (zero-hurdle model: Estimate = –0.12, SE = 0.06, $z = –2.1$, $P = 0.037$), although there was no evidence that the amount of direct care provided by the male was influenced by the amount of direct care provided by the female (count model: $z = 0.19$, $P = 0.85$). However, I found no evidence that negotiation accounted for the focal parent's adjustment to its partner's size, as focal parents mated to small partners still spent significantly less time providing care compared to parents mated to large partners when the amount of direct care provided by the partner was included in the model (male direct care: $z = –2.2$, $P = 0.028$; female direct care: $z = –2.4$, $P = 0.018$). Thus, the adjustment by the focal parent to its partner's size was independent of the partner's behaviour, as expected if this adjustment was mediated through a sealed-bid decision.

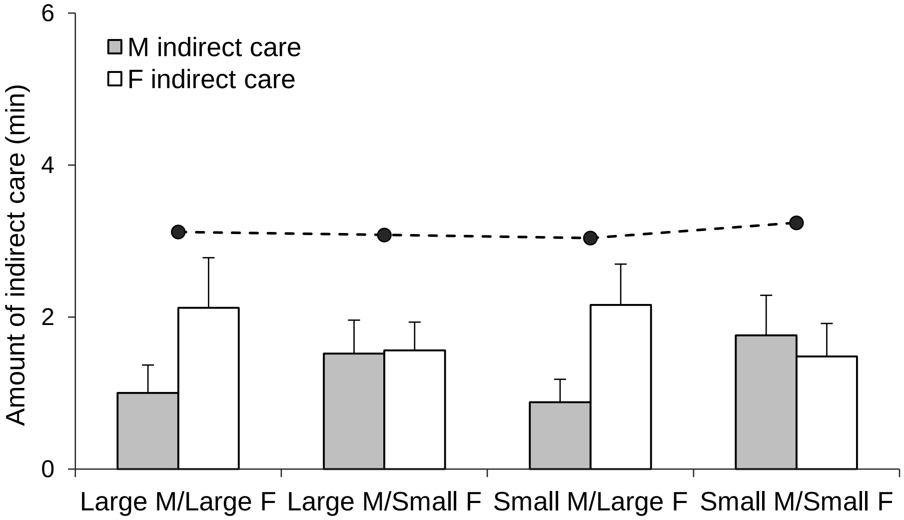

Figure 2.2: Amount of time spent providing indirect care (mean ± SE) by small or large males (grey bars) and small or large females (white bars) during a 30-min observation conducted 24 hours after providing the parents with an experimental mixed brood. Indirect care behaviours comprise guarding and carcass maintenance. The filled circles indicate the mean total indirect care provided by the two parents in each treatment group. The line connecting the filled circles illustrates the level of compensation. In this case, the line is straight across the four treatments, indicating that the total amount of care is similar regardless of male and female body size.

My experimental design also allowed me to test for an effect of the interaction between the focal parent's size and the size of its partner. There was a significant interaction on the amount of direct care provided by females, which reflected that small females spent more time providing direct care when they were mated to a small male, whereas large females provided a similar amount of care regardless of whether they were mated to a small or large male (Table 2.1, Figure 2.1). There was no evidence for such an interaction effect on male direct care, male indirect care, or female indirect care (Table 2.1).

I finally tested for effects of the parents' size on components of the offspring's fitness. I found that larval growth during the first 24 hours on the carcass was higher when the female was large, but there was no effect of male size (Table 2.3). I also found that larval growth rate during the first 24 hours on the carcass was higher in large broods (Estimate = 0.046, SE = 0.008, $t = 6.41$, $P < 0.0001$). Similarly, average larval mass at dispersal was higher in large broods (Estimate = 0.0014, SE = 0.0007, $t = 2.15$, $P = 0.035$) and there was a nonsignificant effect of total direct care on larval mass at dispersal ($t = 1.95$, $P = 0.055$). There were no effects of male or female size on time to dispersal, larval mass at dispersal, or number of larvae surviving to dispersal (Table 2.3).

Table 2.3: Effects of parental body size on offspring fitness. Data on early larval growth, larval mass at dispersal, and number of larvae were analysed using general linear models. Data on time to dispersal were analysed using a GLM fitted with a negative binomial distribution.

	Male size		Female size		Interaction	
Offspring trait	*t/z*	*P*	*t/z*	*P*	*t/z*	*P*
Early larval growth	0.41	0.69	-2.1	0.039	-0.67	0.51
Time to dispersal	-0.08	0.94	0.32	0.75	0.38	0.71
Larval mass	1.94	0.056	0.35	0.73	-0.64	0.52
Number of surviving larvae	0.55	0.58	-0.65	0.52	0.51	0.61

2.4 Discussion

Here, I report evidence from a burying beetle with biparental care showing that individual parents adjust their contribution towards care based on both their own body size and that of their partner. More specifically, I found that small females provided less direct care than large ones and that both males and females provided less direct care when paired with a small partner than with a large one. As a consequence, the amount of total direct care provided by the two parents was lower when at least one of the parents was small. The difference in the amount of care between parents mated to different-sized partners was not related to variation in the amount of care provided by the partner. This suggests that the adjustment in care made by parents mated to a small partner was independent of the amount of care provided by the partner, as predicted by sealed-bid models for the resolution of sexual conflict (Houston and Davies 1985). There was also an effect of the interaction between the size of the focal parent and its partner, as small females provided more care when paired with a small male, while large females provided the same amount of care regardless of whether they were paired with small or large males. Below I provide a detailed discussion of the wider implications of my results for our understanding of biparental cooperation.

My first main finding was that small females provided less direct care than large ones, while there was a non-significant trend in the same direction for males. This finding confirms that female parents adjust their contribution towards parental care based on variation in their own body size. Previous work on the same species shows that small females have lower reproductive success than large females (Steiger 2013). Thus, my results and the results from this previous study show that small females provide less parental care, presumably reflecting some kind of physiological or anatomical constraint on small females. For example, small females might provide less care and have lower reproductive success because they have a reduced capacity to predigest carrion for the larvae and/or produce antimicrobials.

There is mounting evidence showing that cooperating parents adjust their contributions towards offspring care based on variation in components of their own state. In addition to evidence showing that handicapped parents provide less care than control parents (Wright and Cuthill 1989, Harrison et al. 2009, Suzuki and Nagano 2009), there is evidence that the amount of care that a parent provides is dependent on its age (Benowitz et al. 2013), testosterone level (Saino and Møller 1995), and inbreeding status (Pooley et al. 2014, Mattey and Smiseth 2015). Given that parents vary with respect to multiple state components, such as nutritional condition and health, there is now a need for further work to explore how male and female parents adjust their level of parental care based on variation in their own and their partner's state.

My second main finding was that both males and females provided less care when they were mated to small partners than when they were mated to large ones. This result confirms that parents of both sexes adjust their contribution based on the body size of their partner. However, in contrast to what I predicted, parents reduced the amount of care they provided when mated to a small partner. This finding is surprising given that small parents provided less care than large ones and that theoretical models for the evolution of biparental cooperation predict that parents should either compensate (incompletely) or not alter the amount of care that they provide in response to a reduction in the amount of care provided by its partner (Houston and Davies 1985, McNamara et al. 1999). Indeed, previous empirical work on *N. vespilloides* and other species in the genus *Nicrophorus* provides good evidence that parents respond to mate removal or mate handicapping by either increasing or not altering the amount of care that they provide (Smiseth and Moore 2004, Smiseth et al. 2005, Suzuki and Nagano 2009, Creighton et al. 2015, Mattey and Smiseth 2015). Similar results have been reported in birds (Wright and Cuthill

1989, Harrison et al. 2009). One potential explanation for this result is that parents respond to their partner's state not only to adjust for variation in the expected amount of care provided by their partner but also to adjust for their partner’s attractiveness or parental ability (Houston et al. 2005). For example, there is evidence that small parents are less capable of defending their brood against infanticidal intruders (Trumbo 2007). If so, parents mated to small partners might be more at risk from takeovers by intruders, in which case they might reduce their investment in the current brood due to its lower reproductive value. Further work is needed to examine whether parents mated to small partners reduce their investment in the current brood in order invest more in future reproductive attempts.

A key aim of this study was to identify the potential mechanisms by which the focal parent adjusted its contribution based on its partner’s size. I predicted that such responses would be mediated through negotiation, matching, or sealed-bid responses. I found evidence for negotiation as both males and females provided more direct care when their partner provided less direct care (see also Smiseth and Moore 2004, Mattey and Smiseth 2015). Nevertheless, including the partner’s behaviour in the models did not remove or reduce the initial effect of the partner’s size on the amount of care provided by the focal parent. This suggests that the way in which parents responded to their partner’s size was not mediated through a response to the amount of care provided by the partner as predicted by negotiation or matching models (McNamara et al. 1999, Johnstone and Hinde 2006). Instead, the focal parent's response was independent of its partner’s behaviour as predicted by sealed-bid models (Houston and Davies 1995). This finding has important implications for our understanding of the behavioural mechanisms mediating the resolution of sexual conflict over parental care. Negotiation, matching, and sealed-bid responses have traditionally been considered mutually exclusive mechanisms. However, this work provides evidence for both negotiation and sealed-bid responses, as described above. These results are consistent with a previous study investigating the effects of inbreeding on biparental cooperation in the same species (Mattey and Smiseth 2015).

I suggest a simple graphical model based on behavioural reaction norms to illustrate the difference between sealed-bid responses and negotiation and how these two mechanisms might coexist (Figure 2.3). In this model, the intercept depicts a sealed-bid decision, while the slope depicts negotiation between the two parents. Sealed-bid decisions represent a parent's initial decision about how much care to provide to the current brood, which may or may not depend on its own state or its partner's state (Figure 2.3a). In contrast, negotiation represents subsequent changes in the parent's decision about how much care to provide based on information about the actual amount of care provided by the partner (Figure 2.3b). This simple model suggests that these two mechanisms can coexist, and that variation in the amount of care provided by a focal parent might reflect variation in its initial decision about how much care to provide (i.e., the intercept), and its subsequent responses to variation in the amount of care provided by its partner (i.e., the slope; Figure 2.3c). I also argue that we now need to recognise different types of sealed-bid decisions. In Houston and Davies's (1985) classic sealed-bid model, the levels of male and female care were allowed to change over evolutionary time, but there was no scope for facultative adjustments in parental care based on either the parent's own state or its partner's state. These results provide evidence for facultative sealed-bid responses adjusted to both the parent's own state and its partner's state. I therefore distinguish between three types of sealed-bid responses: (*i*) classic non-facultative sealed-bid responses as modelled by Houston and Davies (1985); (*ii*) facultative sealed-bid responses where the focal parent adjusts its level of care to its own state; and (*iii*) facultative sealed-bid responses where the focal parent adjusts its level of care to both its own state and that of its partner. I encourage further theoretical and empirical work to consider different types of sealed-bid responses and the coexistence of sealed bid responses and negotiation.

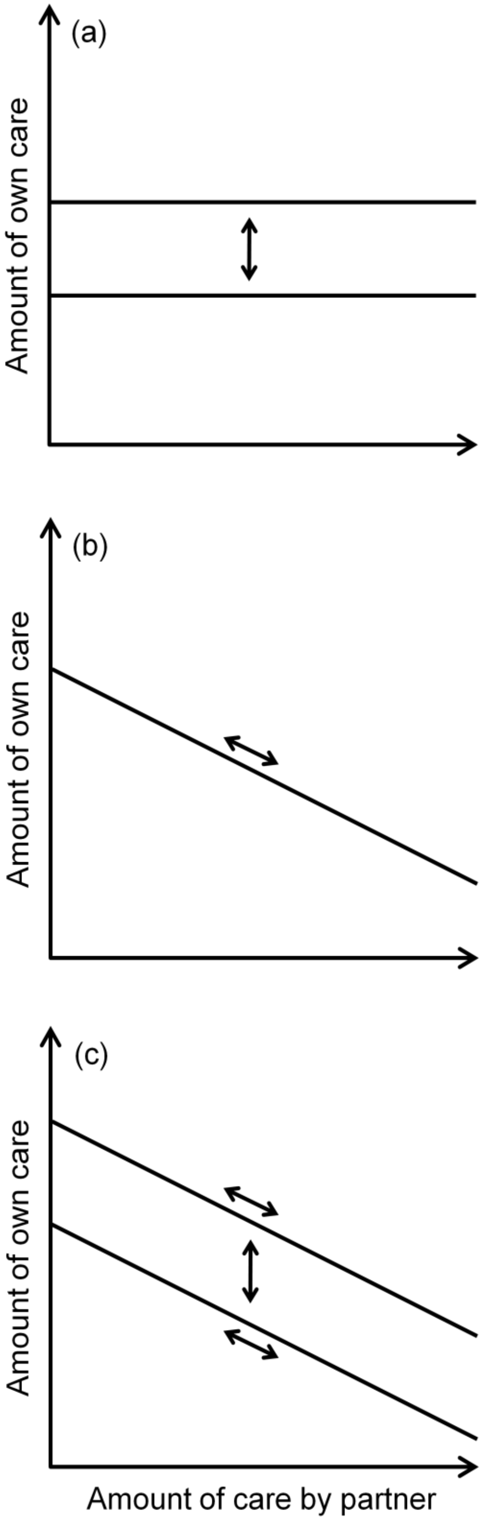

Figure 2.3: Graphical model illustrating sealed-bid decisions (a), negotiation (b), and a combination of sealed-bid decisions and negotiation (c). In all cases, the intercept represents a fixed initial decision that is independent of the amount of care provided by the partner as assumed by sealed-bid models, while the slope represents a flexible adjustment in care based on the amount of care provided by the partner as assumed by negotiation models.

I also found evidence for an effect of the interaction between the parent's own state and the state of its partner on the amount of direct care provided by females. Such an interaction effect might reflect that the focal parent's ability to adjust its contribution to its partner's state is dependent on its own state. For example, if small parents are working closer to their maximum capacity, their ability to adjust their contribution when mated to a small partner might be constrained by their own state. I found no support for this suggestion as small females provided more care when mated to a small male than when mated to a large one, while large females provided the same amount of care regardless of whether they were mated to a small or large male. Thus, there is no evidence that the observed interaction effect is due to constraints on the focal parent's ability to adjust their contribution towards care. Instead, visual inspection of my results suggests that small females reduce their contribution when mated to a large male, while they provide as much care as large females when they are mated to a small male (Figure 2.1). One potential explanation is that small females increase their contribution to care when mated to a small male in order to prevent detrimental effects on the offspring that otherwise might occur when both parents are small. Future work should investigate whether an increase in the workload of small females mated to a small male has a greater beneficial effect on the offspring's fitness as compared to an increase in the workload of small females mated to a large male.

Finally, I found little evidence that variation in the state of the parents had any consequences for components of the offspring's fitness. The larvae of small females grew less in the first 24 hours after hatching, but this difference did not persist until the time of larval dispersal from the carcass. This suggests that the lower amount of care provided by small females is associated with reduced larval growth in the early stages of development, but that parents and/or larvae are capable of compensating for this during later stages of development. In *N. vespilloides*, larval size at dispersal determines adult body size (Lock et al. 2004), which is an important determinant of the reproductive success of adults during fights for possession of carcasses (Otronen

1988). Thus, there would be strong selection on any mechanism that would compensate for reduced early growth, including an extended period of food provisioning by parents and an extended period of self-feeding by larvae.

In conclusion, I report evidence that in a species with biparental cooperation, each parent adjusts its contribution toward care based not only on the amount of care provided by its partner but also on its own state and that of its partner. These results highlight the need for future theoretical and empirical work on biparental cooperation to consider the two parents' state and its implications on the amount of care provided by each parent.

Chapter 3: Parental body size and sexual conflict over consumption from a shared resource

This chapter has been published as referenced below, and this publication appears as Appendix B in this thesis:

> Pilakouta N, Richardson J, Smiseth PT (2015) If you eat, I eat: resolution of sexual conflict over consumption from a shared resource. *Animal Behaviour* 111:175–180.

Abstract

Sexual conflict arises whenever males and females have divergent reproductive interests. The mechanisms mediating the resolution of sexual conflict have been studied extensively in the context of parental care, where each parent adjusts its decision about how much care to provide based on its partner's workload. However, there is currently no information on the mechanisms mediating the resolution of sexual conflict over personal consumption from a shared resource. I address this gap in the burying beetle *Nicrophorus vespilloides*, which breeds on small vertebrate carcasses. The carcass serves as a source of food for both the developing larvae and the caring parents, and parents feed from the carcass for self-maintenance. To study the mechanisms mediating conflict resolution, I experimentally varied the two parents' body size to create variation in carcass consumption. I then assessed whether each parent adjusted its consumption based on its own size, its partner's size, and its partner's consumption. As expected, large parents gained more mass than small parents. Furthermore, males paired to large females gained more mass than males paired to small females, and females responded to their partner's mass change, gaining more mass when their partner did. This study provides insights into the resolution of a new form of sexual conflict, showing that it is mediated through both matching and sealed-bid responses. These findings also suggest that the resolution models developed in the context of sexual conflict over biparental care may apply more generally than previously thought.

3.1 Introduction

Sexual conflict arises whenever males and females have divergent reproductive interests and can occur in various contexts before mating (e.g. male harassment and female resistance; Arnqvist and Rowe 2005), during mating (e.g. duration of copulation; Schneider et al. 2006), or after mating (e.g. contribution to parental care; Houston et al. 2005). Even though previous research has examined many types of sexual conflict (Arnqvist and Rowe 2005, Houston et al. 2005, Parker 2006), one type of conflict that so far has been neglected is that over the consumption of a food resource that is shared by the two parents and their offspring.

Sexual conflict over the consumption of a shared food resource might be common across animal taxa. For example, in many birds, the two parents share a breeding territory where parents search for food, some of which is used for their own consumption and some is used to provision its nestlings. Also, in many insects with biparental care, the two parents share resources in the form of dung, carrion, or wood that serve as food for the parents as well as the developing larvae (Tallamy and Wood 1986). Each parent benefits personally by consuming from the shared resource, as it allows that parent to invest in self-maintenance and thereby enhance its future reproductive potential (Creighton et al. 2009, Billman et al. 2014).

However, given that resources are finite, increased consumption by one parent leaves less of the resource for the offspring and the partner. A study on the burying beetle *N. vespilloides* suggested that sexual conflict over shared resources during the breeding attempt may negatively affect female longevity (Boncoraglio and Kilner 2012). Thus, there is evidence for a conflict battleground between the two sexes over personal consumption from the shared resource, with each parent preferring to consume more resources than would be optimal from its partner's perspective. Nevertheless, the mechanisms underlying the resolution of this form of conflict are still unexplored.

I suggest four mechanisms that might be involved in the resolution of sexual conflict over consumption from a shared resource. The first potential mechanism is coercion, which is based on physical aggression between the two parents. If coercion is mediating the resolution of this conflict, consumption of the resource should depend on asymmetries in fighting ability between the two parents, as the stronger parent might be in a position to control the feeding behaviour of its partner. The other three possible mechanisms (negotiation, matching, and sealed-bid decisions) derive from theoretical models for the resolution of sexual conflict over contribution towards parental care. Negotiation and matching occur when each parent adjusts its own contribution in direct response to its partner's contribution (McNamara et al. 1999, Johnstone and Hinde 2006). When there is negotiation, the focal parent responds to a reduction in the amount of care provided by its partner by increasing its contribution (McNamara et al. 1999). When there is matching, the focal parent matches any increase or reduction in its partner's contribution (Johnstone and Hinde 2006). Sealed-bid decisions occur when each parent makes an initial fixed decision about how much to contribute that is independent of its partner's contribution (Houston and Davies 1985). I suggest that these mechanisms might also apply to the resolution of sexual conflict over consumption from a shared resource because there are clear analogies between these two forms of conflict. Sexual conflict over contributions to parental care occurs because the benefits of care are shared between the two parents while the costs of care are personal (Lessells 2012), and sexual conflict over consumption from a shared resource occurs because the costs of consumption are shared between the parents while the benefits of consumption are personal.

In this study, I investigate the mechanisms underlying the resolution of sexual conflict over carrion consumption in *N. vespilloides*, an insect that breeds on carcasses of small vertebrates (Eggert et al. 1998). The carcass serves as a source of food for the two parents and their offspring during larval development, so the more each parent consumes from the resource, the less will be left for its partner and the offspring (Scott 1989, Boncoraglio and Kilner 2012). Previous work in the burying beetle *N. orbicollis* has shown that there is substantial variation in the parents' mass change over the breeding attempt and that this mass change is a proxy for investment in future reproduction (Creighton et al. 2009, Billman et al. 2014). Because I was interested in whether each parent adjusts its carrion consumption in response to that of its partner, I experimentally varied the body size of the two parents on the assumption that larger individuals consume more carrion. This asymmetry in body size inadvertently introduced asymmetry in the physical strength of the two parents (Otronen 1988), allowing the possibility that the larger parent might enforce their feeding optimum by eating more while interfering with its partner’s access to the carcass. Evidence for physical interference between partners has been observed in the closely related *N. defodiens*. In this species, females behave aggressively towards their male partner to prevent their partner from attracting additional females (Eggert and Sakaluk 1995).

To study the mechanisms mediating conflict resolution and to assess whether each parent adjusts its consumption based on its own size, its partner's size, and its partner's consumption, I recorded (*i*) the amount of time spent feeding on the carcass by each parent during a 30-min observation and (*ii*) the change in the mass of each parent over the reproductive attempt (Creighton et al. 2009, Billman et al.

2014). If sexual conflict over carrion consumption is resolved through negotiation, I predicted that the focal parent would reduce its consumption in response to an increase in consumption by its partner. If it is resolved through matching, I predicted that the focal parent would increase its consumption in response to an increase in consumption by its partner. If the conflict is resolved through sealed-bid decisions, each parent's decisions about how much to consume should be independent of its partner's consumption. Lastly, if the conflict is resolved through coercion, I predicted that a larger parent would prevent a smaller partner from feeding on the carcass.

3.2 Methods

I used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. These beetles were sixth-, seventh-, and eighth-generation beetles from lines originally collected in Edinburgh, UK and Warmond, The Netherlands. They were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20 °C and constant light. Non-breeding adults were fed raw organic beef twice a week.

3.2.1 Experimental design

To induce variation in carcass consumption by the parents, I first generated small and large beetles using a full-sib design based on previously established methodology (Steiger 2013). For each of these 90 broods, I removed half of the brood from the carcass once the larvae reached the third instar, leaving the remaining larvae on the carcass until right before dispersal. I recorded the mass of each larva and kept the larvae in individual containers with moist soil. Larvae weighing less than 150 mg were categorised as small (mean ± SD: 111 ± 14 mg), while larvae weighing more than 150 mg were categorized as large (203 ± 24 mg). Larval mass at dispersal determines adult size, as larvae do not feed after dispersal from the carcass and before eclosion (Bartlett and Ashworth 1988, Lock et al. 2004).

All beetles were bred within two weeks after sexual maturity (10–24 days after eclosion) using a 2 × 2 factorial design: a large male paired with a large female (*n* = 25), a large male paired with a small female (*n* = 25), a small male paired with a large female (*n* = 25), and a small male paired with a large female (*n* = 25). Paired beetles were virgins and did not share common ancestors for at least two generations. The pairs were transferred to transparent plastic containers (17 × 12 × 6 cm) with moist soil and were provided with freshly thawed mouse carcasses (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (22–25 g). For each of these matings, I recorded the mass of the carcass and the pre-breeding mass of each parent. Immediately after eggs were laid, I moved the parents and the carcass to a new container. When the eggs started hatching, I generated experimental broods of 15 larvae by pooling larvae from eggs across all treatments (Mattey and Smiseth 2015). This design ensured that there was no parent-offspring co-adaptation (Lock et al. 2004) and that any differences in the parents' consumption of the carcass were not mediated through differences in brood size.

Twenty-four hours after providing the parents with a brood, I conducted behavioural observations using instantaneous sampling every 1 min for 30 min (Martin and Bateson 1986, Smiseth and Moore 2002, Smiseth et al. 2003). During this time, I recorded the number of scans that each parent spent feeding on the carcass. Parents were then allowed to care for the brood undisturbed until the larvae dispersed from the carcass about four days later. At dispersal, which corresponds to the end of the parental care period, I recorded the post-breeding mass of each parent. I calculated each parent's change in mass during the breeding period, by subtracting its pre-breeding mass from its post-breeding mass.

3.2.2 Data analysis

Data were analysed using R version 3.2.0. In all analyses for mass change, I used absolute rather than relative changes in mass, because I was specifically interested in examining differences in the amount of carrion consumed. Mass change data had a normal error structure, so I used general linear models for those analyses. Because the behavioural data (time spent feeding on carcass) were zero-inflated, I ran zero-adjusted negative binomial (ZANB) regressions, using the hurdle function in the pscl package (Jackman 2014), which splits the data into two components. Significant values on the zero-hurdle model indicate that a given variable influenced the probability of consuming carrion, whereas significant values on the count model indicate that a given variable influenced how much time was spent consuming carrion. All models included male size, female size, the interaction between male and female size, as well as time spent feeding or mass change by the partner. Previous studies on the same species investigating the resolution of sexual conflict over biparental care found that the focal parent's response to the partner's behaviour and the partner's state were independent (Mattey and Smiseth 2015), so I included both variables in my starting models.

Carcass size was included as a covariate in all models because resource availability may influence the parents' consumption. Males but not females spent more time feeding on larger carcasses (male: $z = 2.03$, $P = 0.042$; female: $z = 1.54$, $P = 0.12$), but carcass size had no effect on mass change in either sex (male: $t = –0.70$, $P = 0.48$; female: $t = –0.91$, $P = 0.36$). I also added brood size at the time of the observation as a factor, because although I provided all parents with a brood of 15 newly hatched larvae, there was some variation in the number of larvae alive at the time of the observation. Nevertheless, brood size did not have a significant effect on the amount of time parents spent feeding on the carcass (male: $z = –1.89$, $P = 0.059$; female: $z = –1.61$, $P = 0.11$) or the parents' change in mass (male: $t = –0.78$, $P =$

0.44; female: $t = -1.40$, $P = 0.17$). Decisions about which variables to include in the final models were based on AIC model selection criteria. I also tested for multicollinearity in all models by estimating variance inflation factors using the vif function in the car package (Fox and Weisberg 2011). The largest variance inflation factors were ≤3, indicating absence of multicollinearity.

3.3 Results

Large parents spent more time feeding from the carcass (Table 3.1, Figure 3.1) and also gained more mass over the reproductive attempt (Table 3.2, Figure 3.2) than small parents. Males spent more time feeding from the carcass and gained more mass when they were paired to a large female than when paired to a small female (Tables 3.1 and 3.2, Figures 3.1 and 3.2). However, there was no significant difference in the time spent feeding or mass change by females paired to large and small males (Tables 3.1 and 3.2, Figures 3.1 and 3.2).

Interestingly, there was a significant effect of the interaction between male and female size on male feeding behaviour and mass change (Tables 3.1 and 3.2). This interaction effect reflected that large males spent more time feeding and gained more mass when paired to a large female, whereas small males spent a similar amount of time feeding and gained the same mass regardless of the size of their partner (Figures 3.1 and 3.2). There was no effect of the interaction between male and female size on female mass change or feeding behaviour (Tables 3.1 and 3.2).

Lastly, I tested whether parents adjust their personal consumption based on that of their partner. Males were more likely to feed from the carcass when their partner was feeding less (zero-hurdle model: Estimate = –0.12, SE = 0.05, $z = -2.2$, $P = 0.027$), but males that fed from the carcass did not adjust the amount of time they spent feeding based on their partner's feeding behaviour (Table 3.1). Females did not adjust their feeding behaviour to that of their partner (zero-hurdle model: Estimate = –0.34, SE = 0.19, $z = -1.8$, $P = 0.070$; count model: Table 3.1). Females responded to their partner's mass change, gaining more mass when their partner gained more mass (Table 3.2). Males, on the other hand, did not adjust their mass change in response to that of their partner (Table 3.2).

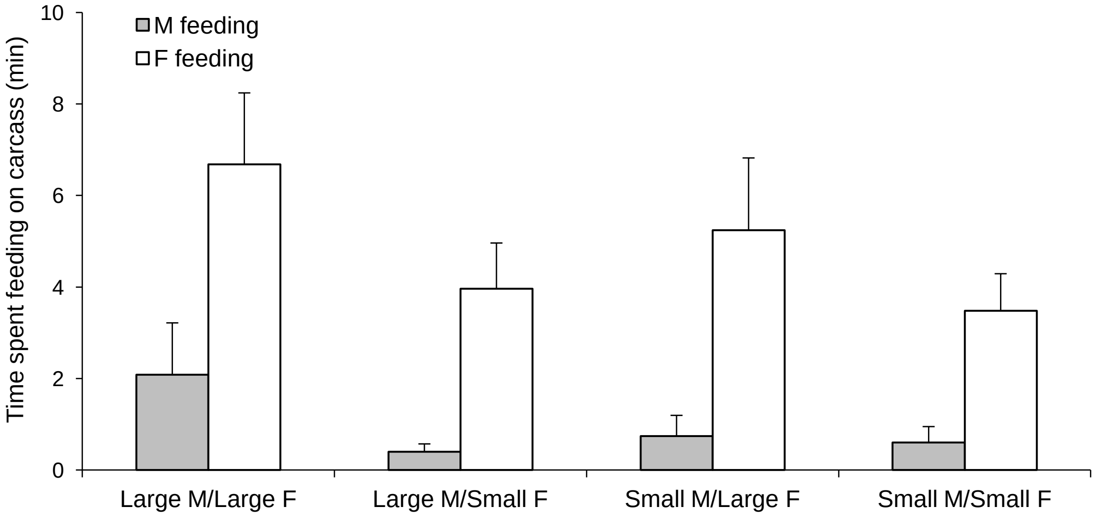

Figure 3.1: Means ± SE for amount of time spent feeding on the carcass (min) by small or large males (M: grey bars) and small or large females (F: white bars) during a 30-min observation.

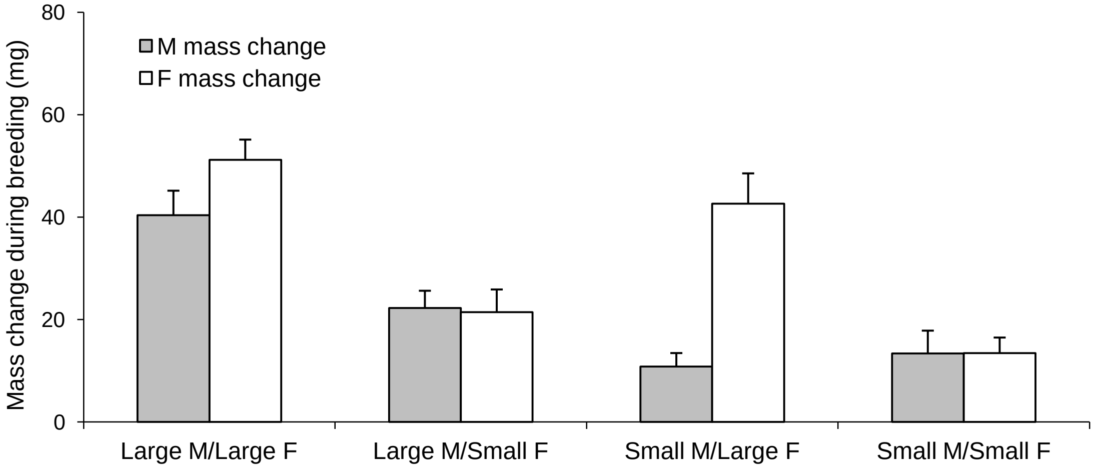

Figure 3.2: Means ± SE for mass change (mg) over the reproductive attempt for small or large males (M: grey bars) and small or large females (F: white bars).

Table 3.1: Effects on the amount of time spent feeding from the carcass by male (M) and female (F) parents during a 30-min observation. Data were analysed using ZANB regressions. For simplicity, I present the results for the count model (see text for zero-hurdle model results).

	M size				F size				Interaction				Partner's feeding rate			
	Est	SE	*z*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*
M feeding	-1.2	0.5	-2.2	0.03	-1.6	0.6	-2.7	0.01	2.0	0.8	2.5	0.01	-0.001	0.1	-0.01	0.99
F feeding	-0.21	0.26	-0.8	0.43	-0.5	0.27	-1.9	0.06	-0.4	0.5	-0.7	0.47	-0.36	0.2	-1.9	0.054

Table 3.2: Effects on male (M) and female (F) mass change (mg) over the course of the breeding attempt.

	M size				F size				Interaction				Partner's mass change			
	Est	SE	*T*	*P*	Est	SE	*t*	*P*	Est	SE	*t*	*P*	Est	SE	*t*	*P*
M mass change	-29	5.6	-5.2	<0.0001	-18	5.7	-3.2	0.002	21	7.9	2.7	0.01	0.13	0.10	1.4	0.17
F mass change	-5.1	5.1	-1.0	0.32	-28	4.6	-6.1	<0.0001	-3.1	9.5	-0.3	0.74	0.22	0.11	2.0	0.047

3.4 Discussion

In this study, I examined the mechanisms that mediate the resolution of sexual conflict in a previously neglected context: conflict over personal consumption from a resource that is shared by the two parents and their dependent offspring. I found some evidence for sealed-bid decisions (i.e., decisions that are independent of the partner’s behaviour) as parents of both sexes adjusted their consumption of carrion based on their own size, and males adjusted their consumption based on the size of their partner. I also found some evidence for matching as females gained more mass when their partner gained more mass. I found no evidence for size-dependent coercion, as parents did not feed less when paired to large partners. My results therefore suggest that the resolution models developed in the context of sexual conflict over biparental care may apply more generally than previously thought. I provide a more detailed discussion of my results below.

The main aim of my experimental design was to induce variation in the parents' consumption of carrion by experimentally varying the body size of the focal parent and its partner. As expected, large parents of both sexes consumed more carrion than small parents. This result confirms my initial assumption that large individuals need more food to replenish their energy reserves and also provides some evidence for sealed-bid decisions, whereby a parent's decision about how much to consume is independent of its partner’s behaviour. An inadvertent consequence of this size manipulation was that I introduced asymmetry in the physical strength of the two parents (Otronen 1988). However, I found no evidence that large parents used their physical superiority to prevent a small partner from feeding on the carcass. My results thus do not support the hypothesis that size asymmetry between parents can influence the resolution of sexual conflict through coercion or punishment. The absence of coercion in this context might be due to its potential costs; attacking a partner that contributes towards providing care for the offspring may incur costs to the focal parent in terms of receiving less assistance from the partner in the future.

Another key finding in this study was that each parent adjusted its consumption of carrion based on attributes of its partner. Females gained more mass when their partner gained more mass, while males adjusted their mass gain based on their partner’s body size rather than its consumption of carrion. These results suggest that there is a sex difference in how parents respond to attributes of their partner: females match their consumption to that of their partner as predicted by matching models (Johnstone and Hinde 2006), while males make decisions that are independent of the behaviour of their partner as predicted by sealed-bid models (Houston and Davies 1985). Previous work on the same species has reported sex differences in how caring parents respond to mate removal: males provide more care following the removal of the female, while females provide a similar amount of care regardless of whether the male is present or absent (Smiseth et al. 2005). The sex difference in personal consumption reported here may reflect that females spend more time on the carcass than males (Smiseth and Moore 2004, Smiseth et al. 2005) and that females therefore have better access to information about their partner's feeding rate. In contrast, males spend more time away from the carcass and may adjust their mass change to the expected feeding rate of their partner based on their partner's size. Indeed, I found that males spent more time feeding on the carcass and gained more mass over the reproductive attempt when they were paired to a large female, which consumed more carrion compared to a small female. The finding that females match their consumption to that of the male is interesting given that the limited empirical evidence for matching in the context of biparental care (Hinde 2006). I suggest that the matching model (Johnstone and Hinde 2006) might be better suited for the resolution of sexual conflict over foraging from a shared resource.

For males, the observed pattern for feeding behaviour (Table 3.1) closely matched the pattern for mass change over the reproductive attempt (Table 3.2); both male feeding behaviour and male mass change were influenced by the male's own size, his partner’s size, and the interaction between the two (Tables 3.1 and 3.2). In contrast, female mass change was influenced by her own size and her partner's mass change (Table 3.2), but this pattern was not reflected in the female's feeding behaviour (Table 3.1). One potential explanation for this discrepancy is that, when both parents provide care, females are typically much more involved in provisioning food to the larvae than are males (Smiseth and Moore 2004, Walling et al. 2008). Thus, females may regurgitate most of the carrion they consume to the larvae, whereas males may consume carrion primarily to replenish their own energy reserves. This interpretation is supported by visual inspection of my data, which suggest that the overall mass change was very similar for males and females in most treatments (Figure 3.2) even though females spent significantly more time feeding on the carcass (Figure 3.1). An alternative explanation is that females appear to be spending more time feeding but instead they are making the carcass more accessible to the larvae. In this species, the larvae obtain some of their food by self-feeding from the day of hatching (Smiseth et al. 2003), and parents may enhance the larvae’s ability to self-feed by cutting it open. It is not possible to discriminate between feeding and cutting the carcass open during behavioural observations. Another plausible explanation for the difference between the results for female feeding behaviour and mass change is that females incurred high energetic costs during egg production and laying, and they were consuming carrion to compensate for this initial energy cost. It is not possible to differentiate between these explanations based on the results from my experiment.

Overall, I found some evidence for sealed-bid decisions, as parents adjusted their consumption of carrion based on cues that were independent of their partner’s behaviour: their own size (males and females) and their partner's size (males). However, I also found some evidence for matching, as females gained more mass when their partner gained more mass. The resolution of conflict over feeding from a shared resource is thus mediated through both matching and sealed-bid responses in this species. Interestingly, a recent paper in *N. vespilloides* showed that outbred and inbred parents resolve conflict over parental care contributions using negotiation and sealed-bids (Mattey and Smiseth 2015). The same pattern was reported in Chapter 2 for small and large parents. These findings suggest that, even within the same species, different mechanisms may be involved in mediating the resolution of different forms of sexual conflict. I encourage future studies to explore whether this might also be the case in other taxa.

My results also raise interesting questions as to whether sexual conflict over consumption from a shared resource could influence sexual conflict over contributions to parental care. For example, if a parent is prevented from feeding by a physically superior partner, it may retaliate by providing less care. On the other hand, if a parent is providing a disproportionate amount of care, its partner may be more tolerant of that parent feeding more from the resource. I am not aware of any studies investigating how the resolution of one type of sexual conflict may interact with the resolution of a different type of conflict in the same system. Such interactions might be expected whenever there are multiple types of sexual conflict occurring either simultaneously or sequentially over the reproductive bout, and I encourage future research to address this gap.

In summary, this study provides novel insights into the resolution of a largely ignored form of sexual conflict by showing that parents use information on their partner to decide how much food to consume from a shared resource. These adjustments in feeding are directly related to the parents' future reproductive potential, since consumption of the breeding resource is a proxy for investment in future reproduction (Creighton et al. 2009, Billman et al. 2014). My findings also suggest that parents use different mechanisms for the resolution of different forms of sexual conflict, and they raise the possibility of interactions between different sources of conflict occurring over the breeding attempt.

Chapter 4: Size-dependent fighting success and parental care

This chapter has been published as referenced below, and this publication appears as Appendix C in this thesis:

> Pilakouta N, Halford C, Rácz R, Smiseth PT (2016) Effects of prior contest experience and contest outcome on female reproductive decisions and offspring fitness. *American Naturalist* 188:319–328.

Abstract

Winning or losing a prior contest can influence the outcome of future contests, but it might also alter subsequent reproductive decisions. For example, losers may increase their investment in the current breeding attempt if losing a contest indicates limited prospects for future breeding. Using the burying beetle *Nicrophorus vespilloides*, I tested whether females adjust their pre-hatching or post-hatching reproductive effort after winning or losing a contest with a same-sex conspecific. Burying beetles breed on carcasses of small vertebrates for which there is fierce intrasexual competition. I found no evidence that winning or losing a contest influenced reproductive investment decisions in this species. Instead, I show that a female's prior contest experience (regardless of its outcome) influenced the amount of posthatching care provided, with downstream consequences for the female’s reproductive output; both winners and losers spent more time provisioning food to

their offspring and produced larger broods than females with no contest experience. I discuss the wider implications of my findings and present a conceptual model linking contest-mediated adjustments in parental investment to population-level processes. I propose that the frequency of intraspecific contests could both influence and be influenced by population dynamics in species where contest experience influences the size and/or number of offspring produced.

4.1 Introduction

Winner-loser effects occur when an individual's prior experience with a fighting contest influences the outcome of its subsequent contests. Previous work in a wide range of taxa has shown that the winner of a contest is more likely to win a future fight, whereas the loser is more likely to lose again (Chase et al. 1994, Safryn and Scott 2000, Hsu et al. 2006, Rutte et al. 2006, Kasumovic et al. 2010, Fawcett and Johnstone 2010). Two recent studies have challenged this idea by showing that both winners and losers have increased fighting success in future contests (Benelli et al. 2015a, 2015b). This suggests that prior experience with a fight, regardless of its outcome, may give individuals an advantage over inexperienced individuals.

Despite an extensive literature on winner-loser effects, little is known about the wider implications of winning or losing a fight beyond an effect on success in future fights. For example, the outcome of a contest may influence an individual's subsequent reproductive decisions by providing information about its size and condition relative to its competitors (Hsu and Wolf 2001, Walling et al. 2008, Okada et al. 2010). I am aware of only one study that has investigated the effects of contest outcome in the context of reproduction. Okada et al. (2010) compared male flour beetles (*Gnatocerus cornutus*) that lost or won a prior contest and found that losers transferred more sperm during a subsequent copulation compared to winners. Although this study shows that losing a contest can affect sperm allocation in males, there is no information as to whether contest outcome may also influence decisions over parental effort. If losing indicates limited prospects for success in future breeding attempts, a loser might increase its parental effort in the current breeding attempt, thereby altering both its own fitness as well as the fitness of its offspring. Alternatively, individuals might base their reproductive investment decisions on whether they have participated in a fighting contest rather than whether they won or lost such a contest. This could occur when encounters with conspecific competitors serve as a cue for the intensity of competition in the population and therefore the likelihood of future breeding opportunities.

This study addresses this gap in our knowledge using the burying beetle *Nicrophorus vespilloides*. A number of attributes make this species particularly well suited for studying how contest outcome and contest experience might affect parental care decisions. First, there is fierce intrasexual competition over the possession of a carcass, which is an ephemeral, high-value resource (Safryn and Scott 2000). Body size is the strongest determinant of the outcome of these contests with larger beetles being more successful at both acquiring and defending a carcass (Bartlett and Ashworth 1988). A study on the related *N. humator* reported evidence for winner-loser effects, as the outcome of a prior contest affected the likelihood of success in subsequent contests (Otronen 1990). Secondly, *N. vespilloides* females (sometimes assisted by a male) provide elaborate parental care that enhances larval growth and survival (Eggert et al. 1998, Smiseth and Moore 2002, Smiseth et al. 2003). Pre-hatching care includes preparation of the carcass and investment of nutrients in eggs (Rozen et al. 2008, Monteith et al. 2012), while post-hatching care includes brood defense, secretion of antimicrobials, and food provisioning (Eggert et al. 1998, Smiseth et al. 2003, Rozen et al. 2008). Lastly, there is evidence for a trade-off between investment in current and future reproduction in *N. vespilloides* and the related *N. orbicollis*: females that overproduce offspring in the first breeding attempt suffer a reduction in fecundity in future breeding attempts (Creighton et al. 2009, Ward et al. 2009, Billman et al. 2014).

The aim of my study was to investigate whether females adjust their parental effort in the current brood depending on whether they won or lost a prior contest. I focused on female reproductive decisions because females are more involved in care and stay on the carcass longer than males (Fetherston et al. 1994, Eggert et al. 1998, Smiseth and Moore 2002, Rauter and Moore 2004, Smiseth et al. 2005). Furthermore, there is no evidence that the additional presence of a male has a positive effect on larval growth or survival under laboratory conditions (Smiseth et al. 2005). To avoid a possible confounding effect of body size on the reproductive decisions of females (Steiger 2013), I compared medium-sized females with no fighting experience (controls) with medium-sized females that either won or lost a prior contest to small or large females, respectively. Although I hypothesized that the outcome of a prior contest would influence subsequent reproductive decisions, females might also adjust their reproductive investment simply due to their experience with a contest, regardless of its outcome. I thus used an experimental design that allowed me to disentangle the potential effects due to experience with a prior contest and the outcome of that contest (see Methods).

I first tested whether females adjusted their pre-hatching investment (clutch size and egg size) and post-hatching investment (amount of direct care and amount of indirect care) based on contest outcome or contest experience. I then tested whether any adjustments in investment had fitness consequences for the mother by measuring brood size (a measure of reproductive output) and maternal post-breeding longevity (a measure of residual reproductive value). Lastly, to determine whether contest outcome or contest experience had consequences for offspring fitness, I measured larval begging rate during early development and average larval mass at the dispersal stage, which corresponds to the end of the parental care period. I predicted that losers would increase their investment in current reproduction and consequently suffer higher mortality after breeding. As a result of such an increase in parental investment, I also expected that losers would have more and/or larger offspring at the end of the parental care period.

4.2 Methods

I used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. The beetles used in this study comprised fourth-, fifth-, and sixth- generation beetles from lines originally collected in Edinburgh, UK and Warmond, The Netherlands. All adult beetles were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20 °C and constant light (Mattey and Smiseth 2015a). All non-breeding adults were fed small pieces of raw organic beef twice a week.

4.2.1 Experimental design

Given that the outcome of contests over the possession of a carcass is largely determined by body size (Bartlett and Ashworth 1988) and that larger females provide better care to their offspring (Steiger 2013), it was essential to use an experimental design that would allow me to separate effects due to winning or losing a contest from effects due to body size. To this end, I compared medium-sized females that had different experiences from a prior contest; these medium-sized females competed with either larger or smaller females and thus lost or won the contest, respectively. This design excluded confounding effects of focal female size but not of competitor size, and it did not separate the effects of contest experience from effects of merely encountering a conspecific.

Because adult body size is determined by larval mass at the dispersal stage (Bartlett and Ashworth 1988, Lock et al. 2004), it is possible to generate different-sized beetles by removing larvae from the carcass at various times after hatching (Steiger 2013). Thus, for each of 100 broods, I removed third-instar larvae weighing 80–100 mg, 140–160 mg, and 200–220 mg to generate small, medium-sized, and large adults, respectively. I kept each of these larvae in individual containers (12 × 8 × 2 cm) filled with moist soil until sexual maturity. After eclosion, I measured the pronotum length of all females to confirm that adults from the three groups differed in body size. As intended, there were clear differences in the mean (± SD) pronotum length (mm) for the three groups: 2.89 (± 0.14) for small females, 3.65 (± 0.14) for medium-sized females, and 4.30 (± 0.11) for large females.

I then set up contests over the possession of a carcass by pairing medium-sized females with either a small or large female competitor, which provided me with winners and losers of the same size. I expected the medium-sized female to win the contest if she was paired with a small female, whereas I expected her to lose the contest if she was paired with a large female. I only used females that had been sexually mature for up to two weeks (i.e., 10–24 days after eclosion), because female age has been shown to influence contest outcome in the closely related *N. orbicollis* (Trumbo 2012). At the start of the experiment, I transferred pairs of females to transparent plastic containers (17 × 12 × 6 cm) with 1 cm of moist soil and a freshly thawed mouse carcass of a standardized size (20–22 g). I left the pairs undisturbed for three days, at which point I determined the winner by checking which beetle was present on the carcass (Safryn and Scott 2000; Trumbo 2012). In the vast majority of cases, the outcome of these contests was consistent with what I expected. However, when a medium-sized female won the contest to a large female or a medium-sized female lost the contest to a small female, she was excluded from the rest of the experiment. For the next part of the experiment, I allowed winners to breed on the mouse they had successfully competed for to mimic the outcome of winning a contest in the wild. In contrast, I prevented losers from breeding on the same mouse that was used for the contest, because losers would be driven away by the winner under natural conditions. Allowing losers to breed on the same mouse would have introduced uncertainty as to whether the female perceived herself to be a winner or loser of the contest. I therefore transferred losers to a new container (17 × 12 × 6 cm) with 1 cm of moist soil and a new mouse of the same size (20–22 g). Winners and losers were mated with an unrelated, virgin male immediately following the contest; that is, three days after they were paired up with a small or a large female, respectively.

In addition to the winner and loser treatments, I added two control treatments of medium-sized females that had no prior experience with a contest. Because losers had been exposed to two carcasses and winners had been exposed to only one, one of the control treatments matched the winner treatment while the other control treatment matched the loser treatment. To this end, winner-control females were exposed to only one carcass, and a male was added to the box three days later. Meanwhile, loser-control females were exposed to two carcasses; they stayed on the first carcass for three days, at which time they were transferred to a different box with a new carcass of the same size and an unrelated, virgin male. I also collected data on the females' pre-breeding mass to be added as a covariate in my statistical models. For females exposed to only one carcass (winners and winner-controls), I measured pre-breeding mass before placing the females on the carcass. For females exposed to two carcasses (losers and loser-controls), I measured pre-breeding mass before placing the females on the second carcass (which was the one they bred on). The total sample size for this experiment was n = 224 females, and the sample size for each treatment was as follows: n = 56 for loser females, n = 57 for loser-control females, n = 58 for winner females, and n = 53 for winner-control females.

To test for effects of contest outcome on pre-hatching reproductive effort, I recorded the number of eggs laid by each female and measured average egg size in each clutch. To do this, I checked the containers twice a day after mating for the presence of eggs. When the first eggs were laid, I removed the male to remove any effects of male presence on the female's post-hatching reproductive decisions. Immediately before hatching, I scanned the bottom of each container using a CanoScan 9000F Mark II scanner to calculate egg size. For each image, I measured the length and width of six randomly selected eggs in pixels using ImageJ (Abramoff et al. 2004, Monteith et al. 2012). I then converted these measurements to metric length (mm) and calculated a prolate spheroid volume V for each egg using the equation $V = (1/6)\pi w^2 l$, where w is the width and l the length of the egg (Berrigan 1991).

To test for effects of contest outcome on post-hatching reproductive effort, I conducted behavioural observations for a random subset of broods (n = 15 for loser females, n = 15 for loser-control females, n = 17 for winner females, and n = 18 for winner-control females). This was done 24 hours after the first larva hatched in each brood, as this stage in larval development corresponds to the peak in post-hatching care in this species (Smiseth et al. 2003). I used instantaneous sampling every 1 min for 30 min in accordance with established protocols (Smiseth and Moore 2002, Mattey and Smiseth 2015b). I recorded the number of scans that a female spent providing (*i*) direct care, defined as food provisioning to the larvae (i.e., mouth-to-mouth contact with at least one larva) or interacting with the larvae (i.e., being inside or around the crater and allowing larvae to beg), and (*ii*) indirect care, defined as carcass maintenance (i.e., deposition of secretions to the surface of the carcass or excavation of the crypt) or guarding (i.e., standing still in a position where it could defend the brood from predators or interspecific competitors). I also measured the larvae's begging rate as a proxy for offspring condition during early development (Smiseth and Moore 2004). To this end, I recorded the number of larvae begging at each scan, defined as larvae raising their head towards the parent while waving their legs or touching the parent with their legs (Smiseth and Moore 2002). From these data, I calculated an average begging rate for each brood, adjusting for brood size and the amount of time the parent spent in proximity to the larvae, given that larvae only beg in the presence of a parent (Rauter and Moore 1999, Smiseth and Moore 2002). This rate was based on the equation $b_i = \sum b / L \times 100/p$, where b_i is the percentage of time spent begging by each larva in the brood, $\sum b$ is the total number of larval begging events counted during the 30 scans of an observation session, L is the brood size, and p is the number of scans the parent was in close proximity to the larvae (Smiseth and Moore 2002, Smiseth and Moore 2004).

At the end of the 30-min observation, I counted the number of larvae and weighed the whole brood. The larvae were returned to the carcass, and the female was allowed to care for the brood undisturbed until the larvae dispersed from the carcass about four days later.

At dispersal from the carcass, I recorded the size of the brood and the total brood mass. I calculated average larval mass by dividing total brood mass by the number of larvae in that brood. Brood size was used as a measure of the female's reproductive output. Females were transferred to individual containers filled with moist soil and were checked twice a week until death to record their post-breeding longevity, which was used as a measure of residual reproductive value (Boncoraglio and Kilner 2012).

4.2.2 Data analysis

All analyses were performed using R version 3.2.0. I used general linear models for traits with a normal distribution (egg size, larval begging rate, average larval mass at dispersal, and post-breeding longevity) and generalized linear models for traits with a Poisson distribution (amount of direct and indirect care) or a negative binomial distribution (egg number and brood size at dispersal). My initial hypothesis was that the outcome of a prior contest would influence female reproductive decisions. Nevertheless, another possibility was that breeding females would adjust their reproductive investment simply due to the experience of a prior contest, regardless of its outcome. To disentangle potential effects due to experience with a contest and the outcome of that contest, all starting models included the following three factors: (*i*) prior contest experience, which compared the winners and losers to the controls (contest experience versus no contest experience); (*ii*) number of mice encountered, which compared the winners and winner-controls to the losers and loser-controls (one mouse versus two mice); and (*iii*) the interaction between these two factors, which reflected the effect of contest outcome (winning versus losing). I also added female pre-breeding mass and age at the time of mating as covariates in the models for clutch size, egg size, brood size at dispersal, and larval mass at dispersal. The models for behavioural data (direct care, indirect care, and larval begging rate) included carcass size, brood size and average larval mass at the time of the observation as covariates, because parents might adjust the amount of care they provide based on the size of the carcass, the brood size, and the developmental stage of the larvae (Smiseth et al. 2003). Lastly, I included female age at the dispersal stage in the model for female post-breeding longevity. Decisions on whether to include covariates in the final models were based on the lowest AIC score.

4.3 Results

I found no evidence that prior experience with a contest or the outcome of that contest influenced pre-hatching investment, as measured by clutch size and egg size (Table 4.1). However, prior experience with a contest had a significant effect on post-hatching investment, with winners and losers providing more direct care (food provisioning and interactions with the larvae) than control females (Table 4.1, Figure 4.1a). There was no additional effect of the outcome of the contest on post-hatching investment (see Interaction in Table 4.1); winners and losers spent a similar amount of time providing direct care to the developing larvae (Figure 4.1a). There were no effects of either contest experience or contest outcome on the amount of time females spent providing indirect care (Tables 4.1). However, females provided more indirect care when breeding on smaller carcasses (LR $\chi^2_1 = 6.25$, $P = 0.01$) and when the larvae were larger at the time of observation (LR $\chi^2_1 = 21.0$, $P < 0.0001$). There were no effects of the number of mice encountered on either pre-hatching or post-hatching investment (Table 4.1).

Table 4.1: Effects of contest experience (yes or no), the number of mice encountered by the female (one or two), and the interaction between these two factors (reflecting the effect of contest outcome) on female pre-hatching and post-hatching investment.

	Contest experience		Number of mice		Interaction	
	LR χ^2_1	*P*	LR χ^2_1	*P*	LR χ^2_1	*P*
Pre-hatching investment						
Egg number	0.05	0.82	0.41	0.52	0.47	0.50
Egg size	1.06	0.30	0.01	0.92	0..28	0.60
Post-hatching investment						
Amount of direct care	52.77	<0.0001	0.55	0.46	0.48	0.49
Amount of indirect care	0.42	0.52	3.21	0.07	0.29	0.59

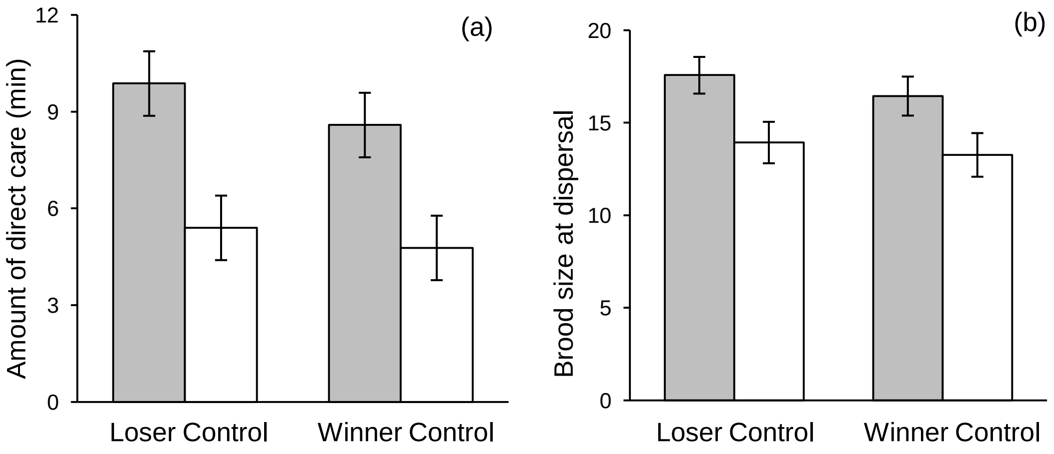

Figure 4.1: Amount of direct care (a) and brood size at dispersal (b) for females that either lost or won a prior contest (grey bars) and their corresponding controls (white bars). Error bars indicate SE.

Prior experience with a contest had a significant effect on the reproductive output of females, as winners and losers had larger broods at the dispersal stage than control females (Table 4.2, Figure 4.1b). However, brood size was not influenced by the number of mice encountered by a female or the outcome of the contest (i.e., the interaction between number of mice and contest experience) (Table 4.2, Figure 4.1b). Female post-breeding longevity was not influenced by prior experience with a contest, the outcome of that contest, or the number of mice encountered by the female (Table 4.2).

Table 4.2: Effects of contest experience (yes or no), the number of mice encountered by the female (one or two), and the interaction between these two factors (reflecting the effect of contest outcome) on the mother's reproductive output (brood size) and residual reproductive value (post-breeding longevity), offspring condition during early development (larval begging rate), and offspring size at the end of the parental care period (larval mass at dispersal). LR refers to likelihood ratio.

	Contest experience		Number of mice		Interaction	
	LR χ^2_1	*P*	LR χ^2_1	*P*	LR χ^2_1	*P*
Consequences for mother						
Brood size at dispersal	8.01	<0.01	<0.01	>0.99	0.08	0.78
Post-breeding longevity	1.34	0.25	0.49	0.48	0.01	0.91
Consequences for offspring						
Larval begging rate	0.92	0.34	1.36	0.24	0.02	0.89
Larval mass at dispersal	0.83	0.36	10.88	<0.01	2.27	0.13

With respect to offspring fitness, none of the main effects had a significant effect on larval begging rate, but larger larvae spent more time begging than smaller larvae (LR χ^2_1 = 11.2, P < 0.001) and there was a nonsignificant trend for larvae to beg more on larger carcasses (LR χ^2_1 = 3.62, *P* = 0.06). Similarly, neither contest experience nor contest outcome had a detectable effect on average larval mass at dispersal (Tables 4.2, Figure 4.2a). However, females that had encountered two mice (losers and loser-controls) produced heavier offspring than females that encountered one mouse (winners and winner controls). This pattern may have been driven by differences in pre-breeding mass between these females (Figure 4.2b), given the correlation between the mother's pre-breeding mass and the offspring's average larval mass (Pearson correlation: r = 0.31, *P* < 0.0001). Female pre-breeding mass (LR χ^2_1 = 4.65, *P* = 0.03) and female age (LR χ^2_1 = 4.88, *P* = 0.03) were included in the final model for average larval mass. Because of the correlation between female pre-breeding mass and number of mice encountered (Figure 4.2b), I tested for multicollinearity in this model. I estimated variance inflation factors using the vif function in the car package (Fox and Weisberg 2011). The largest variance inflation factors were <2 indicating absence of multicollinearity.

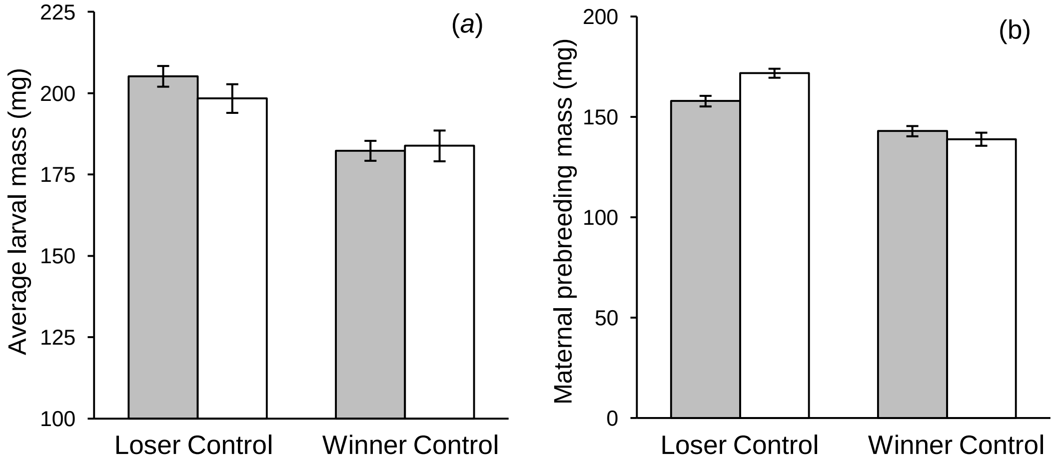

Figure 4.2: Average larval mass (a) and female pre-breeding mass (b) for females that either lost or won a prior contest (grey bars) and their corresponding controls (white bars). Error bars indicate SE. For females exposed to only one carcass (winners and winner-controls), I measured pre-breeding mass before placing the females on the carcass. For females exposed to two carcasses (losers and loser-controls), I measured pre-breeding mass before placing the females on the second carcass, which was the one they bred on.

4.4 Discussion

Here, I tested the hypothesis that females increase their investment in current reproduction after losing a contest with a larger competitor and that such adjustments in investment alter the female's own fitness and the fitness of her offspring. I found no evidence to support this hypothesis, as contest outcome had no effect on either pre-hatching or post-hatching investment (Figure 4.1a) and had no fitness consequences for either the female or her offspring (Figure 4.1b, 4.2a).

Instead, my key finding was that experience with a prior contest, regardless of its outcome, influenced subsequent reproductive decisions, thus altering reproductive output. Both winners and losers spent almost twice as much time providing direct care (food provisioning and interactions with larvae) than females with no contest experience, and they had larger broods at the dispersal stage. These findings are in line with two recent studies on winner-loser effects showing that contest experience might be more important than contest outcome in determining fighting success in future contests (Benelli et al. 2015a, 2015b).

One potential explanation for why both winners and losers increased their investment in the current brood is that females use the presence or absence of conspecific competitors as an indicator of the intensity of competition over limited breeding resources in the population. For example, if the absence of conspecific competitors indicates a low population density, control females may have invested less in their current brood in order to take advantage of additional breeding opportunities in the future (McNamara et al. 2009). An alternative explanation is that involvement in a contest might have resulted in injuries given the fierce competition over carcasses in *Nicrophorus* (Trumbo 1991, Cotter et al. 2010), and injured females might have increased their investment in the current brood due to a higher risk of infection. A previous study on *N. vespilloides* found that immunologically challenged females produced heavier broods than control females (Cotter et al. 2010). Nevertheless, I only observed visible injuries in the form of missing antennae or legs in one loser and I never observed such injuries in any of the winners. Thus, the most likely explanation for the observation that winners and losers increased their investment in current reproduction is that they responded to the presence of a competitor, which served as a cue about the intensity of competition in the population.

As a result of this increased investment, winners and losers produced more offspring at the dispersal stage than control females. To my knowledge, this is the first evidence that a female's prior experience with a contest influences her reproductive output by altering her subsequent parental investment decisions. On the other hand, I found no evidence for an effect of either contest experience or contest outcome on the offspring's fitness. This result was somewhat surprising given that larval mass determines adult size, which in turn determines the likelihood of acquiring a carcass for breeding (Bartlett and Ashworth 1988, Safryn and Scott 2000, Lock et al. 2004). I thus expected an adjustment in offspring size by females that had prior contest experience, due to an anticipatory maternal response to the intense levels of competition at high population density (Creighton 2005). Instead, I found that offspring size depended on the number of mice females had encountered. Losers and loser-controls, which had encountered two mice, produced larger larvae compared to winners and winner-controls, which had encountered only one mouse. This pattern might have been driven by the higher mass of losers and loser-controls (Figure 4.2b), which had access to an additional carcass before being placed on the mouse they eventually bred on. Mouse carcasses are a highly nutritional food resource for burying beetles, and parents feed on the carcass before and during a breeding attempt to replenish their energy reserves (Chapter 3). If losers and loser-controls had more energy reserves at the start of their breeding attempt, they might have consumed less of the second carcass, leaving more food for the larvae.

Overall, my results show that females with prior contest experience invest more in current reproduction and produce larger broods, but they do not adjust their offspring's size. This finding is in contrast to studies in other species showing that mothers produce fewer but larger offspring at high densities (Both 2000, Creighton 2005, Goubault et al. 2007, Plaistow et al. 2007, Allen et al. 2008, Leips et al. 2009). Thus, there is mixed empirical evidence with respect to how females adjust the number versus size of their offspring in response to intense levels of competition. This highlights the need for further work on this topic, given that different scenarios for how females respond to high population density could have divergent consequences for population dynamics.

To illustrate this issue, I propose a conceptual model based on the simple assumption that variation in population density determines the intensity of intraspecific competition over resources used for breeding (Creighton 2005). When population density is low, there will be little competition over resources. Under these conditions, most breeding individuals will have no experience with a prior contest, in which case they may show reproductive restraint because they have a relatively high reproductive potential. Conversely, when population density is high, the majority of breeding individuals will encounter competitors, leading to potential adjustments in their subsequent reproductive decisions. If females produce more offspring of the same size by increasing their investment in current reproduction, a greater number of offspring will be recruited into the breeding population at high densities. On the other hand, if females produce fewer but larger offspring without increasing their overall investment in the current brood, a smaller number of offspring will be recruited into the breeding population at high densities. Given that offspring recruitment into the breeding population is inextricably linked to population dynamics, these interactions could create a feedback loop between population density, intraspecific competition, investment in current reproduction, and offspring recruitment into the population.

Such feedback loops could occur in any species where there is size-dependent competition over resources and parents make reproductive investment decisions based on cues about the population density. Evidence for density-dependent adjustments in the number and/or size of offspring has been documented in a variety of taxa, including birds, fishes, insects, and aquatic invertebrates (Both 2000, reighton 2005, Goubault et al. 2007, Plaistow et al. 2007, Allen et al. 2008, Leips et al. 2009, Rauter et al. 2010). In most of these studies, parents produced fewer, larger offspring at high densities and more, smaller offspring at low densities. Under this scenario, I would expect a negative feedback loop, where the population density fluctuates around a stable equilibrium. In contrast, if parents produce more offspring (of the same size) at high densities as shown in the present study, this would lead to a positive feedback loop. These two scenarios focus on how parents may adjust their investment during the current breeding attempt, but these adjustments might also have implications for future reproduction, which will in turn contribute to population dynamics. Parents that increase their overall investment in current reproduction are expected to suffer future costs in the form of a smaller second brood (Creighton et al. 2009, Ward et al. 2009, Billman et al. 2014), whereas there might not be any future costs associated with merely adjusting the trade-off between number and size of offspring. Theoretical modeling and long-term field studies are now needed to better understand these dynamics. A suitable dataset would provide multi-generation information on parental investment patterns (e.g., incubation time or nestling provisioning rate for birds), clutch size, offspring size, offspring recruitment into the population, and estimated population size.

In summary, I propose that in species where contest experience mediates parental adjustments in the size and/or number of offspring, the frequency of intraspecific contests could both influence and be influenced by population dynamics. This potential link between individual-level behavioural changes and population-level processes has so far been overlooked in the literature. More generally, previous studies have largely ignored the wider fitness consequences of contest experience and contest outcome beyond an effect on success in subsequent fights. My finding that contest experience can alter a female's reproductive decisions highlights the need for further empirical work in this area. Even though I did not find evidence for an effect of contest outcome on reproductive investment in this study, such an effect may still exist in other systems, making this an interesting question for future studies to explore. Lastly, although my study focused on parental investment, participation in a fighting contest might influence a variety of other behaviours and life-history trade-offs, and I encourage future research to consider these effects.

Chapter 5: Inbreeding and mate choice

A revised version of this chapter has been published as referenced below, and this publication appears as Appendix D in this thesis:

> Pilakouta N, Smiseth PT (2017) Female mating preferences for outbred versus inbred males are conditional upon the female's own inbreeding status. *Animal Behaviour* 123:369–374.

Abstract

Inbreeding, which occurs when relatives mate with each other, has detrimental effects for the fitness of the resulting offspring. Inbreeding may shape mate choice through (*i*) the avoidance of related, outbred individuals in order to prevent inbreeding or (*ii*) the avoidance of unrelated, inbred individuals that have been produced through inbreeding. Although inbreeding avoidance has been studied extensively, relatively little is known about mating preferences based on the inbreeding status of potential partners. Furthermore, it is still unclear whether such mating preferences are influenced by the inbreeding status of the choosing sex. In this study, I examined female mating preferences for outbred versus inbred males using dichotomous choice tests in the burying beetle *Nicrophorus vespilloides*. I show that mating preferences for outbred versus inbred males are conditional upon the females' own inbreeding status: inbred females preferentially mated with outbred

males, whereas outbred females did not show such a preference. In addition to being choosier, inbred females were also less likely to mate with both males during the choice test, which indicates that they were less promiscuous than outbred females. My findings suggest that inbred males suffer reduced mating success only when interacting with inbred females. In species where this is the case, the fitness costs of inbreeding with respect to male mating success may therefore depend on the frequency of inbred females relative to outbred females, which is determined by the rate of inbreeding in the population.

5.1 Introduction

Inbreeding is a process that occurs over two generations; that is, the mating between close relatives in the parental generation and the production of inbred offspring in the subsequent generation. Inbreeding is often associated with a reduction in the fitness of any resulting offspring, known as inbreeding depression (Charlesworth and Charlesworth 1987). These fitness costs are due to a general loss of heterozygosity, which increases the likelihood that recessive, deleterious alleles are expressed (Charlesworth and Charlesworth 1987). Given its detrimental effects on the fitness of inbred offspring, inbreeding has the potential to affect mate choice. Mate choice is the outcome of interactions between males and females, with females usually being the choosing sex while males are the competing sex (Andersson 1994). Inbreeding may influence mate choice in both generations involved in the process of inbreeding. Firstly, the costs of inbreeding may lead to active mate preferences for unrelated versus related individuals in the parental generation. Active mate choice is one of the key mechanisms for inbreeding avoidance, whereby individuals avoid mating with a relative in order to reduce the risk of producing inbred offspring (Frommen and Bakker 2006, Gerlach and Lysiak 2006, Hansson et al. 2007). Secondly, when inbreeding does occur, the costs of inbreeding may lead to active mate preferences for outbred versus inbred individuals in the subsequent generation if inbred offspring are less attractive or of lower quality than outbred individuals (Ilmonen et al. 2009). Thus, inbreeding may shape mate choice through the avoidance of related individuals in order to prevent inbreeding and/or through the avoidance of low-quality inbred individuals produced through inbreeding.

Inbreeding avoidance by active mate choice has been studied extensively across a wide range of taxa (Pusey and Wolf 1996, Tregenza and Wedell 2000, Szulkin et al. 2013). This work has focused on when and why animals may avoid, tolerate, or in some cases prefer to mate with their relatives (Kokko and Ots 2006, Szulkin et al. 2013). Relatively little is known about whether and when inbred individuals might be less preferred as potential mates, but a growing number of empirical studies in mammals, birds, fishes, and insects have shown that outbred partners are typically preferred over inbred partners (Ilmonen et al. 2009, Bolund et al. 2010, Zajitschek and Brooks 2010, Okada et al. 2011, Polkki et al. 2012, McKee et al. 2014, Ala-Honkola et al. 2015; but also see Drayton et al. 2010, Michalczyk et al. 2010). These mating preferences are unlikely to be driven by indirect (genetic) benefits, given that theoretical models predict only small indirect benefits to mating with an outbred over an inbred partner (Reinhold 2002, Lehmann et al. 2007). This is because offspring heterozygosity will increase when an individual mates with an unrelated partner, regardless of whether that partner is inbred or outbred (Frankham et al. 2002).

An alternative explanation for the avoidance of inbred males is that inbreeding reduces overall male quality and condition such that females gain fewer direct benefits from mating with inbred males (Fox et al. 2012). Some direct benefits that may be affected by the male's inbreeding status are nuptial gift size, the male's ability to contribute to parental care, and sperm number and quality (Fox et al. 2012). For example, in the seed beetle *Callosobruchus maculatus*, females mated to inbred males were less likely to lay eggs, and they also laid fewer eggs; these effects were likely mediated by inbreeding depression in sperm number (Fox et al. 2012).

Similarly, inbred male guppies (*Poecilia reticulata*) transfer less sperm that is less motile and has more abnormalities than that of outbred males (Zajitschek et al. 2009, Fitzpatrick and Evans 2014).

Traditionally, studies on female mate choice have focused on the inbreeding status of males only, giving outbred females a choice between outbred and inbred males. Nevertheless, the females' own inbreeding status might influence their promiscuity and/or choosiness. For example, inbred females may be more promiscuous than outbred females in order to ensure genetic diversity in their offspring (Michalczyk et al. 2011, Dolphin and Carter 2016). Consistent with this hypothesis, Michalczyk et al. (2011) found that inbred female flour beetles (*Tribolium castaneum*) were more promiscuous, and that higher promiscuity led to higher reproductive success. This effect was likely due to higher genetic compatibility mediated through sperm competition and post-copulatory mate selection for sperm conferring greater fitness benefits to the female (Michalczyk et a. 2011). In contrast, Dolphin and Carter (2016) found that inbred *Drosophila melanogaster* females were less promiscuous than outbred females. Lower promiscuity due to inbreeding could be a byproduct of reduced mating activity or eagerness to mate (Ala-Honkola et al. 2014). Because of the scarcity of studies in the area, we currently lack an understanding of why inbreeding promotes female promiscuity in some species but reduces it in other species.

Outbred and inbred females may also differ in terms of their choosiness. If low-quality females cannot afford the costs of being choosy (Hunt et al. 2005, Cotton et al. 2006, Burley and Foster 2006, Ilmonen et al. 2009, McKee et al. 2014), we might expect a stronger mating bias towards outbred males by outbred (high-quality) females than by inbred (low-quality) females. On the other hand, if the benefits gained from being choosy are negatively related to female quality, inbred females may be expected to display a stronger preference for outbred males in order to compensate for their own low quality (Ilmonen et al. 2009). Such differences in female choosiness as a result of inbreeding may indirectly influence the rate and direction of sexual selection in a given population. For example, if inbred females are less choosy than outbred females, the rate of directional selection on male sexual traits will decrease with increasing inbreeding rates. In contrast, if inbred females are choosier, directional selection on male sexual traits will be stronger in populations with high inbreeding rates. I thus argue that it is important to better understand how inbreeding affects female choosiness since it may have important implications for sexual selection dynamics.

In this study, I used the burying beetle *Nicrophorus vespilloides* to test whether females preferentially mate with outbred over inbred males and whether female promiscuity and choosiness are influenced by the females' own inbreeding status. Mattey and Smiseth (2015a) found no evidence for inbreeding avoidance in this species despite severe inbreeding depression in the offspring (Mattey et al. 2013) and heavy investment by both sexes in parental care (Smiseth and Moore 2004, Smiseth et al. 2005). Nevertheless, it is possible that females exhibit mating preferences based on the inbreeding status rather than the relatedness of potential partners. To test this, I conducted dichotomous choice tests during which I recorded the copulation rate of an outbred or inbred female presented with two potential mates, one outbred and one inbred. I used a simultaneous choice test rather than a sequential test based on the natural history of this species: the chosen design simulates a situation where a female burying beetle encounters multiple males on a carcass in the wild (Mattey and Smiseth 2015a). In burying beetles, carcasses of small vertebrates serve as a breeding resource (Scott 1998), and multiple males and females often locate the same carcass. I predicted that females would avoid mating with inbred males, because inbred individuals suffer substantial fitness costs, such as a shorter lifespan and a lower capacity to provide care (Mattey et al. 2013). I also expected that females' choosiness and promiscuity would depend on their inbreeding status, but I did not have an *a priori* prediction about the direction of this effect.

5.2 Methods

I used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. The beetles used in this study comprised of second- and third-generation beetles from lines originally collected in Edinburgh, UK. They were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 22 °C and a 16:8 h light:dark cycle. All non-breeding adults were fed small pieces of raw organic beef twice a week.

5.2.1 Generating outbred and inbred beetles

In the first part of my experiment, I generated outbred and inbred males and females for use in the mate choice trials. To produce outbred individuals, I paired outbred beetles that had no common ancestors for at least two generations. To produce inbred individuals, I paired outbred beetles that were full siblings. Each pair was placed in a transparent plastic container (17 × 12 × 6 cm) filled with 1 cm of moist soil. I provided these pairs (n = 25) with a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, UK) as a resource for breeding. I did not disturb them until the larvae started dispersing from the carcass, which occurs approximately five days after hatching.

At the dispersal stage, I placed five larvae from each brood into individual containers (12 × 8 × 2 cm) filled with moist soil. The inbred and outbred offspring eclosed as adults about 20 days later, at which point they were all sexed based on differences in the terminal segments of their abdomen (Trumbo 1996). I also recorded the body size of all individuals by measuring their pronotum width using a digital caliper with a precision of 0.01 mm (Bartlett and Ashworth 1988).

5.2.2 Dichotomous choice tests

Adult beetles become sexually mature around 10 days after eclosion. For my mate choice trials, I only used virgin beetles aged between 10 and 20 days after eclosion to minimize variation in male and female age and prevent variation due to previous mating experience. Each trial consisted of a single outbred or inbred female, which was given a choice between an outbred and an inbred male. This design simulates a situation where a female encounters multiple males on a carcass in the wild (i.e., simultaneous mate choice). In half of the trials, I used an outbred female ($n = 15$) and in the other half I used an inbred female ($n = 15$). The two males used in a given pair were size-matched based on their pronotum width (difference < 0.1 mm) to exclude differences in female mating preferences due to male size. I always used unrelated individuals in each trial.

Mate choice trials took place in a transparent container (17 × 12 × 6 cm) filled with 0.5 cm of moist soil and a freshly thawed mouse carcass of a standardized size (27–30 g). I first tethered each male by tying one end of a string of dental floss around the male's pronotum and taping the other end to the side of the box. The two males were tethered to opposite sides of the box to prevent competition between the two males, which otherwise would restrict the female's ability to choose between them (Otronen 1988). I tied the string such that there was about 3 cm of give to ensure that I did not limit the males' ability to mount and mate with the female. Both males could reach the carcass, which was placed in the middle of the box, but they could not come in direct contact with each other. I alternated between trials whether it was the outbred or inbred male that was tethered on the side close to the front versus the back of the mouse (Mattey and Smiseth 2015a).

At the start of the trial, I placed the female at the center of the carcass such that she was equidistant from the two males. I recorded the time when the female first came into contact with the outbred and the inbred male and the number of copulations she had with each male over the next 45 minutes. Successful copulations occurred when the male inserted his aedeagus (intromittent organ) into the female's vagina (House et al. 2008). Each copulation typically lasts about 90 s, and females do not have a refractory period (House et al. 2008). Thus, during my 45-min observations, it was possible for females to mate repeatedly with the same male or both males.

5.2.3 Data analysis

All analyses were run in R version 3.3.1. I first tested for a negative correlation between the number of times the female copulated with the outbred and inbred male in a given trial, which would indicate that mating with one male reduced the likelihood of mating with the other male. After confirming the absence of such a correlation (Spearman's rank test: $\rho = 0.063$, $P = 0.74$), I tested whether females copulated more frequently with the outbred or the inbred male, using a generalized linear mixed model (GLMM) with a Poisson error distribution ('glmer' function in the 'lme4' package). The starting model included the following factors: male inbreeding status (outbred or inbred), female inbreeding status (outbred or inbred), female pronotum width, the male's position relative to the carcass (front or back), and whether that male was the first the female interacted with (yes or no). Female identity was added as a random effect to account for the non-independence between the observations on the two males in the same trial. The model was fitted using maximum likelihood methods.

To determine whether outbred or inbred females were more promiscuous, I used a generalized linear model (GLM) where the response variable indicated whether a female mated with only one male or both males during the dichotomous choice test. The two explanatory variables were female inbreeding status (outbred or inbred) and female pronotum width. This model was fitted using a binomial error distribution with a complementary log-log link function. Lastly, I used a two-sample t-test to compare the total number of copulations by outbred and inbred females, as a measure of female mating activity or eagerness to mate.

5.3 Results

Female mating preferences for inbred versus outbred males were conditional upon the female's own inbreeding status (Figure 5.1). Inbred females preferentially mated with outbred males, while outbred females showed no preference between outbred and inbred males (GLMM: male inbreeding status: LR $\chi_1^2 = 5.47$, $P = 0.02$; female inbreeding status: LR $\chi_1^2 = 0.87$, $P = 0.35$, interaction: LR $\chi_1^2 = 9.01$, $P < 0.01$). These mating preferences were not influenced by female pronotum width (GLMM: LR $\chi_1^2 = 0.15$, $P = 0.70$), whether the male was tethered to the side closest to the front or back of the carcass (GLMM: LR $\chi_1^2 = 0.54$, $P = 0.46$), or which male the female interacted with first (GLMM: LR $\chi_1^2 = 0.34$, $P = 0.56$).

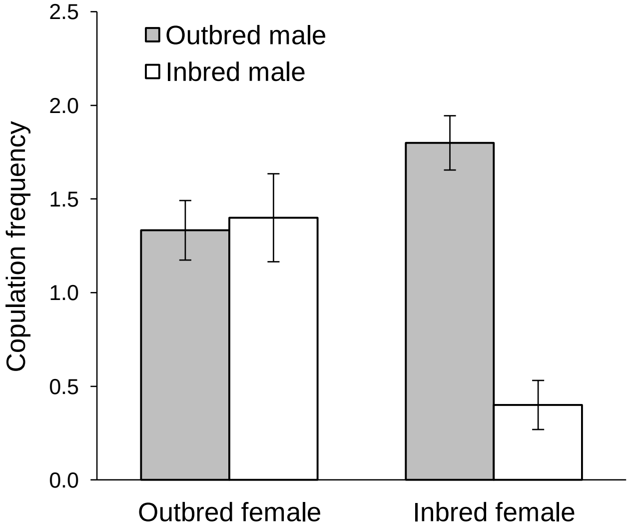

Figure 5.1: Mean (±SE) number of times an outbred or inbred female mated with the outbred male (grey) and the inbred male (white) during a 45-min mate choice trial.

Outbred and inbred females were equally eager to mate, as measured by the number of total copulations (two-sample t-test: $t = 1.38$, $P = 0.17$). However, inbred females were less promiscuous than outbred females, as indicated by their lower likelihood of mating with both males during the dichotomous choice test (GLM: $\chi^2_1 = 4.32$, $P = 0.038$; Figure 5.2). Female pronotum width did not influence promiscuity (GLM: $\chi^2_1 = 0.42$, $P = 0.52$).

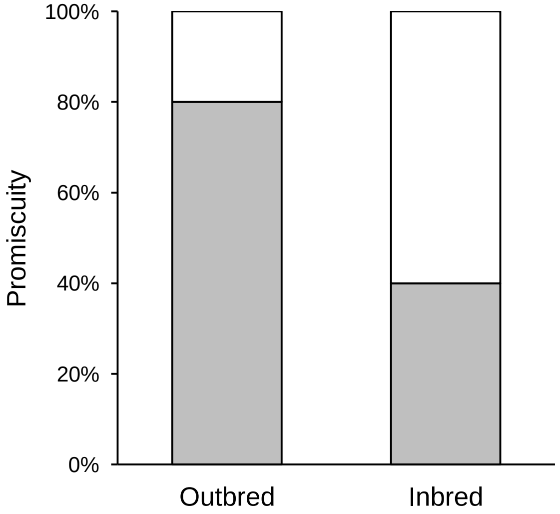

Figure 5.2: Percentage of outbred and inbred females that mated with only one of the two males (white) or both males (grey) over the course of the 45-min mate choice trials. The likelihood of mating with both males was used as a measure of promiscuity.

5.4 Discussion

Here, I have shown that female mating preferences between outbred and inbred males are conditional upon the females' own inbreeding status: inbred females preferentially mated with outbred males over inbred males, whereas outbred females did not show a preference between outbred and inbred males (Figure 5.1). In addition to being choosier, inbred females were also less promiscuous, as they were less likely to mate with both males during the dichotomous choice test. This indicates that inbred females not only copulated with the outbred male more often than with the inbred male but also that the majority of inbred females mated only with the outbred male (Figure 5.2). My results highlight the potential importance of male inbreeding status as a factor influencing female choice but also indicate that this effect may depend on the female's own inbreeding status. Below, I discuss possible explanations for my findings and their potential implications for female mate choice and male mating success in this species and more generally.

I found that inbred females showed a mating preference for outbred males over inbred males, whereas outbred females showed no such preference. Given that choosiness is thought to be costly (Pomiankowski 1987), my results suggest that inbred females might be prepared to pay the costs of being choosy in order to gain higher marginal benefits (Mazzi et al. 2004, Bolund et al. 2010). Theoretical models predict only small indirect (genetic) benefits to mating with outbred over inbred males (Reinhold 2002, Lehmann et al. 2007), because mating with an unrelated partner restores offspring heterozygosity regardless of whether that partner is inbred or outbred. Direct benefits may thus be more important than indirect benefits in this context (Fox et al. 2012). For example, inbred males have been shown to transfer less sperm during copulations and their sperm is less motile and has more abnormalities, leading to lower fertilization success (Zajitschek et al. 2009, Fitzpatrick and Evans 2009, Ala-Honkola et al. 2013). This lower sperm quality of inbred males might be more detrimental to fertilization success if the female herself is inbred than if she is outbred. Another form of direct benefits that might be influenced by inbreeding status is parental care. In burying beetles, both parents prepare the carcass by removing its fur or feathers, applying antimicrobials to prevent bacterial and fungal growth, protecting the brood from predators and conspecifics, and provisioning the larvae with pre-digested carrion (Eggert et al. 1998, Rozen et al. 2008, Walling et al. 2008, Arce et al. 2012). If inbred parents have a lower capacity to provide care (Mattey et al. 2013), it may be beneficial for inbred females to mate with outbred partners that might at least partially compensate for the female's lower contributions to parental care. A recent study in *N. vespilloides* found that females had to provide more care when they were mated to inbred males, potentially to compensate for the inbred males' lower-quality care (Mattey and Smiseth 2015b). Future research should further examine mate preferences for outbred versus inbred partners in species with biparental care, where direct benefits related to care are likely to be influenced by both male and female inbreeding status. Previous studies have focused on fertilisation success as a direct benefit driving female mate preferences, but this may underestimate the true costs of mating with an inbred male.

Although the avoidance of inbred males by inbred females might have evolved in direct response to inbreeding, another possibility is that it reflects a general response to an overall decline in condition due to inbreeding depression. Inbreeding is relatively uncommon in most species, so it seems unlikely that the mating preferences I observed evolved in the specific context of inbreeding (Mattey and Smiseth 2015b). Instead, it is more likely that these mating preferences were mediated through pre-existing mechanisms that evolved to serve an adaptive function in a different context. For example, females might have evolved general mating preferences for high-quality males, which may be conditional upon the female's own quality. Nevertheless, all populations are potentially at risk of inbreeding in the future, given increasing habitat loss and other human-induced disturbances that increase the chances of inbreeding (Andersen et al. 2004). Whenever species with no prior history of inbreeding depression become subject to inbreeding, the associated fitness costs may thus be mediated through pre-existing mechanisms that evolved outside this context (Mattey and Smiseth 2015b).

My finding that inbred females preferentially mate with outbred males suggests that females must respond to a cue that differentiates inbred and outbred males, such as cuticular hydrocarbons (CHCs) or other chemical cues (Howard and Blomquist 2005). In insects, CHCs are often used to discriminate between relatives and nonrelatives (Howard and Blomquist 2005, Tsutsui 2004, Weddle et al. 2013). More specifically, in burying beetles, CHCs are used for partner recognition based on information about sex and breeding status (Müller et al. 2003, Steiger et al. 2007), as well as parent-offspring discrimination (Smiseth et al. 2010). Females might have been under selection to differentiate between males based on their CHC profiles specifically as a mechanism to avoid mating with inbred males, or as a more general mechanism to avoid mating with males that are in poor condition or health as a consequence of inbreeding. My suggestion that female burying beetles use CHCs to discriminate between outbred and inbred males is in line with a recent study in the butterfly *Bicyclus anynana* showing that inbreeding reduces the production of a male sex pheromone, thereby allowing females to discriminate between males based on their inbreeding status (van Bergen et al. 2013). Similarly, there is evidence that female discrimination between outbred and inbred males in mealworm beetles is odour-based (Polkki et al. 2012). Given that there is a genetic basis to CHCs (Ferveur 2005, Dronnet et al. 2006, Foley et al. 2007) and that traits with a genetic basis are prone to inbreeding (van Bergen et al. 2013), CHCs are a plausible mechanism for discrimination between outbred and inbred individuals in *N. vespilloides* and other insects.

Regardless of the underlying mechanism, my findings could have important implications for male mating success in the wild. Earlier work has shown that inbred males often suffer reduced mating success (Joron and Brakefield 2003, van Oosterhout et al. 2003, Mariette et al. 2006, Ala-Honkola et al. 2009, Enders and Nunney 2010, Ketola and Kotiaho 2010). Here, I demonstrate that inbred females avoid mating with inbred males while outbred females do not. This suggests that inbred males will suffer reduced mating success only when interacting with inbred females. I therefore propose that, in species where female inbreeding status influences mate choice for outbred versus inbred males, the fitness costs of inbreeding with respect to male mating success may be frequency-dependent. In populations with high rates of inbreeding, a larger proportion of breeding females will be inbred, and I would expect inbred males to experience lower mating success than in populations with low rates of inbreeding. Such social effects of inbreeding depression on male mating success may be widespread, but their occurrence is still unexplored. I encourage future research to further investigate this issue, as it could have important implications for the rate and direction of sexual selection in populations that are subject to inbreeding. For example, under a scenario where inbred females are choosier than outbred females, directional selection on male sexual traits will be stronger when inbreeding rates are high than when they are low.

In summary, I found that a female's mating bias for outbred versus inbred males depends on her own inbreeding status. This is the first example of a species where inbred females discriminate against inbred males while outbred females show no preference between inbred and outbred males. Under this scenario, the fitness costs of inbreeding with respect to male mating success will depend on the frequency of inbred females relative to outbred females and thus the rate of inbreeding in the population. Future studies should examine mate preferences for outbred versus inbred partners in other species with biparental care, where any potential direct benefits are likely to be influenced by both male and female inbreeding status. Previous studies have focused on fertilisation success as a direct benefit driving female mate preferences, but this may underestimate the true costs of mating with an inbred male.

Chapter 6: Inbreeding and sibling competition

This chapter has been published as referenced below, and this publication appears as Appendix E in this thesis:

> Pilakouta N, Sieber D, Smiseth PT (2016) Sibling competition does not exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*. *Journal of Evolutionary Biology* 29:704-710.

Abstract

Inbreeding results from matings between relatives and can cause a reduction in offspring fitness, known as inbreeding depression. Previous work has shown that a wide range of environmental stresses, such as extreme temperatures, starvation, and parasitism, can exacerbate inbreeding depression. It has recently been argued that stresses due to intraspecific competition should have a stronger effect on the severity of inbreeding depression than stresses due to harsh physical conditions. Here, I tested whether an increase in the intensity of sibling competition can exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*. I used a 2 × 3 factorial design with offspring inbreeding status (outbred or inbred) and brood size (5, 20, or 40 larvae) as the two factors. I found a main effect of inbreeding status, as inbred larvae had lower survival than outbred larvae, and a main effect of brood size, as larvae in large broods had lower survival and mass than larvae in medium-

sized broods. However, there was no effect of the interaction between inbreeding status and brood size, suggesting that sibling competition did not influence the severity of inbreeding depression. Since I focused on sibling competition within homogeneous broods of either inbred or outbred larvae, I cannot rule out possible effects of sibling competition on inbreeding depression in mixed paternity broods comprising of both inbred and outbred offspring. More information on whether and when sibling competition might influence the expression of inbreeding depression can help advance our understanding of the causes underlying variation in the severity of inbreeding depression.

6.1 Introduction

Inbreeding results from matings between relatives and can cause a reduction in offspring fitness, known as inbreeding depression (Crnokrak and Roff 1999). These negative fitness effects are due to the higher degree of homozygosity associated with inbreeding, which increases the risk that deleterious recessive alleles are expressed (Charlesworth and Charlesworth 1987). Although there is widespread evidence for inbreeding depression in a range of taxa (Crnokrak and Roff 1999), there is substantial variation both among and within species with respect to the severity of inbreeding depression (Crnokrak and Roff 1999, Keller and Waller 2002, Meunier and Kölliker 2013, Moorad and Wade 2005). This variation may be in part driven by differences in the physical and social environment, which can have profound effects on inbreeding depression (Fox and Reed 2011, Reed et al. 2012). For example, environmental stresses, such as extreme temperatures, starvation, parasitism, and competition, can exacerbate inbreeding depression (Fox and Reed 2011, Meunier and Kölliker, 2013). Although there is growing evidence that physical and social stresses can alter the severity of inbreeding depression (Fox and Reed 2011, Reed et al. 2012), little is known about the mechanisms by which particular environmental stresses influence its expression.

A recent study suggested that stresses due to intense intraspecific competition over limited resources should have a stronger effect on the severity of inbreeding depression than stresses due to harsh physical conditions, such as extreme temperatures (Yun and Agrawal 2014). Intraspecific competition over limited resources can take several forms and can occur at different stages of the life cycle, including competition with siblings during development (Mock and Parker 1997). Sibling competition for resources provided by the parents occurs because parents usually produce an optimistic brood size, thereby creating a mismatch between the supply of resources from the parents and the demand of resources by the offspring (Mock and Parker 1997). Sibling competition is an important determinant of the offspring's growth and survival in many species and may therefore be a key source of environmental stress to the offspring (Mock and Parker 1997, Roulin and Dreiss 2012, Meunier and Kölliker 2013). Thus, sibling competition should be associated with an increase in this mismatch between supply and demand of resources, which in turn may exacerbate inbreeding depression. To my knowledge, the only experimental study to test this hypothesis was conducted on the European earwig, *Forficula auricularia* (Meunier and Kölliker 2013). This study found no effect of the interaction between the intensity of sibling competition and the offspring's inbreeding status on offspring fitness, suggesting that sibling competition did not influence the severity of inbreeding depression (Meunier and Kölliker 2013). However, the absence of such an interaction effect may reflect that there was no evidence for a main effect of inbreeding status on offspring fitness during the early life stages (Meunier and Kölliker 2013). In order to advance our understanding of whether sibling competition can exacerbate inbreeding depression, it is now essential to focus on species in which inbred offspring suffer a significant reduction in fitness and sibling competition negatively affects offspring fitness.

Here, I tested whether sibling competition influences the severity of inbreeding depression in the burying beetle *Nicrophorus vespilloides*. This species is well suited for addressing this question, because previous work has shown that inbred offspring suffer significant fitness costs during the larval stage (Mattey et al. 2013) and that larvae in experimentally enlarged broods suffer a reduction in fitness due to the increased mismatch between supply and demand for resources (Smiseth et al. 2007a). In this species, which breeds on carcasses of small vertebrates, larvae compete for resources by begging for predigested carrion from the parents and by self-feeding directly from the carcass (Smiseth et al. 2003). Earlier work has also shown that sibling competition reduces offspring fitness only in the presence of the parents, reflecting interference competition due to successful larvae excluding their siblings from getting access to the parents (Smiseth et al. 2007a,b). Although the independent effects of inbreeding status and sibling competition on offspring fitness are well established in this species, there is no prior information on the effect of their interaction.

To test for such an interaction, I used a 2 × 3 factorial design with offspring inbreeding status and brood size as the two factors. I assessed the joint effects of inbreeding status and sibling competition on fitness traits previously shown to be affected by these two factors (Smiseth et al. 2007a, Mattey et al. 2013): (*i*) average larval mass at dispersal; (*ii*) survival from hatching to dispersal; (*iii*) survival from dispersal to eclosion; and (*iv*) total survival from hatching to eclosion. If sibling competition exacerbates inbreeding depression, I would expect offspring in larger broods to incur higher fitness costs if they are inbred than if they are outbred.

6.2 Methods

I used virgin beetles from an outbred laboratory population maintained at University of Edinburgh. These were sixth-, seventh-, and eighth-generation beetles from lines originally collected in Edinburgh, UK and Warmond, The Netherlands. Beetles were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20 °C and constant light. Non-breeding adults were fed raw organic beef twice a week.

6.2.1 Experimental design

To test for a causal effect of sibling competition on the severity of inbreeding depression, I used a 2 × 3 factorial design with offspring inbreeding status (outbred or inbred) and brood size (5, 20, or 40 larvae) as the two factors. Inbred larvae were produced by pairing males and females that were full siblings (n = 186), while outbred larvae were produced by pairing unrelated males and females that shared no common ancestors for at least two generations (n = 187). I only used outbred parents in this experiment as inbreeding in the parents has a negative effect on offspring survival (Mattey et al. 2013). These breeding pairs (n = 373) were transferred to transparent plastic containers (17 × 12 × 6 cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (22–25 g). Immediately after the eggs were laid, I removed the male and moved the female and the carcass to a new container with fresh, moist soil. I removed the males because the amount of care provided by the male is highly variable and male removal has no effect on offspring fitness under laboratory conditions (Smiseth et al. 2005). I allowed the females to provide care for the brood because previous work on this species showed that sibling competition reduces offspring fitness only when larvae compete by begging for food from a parent (Smiseth et al. 2007a,b).

When the eggs started hatching, I used the newly hatched larvae to generate inbred and outbred broods comprising of 5, 20, or 40 larvae. All experimental broods included larvae of mixed maternity in accordance with established protocols (Smiseth et al., 2007). This brood size manipulation is within the natural variation of brood size in *N. vespilloides* (mean ± SD: 21 ± 10 larvae, range: 2–47 larvae;

Smiseth and Moore 2002) and corresponds to small, average, and large broods (i.e., low, medium, and high level of sibling competition), respectively. I used a design with more than two levels of stress, because the relationship between stress and inbreeding depression may be nonlinear (Fox and Reed 2011).

Each experimental brood (outbred or inbred) was randomly assigned to an unrelated female who had been mated either to their full-sib brother or to an unrelated male. To account for potential effects of relatedness between the female and her male partner (who was always removed before the female was provided with a foster brood), I added this information as a factor in all of my models (see below). In this species, parents cannot distinguish between unrelated foster broods and their own broods, as long as the larvae are at the same developmental stage (Müller and Eggert 1990). Since parents kill any larvae that arrive on the carcass before their eggs are expected to hatch (Müller and Eggert 1990), I only provided females with a brood once their own eggs had hatched.

Females were left to care for their brood until the larvae dispersed from the carcass about five days later. At dispersal from the carcass, I recorded the number of larvae and total brood mass to calculate larval survival rate and average larval mass. Lastly, to assess survival after independence (i.e., from dispersal to eclosion), I placed all dispersing larvae from each brood into a large transparent container (17 × 12 × 6 cm) filled with moist soil. About 20 days after dispersal, I recorded the number of individuals that eclosed successfully from each brood and calculated the survival rate from dispersal to eclosion.

The total sample size in the experiment was n = 166 broods. The sample sizes for the different treatments were as follows: n = 31 for outbred broods with 5 larvae, n = 32 for outbred broods with 20 larvae, n = 22 for outbred broods with 40 larvae, n = 31 for inbred broods with 5 larvae, n = 30 for inbred broods with 20 larvae, and n = 20 for inbred broods with 40 larvae.

6.2.2 Data analysis

Data were analysed using R version 3.2.0. Larval mass at dispersal was analysed using a linear model. Proportion data (i.e., survival rates from hatching to dispersal, from dispersal to eclosion, and from hatching to eclosion) were analysed using generalized linear models fitted with a binomial distribution corrected for overdispersion. Proportion data were entered into the models using the 'cbind' function.

All models included offspring inbreeding status (outbred or inbred), brood size (small, medium, or large), and the interaction between these two factors. As additional factors, I included carcass mass, the relatedness between the foster mother and her removed male partner (i.e., whether the female rearing the brood had been mated to a full sibling or an unrelated male), the interaction between foster parent relatedness and offspring inbreeding status, and the interaction between foster parent relatedness and brood size. Decisions on which factors to include in the final models were based on AIC scores. For models where brood size was found to have a statistically significant effect, I used the 'glht' function in the 'multcomp' package (Hothorn et al. 2008) to perform a Tukey test for post-hoc pairwise comparisons.

6.3 Results

I found evidence for a main effect of offspring inbreeding status on survival to dispersal, survival to eclosion, and total survival, as inbred larvae suffered lower survival than outbred larvae (Table 6.1, Figure 6.1b-d). There was no significant difference in average larval mass between inbred and outbred larvae (Table 6.1, Figure 6.1a). In addition, I found an effect of sibling competition on offspring fitness: larvae in large broods were smaller and had a lower rate of survival to dispersal than larvae in medium-sized broods (Tables 6.1 and 6.2, Figure 6.1a-b). Sibling competition also had a non-significant effect on total survival (Tables 6.1 and 6.2, Figure 6.1d). In contrast, larvae in small broods had a lower rate of survival to eclosion than larvae in medium-sized or large broods (Table 6.2, Figure 6.1c). Finally, there was no evidence that sibling competition exacerbated inbreeding depression, as there was no effect of the interaction between the offspring's inbreeding status and the size of the brood on any component of offspring fitness (Table 6.1).

Table 6.1: Effects of offspring inbreeding status (inbred or outbred), sibling competition (small, medium-sized, or large brood), and their interaction on offspring fitness traits: average larval mass at dispersal (mg), survival rate from hatching to dispersal, survival rate from dispersal to eclosion, and survival rate from hatching to eclosion.

	Larval mass		Survival to dispersal		Survival to eclosion		Total survival	
	F	*P*	LR χ^2	*P*	LR χ^2	*P*	LR χ^2	*P*
Inbreeding status	2.27	0.13	4.54	0.03	37.79	<0.0001	17.17	<0.001
Brood size	4.93	<0.01	6.72	0.03	12.23	<0.01	6.07	0.048
Interaction	0.09	0.91	0.31	0.86	4.00	0.14	0.38	0.83

Table 6.2: Post-hoc pairwise comparisons (Tukey test) for the effect of sibling competition (small, medium-sized, or large brood) on offspring fitness traits: average larval mass at dispersal (mg), survival rate from hatching to dispersal, survival rate from dispersal to eclosion, and survival rate from hatching to eclosion.

	Larval mass		Survival to dispersal		Survival to eclosion		Total survival	
Brood size	*t*	*P*	*z*	*P*	*z*	*P*	*z*	*P*
Small-Medium	-2.15	0.08	-0.38	0.92	-3.36	<0.01	-1.97	0.11
Small-Large	1.04	0.55	1.13	0.49	-3.40	<0.01	-0.73	0.74
Medium-Large	2.98	<0.01	2.52	0.03	-0.06	>0.99	2.08	0.09

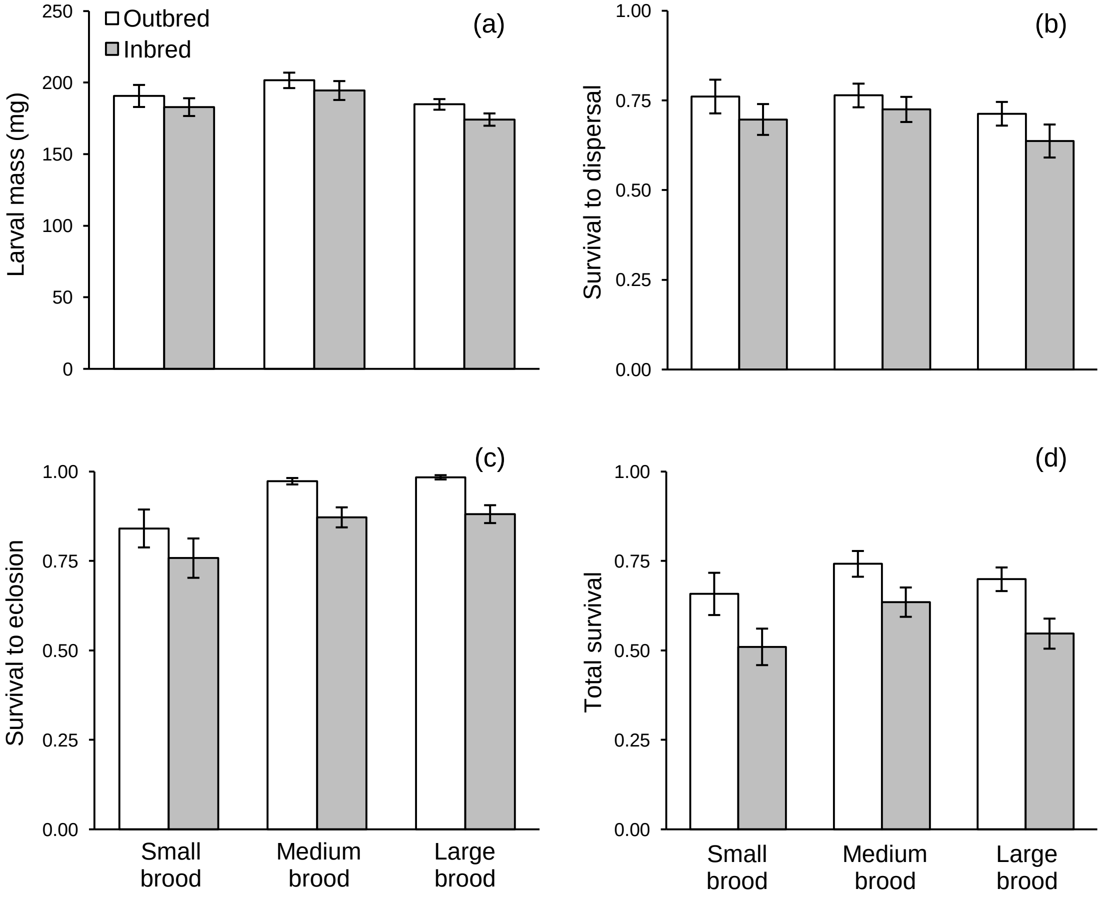

Figure 6.1: Mean (±SE) average larval mass at dispersal (a), survival from hatching to dispersal (b), survival from dispersal to eclosion (c), and survival from hatching to eclosion (d) for outbred (white) and inbred (grey) larvae reared in small, medium-sized, or large broods (5, 20, or 40 larvae, respectively).

6.4 Discussion

I find no evidence for an effect of the interaction between sibling competition and inbreeding status, suggesting that sibling competition does not exacerbate inbreeding depression in *N. vespilloides*. I show that inbreeding status negatively affected offspring fitness, as inbred larvae suffered lower survival during both the larval and pupal stages. These results demonstrate that there is significant inbreeding depression in this species as reported previously (Mattey et al. 2013). Furthermore, I show that sibling competition reduced offspring fitness, as larvae in large broods were smaller and suffered lower survival to dispersal than larvae in medium-sized broods. These results confirm that the intensity of sibling competition has a negative effect on larval fitness as previously reported by Smiseth et al. (2007a). Even though there were significant main effects of both inbreeding status and sibling competition, there was no effect of the interaction between these two factors on offspring fitness.

To my knowledge, the only other study to investigate this question (conducted on the European earwig) found no evidence for a main effect of inbreeding status on offspring fitness in the early life stages (Meunier and Kölliker 2013). Given the absence of inbreeding depression, it was not possible to test whether inbreeding depression becomes more severe when sibling competition increases. Thus, my study is the first to show that sibling competition for resources provided by the parents does not exacerbate inbreeding depression in a species where inbred offspring do suffer substantial fitness costs.

There is accumulating evidence that the negative effects of inbreeding can be intensified by a wide range of environmental stresses, such as parasitism, starvation, population density, extreme temperatures, and exposure to chemicals (Armbruster and Reed 2005, Waller et al. 2008, Fox and Reed 2011, Reed et al. 2012). Nevertheless, we still have a limited understanding of the mechanisms by which particular stresses exacerbate inbreeding depression. Yun and Agrawal (2014) argue that density-dependent stresses caused by intense competition among conspecifics should have a stronger effect on the severity of inbreeding depression than density-independent stresses caused by harsh physical conditions. In their study on *Drosophila melanogaster*, they found a moderate correlation between environmental stress and density dependence, but inbreeding depression was significantly more correlated with density dependence than environmental stress per se (Yun and Agrawal 2014).

I expected that an increase in sibling competition should affect the severity of inbreeding depression because sibling competition is a density-dependent source of environmental stress caused by a mismatch between the supply and demand for resources (Mock and Parker 1997, Roulin and Dreiss 2012). Yet, I found that sibling competition does not exacerbate inbreeding depression in *N. vespilloides*, which appears to contradict the argument made by Yun and Agrawal (2014). One potential explanation for this discrepancy is the difference in experimental designs between my study and that of Yun and Agrawal (2014). I studied the effects of sibling competition within homogeneous broods comprised of either outbred or inbred larvae, while Yun and Agrawal (2014) studied the effects of competition within heterogeneous groups of unrelated inbred and outbred fruit flies. Thus, in my study, any effect of sibling competition on the severity of inbreeding depression would be mediated through an increase in the level of stress. In contrast, in the latter study, such effects would be mediated through direct competitive interactions between inbred individuals (inferior competitors) and outbred individuals (superior competitors). Homogeneous broods comprised of either outbred or inbred larvae are likely to be the norm in *N. vespillodes* given that caring males sire over 90% of the offspring in their brood (Müller and Eggert 1989). In such homogeneous broods, all larvae may be equally disadvantaged by stresses caused by a shortage of resources.

However, I cannot rule out possible effects of sibling competition on the severity of inbreeding depression in mixed paternity broods comprising of both inbred and outbred larvae. Under those conditions, higher-quality (i.e., outbred) offspring may outcompete their lower-quality (i.e., inbred) half-siblings, thus magnifying differences in fitness between them. I encourage future studies to investigate this question in the context of family groups that comprise of both outbred and inbred offspring, as in socially monogamous birds where the female is closely related to her social partner and has extra-pair matings with non-relatives (e.g., Blomqvist et al. 2002, Foerster et al. 2003, Brouwer et al. 2011, Reid et al. 2015).

Understanding the factors that drive the observed variation in the severity of inbreeding depression across species and across environments could have important implications for the conservation of many endangered populations, yet these dynamics are still not well understood. My findings suggest that the intensity of sibling competition may not contribute towards variation in the severity of inbreeding depression, at least within homogeneous broods of inbred offspring. Determining whether and when sibling competition might play a role in the expression of inbreeding depression may help us better understand the causes underlying temporal and spatial patterns of variation in inbreeding depression in natural populations.

Chapter 7: Inbreeding and parental care

This chapter has been published as referenced below, and this publication appears as Appendix F in this thesis:

> Pilakouta N, Jamieson S, Moorad JA, Smiseth PT (2015) Parental care buffers against inbreeding depression in burying beetles. *Proceedings of the National Academy of Sciences* 112:8031-8035.

Abstract

When relatives mate, their inbred offspring often suffer a reduction in fitness-related traits known as inbreeding depression. There is mounting evidence that inbreeding depression can be exacerbated by environmental stresses, such as starvation, predation, parasitism, and competition. Parental care may play an important role as a buffer against inbreeding depression in the offspring by alleviating these environmental stresses. Here, I examine the effect of parental care on the fitness costs of inbreeding in the burying beetle *Nicrophorus vespilloides*, an insect with facultative parental care. I used a 2 × 2 factorial design with the following factors: (*i*) the presence or absence of a caring female parent during larval development and (*ii*) inbred or outbred offspring. I examined the joint influence of maternal care and inbreeding status on fitness-related offspring traits to test the hypothesis that maternal care improves the performance of inbred offspring more than that of

outbred offspring. Indeed, the female's presence led to a higher increase in larval survival in inbred than in outbred broods. Receiving care at the larval stage also increased the lifespan of inbred but not outbred adults, suggesting that the beneficial buffering effects of maternal care can persist long after the offspring have become independent. My results show that parental care has the potential to moderate the severity of inbreeding depression, which in turn may favour inbreeding tolerance and influence the evolution of mating systems and other inbreeding avoidance mechanisms.

7.1 Introduction

Inbreeding is an important issue in evolutionary biology and ecology because of its profound implications for genetic variation and the evolution of mating systems and reproductive strategies (Charlesworth 2003, Charlesworth and Willis 2009, Escobar et al. 2011, Liu et al. 2013, Szulkin et al. 2013). Inbreeding results from matings between related individuals and can cause a reduction in offspring fitness because the higher degree of homozygosity associated with inbreeding increases the risk that deleterious recessive alleles are expressed (Charlesworth and Charlesworth 1987). Evidence for such fitness costs of inbreeding, known as inbreeding depression, has been documented in a wide range of taxa, including mammals, birds, insects, and plants (Crnokrak and Roff 1999). There is, however, substantial variation in the severity of inbreeding depression among species as well as among and within populations of a species (Crnokrak and Roff 1999, Keller and Waller 2002, Moorad and Wade 2005, Meunier and Kölliker 2013). This variation may be in part driven by differences in the physical or social environment, which can have a major effect on the severity of inbreeding depression (Fox and Reed 2011, Reed et al. 2012). For example, environmental stresses, such as starvation and competition, are expected to exacerbate inbreeding depression (Meunier and Kölliker 2013, Fox and Reed 2011).

Parental care is an important component of the social environment in many birds, mammals, and insects (Royle et al. 2012). It is thought to have evolved as a means by which parents enhance their offspring's fitness by neutralizing the detrimental effects of a wide range of environmental stresses, including starvation, predation, parasitism, and competition (Royle et al. 2012). Thus, parental care may indirectly buffer against inbreeding depression by alleviating these stresses (Avilés and Bukowski 2006), but there is currently little empirical evidence in support of this suggestion. A study on a subsocial spider (*Anelosimus* cf. *jucundus,* currently *Anelosimus arizona*) proposed that the absence of detectable inbreeding depression in the offspring of this species could be due to the buffering effects of either parental care or group living (Avilés and Bukowski 2006). The only experimental test of this hypothesis, conducted on the European earwig (*Forficula auricularia*), found no evidence that maternal care reduced the fitness costs of inbreeding depression (Meunier and Kölliker 2013).

Here, I tested whether parental care can buffer against inbreeding depression in the burying beetle *Nicrophorus vespilloides*, an insect with facultative parental care. Although there is no prior information on the effect of the interaction between parental care and the offspring's inbreeding status, the independent effects of parental care and inbreeding status on offspring fitness are well established in this species (Smiseth et al. 2003, Mattey et al. 2013). Parental removal experiments show that larval growth is reduced if the caring parents are removed during the early stages of development when offspring are too young to self-feed efficiently (Smiseth et al. 2003). Furthermore, inbred offspring suffer reduced survival at the larval stage (Mattey et al. 2013).

To test for a causal effect of parental care on the severity of inbreeding depression, I used a 2 × 2 factorial design with the following factors: (*i*) presence or absence of post-hatching maternal care and (*ii*) inbred or outbred offspring. All parents used in this experiment were outbred. Because inbreeding depression can affect traits across the entire life cycle of an organism, I assessed the joint effects of inbreeding status and maternal care on the following fitness-related offspring traits: (*i*) time to dispersal from the carcass (corresponding to the end of the parental care period); (*ii*) larval survival to dispersal; (*iii*) average larval mass at the time of dispersal; (*iv*) survival from dispersal to eclosion as an adult; and (*v*) post-eclosion lifespan. I predicted that if maternal care can buffer against inbreeding depression, the presence of the mother would have a stronger fitness effect on inbred offspring than on outbred offspring.

7.2 Methods

I used beetles from an outbred laboratory population maintained at The University of Edinburgh. To avoid inbreeding in the stock population, I maintained a large population and only mated unrelated or distantly related individuals (i.e., no common ancestors for at least two generations). The beetles used in this study comprised of third- and fourth-generation beetles from lines originally collected in Edinburgh, UK and Warmond, The Netherlands. Similar numbers of beetles were used from each line and there was no evidence of outbreeding depression. They were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20 °C and constant light. Non-breeding adults were fed raw organic beef twice a week.

7.2.1 Experimental design

To examine whether parental care buffers against inbreeding depression, I used a 2 × 2 factorial design with the following treatment groups: (*i*) outbred offspring that received maternal care ($n = 32$), (*ii*) outbred offspring that received no maternal care (*n* = 33), (*iii*) inbred offspring that received maternal care (*n* = 33), and (*iv*) inbred offspring that received no maternal care (*n* = 33). To produce outbred offspring for treatments (*i*) and (*ii*), I paired outbred virgin beetles that did not share ancestors for at least two generations. To produce inbred offspring for treatments (*iii*) and (*iv*), I paired outbred virgin beetles that were full siblings. These experimental pairs (*n* = 131) were randomly assigned to treatments (care or no care). They were then transferred to transparent plastic containers (17 × 12 × 6 cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (24–27 g). In this species, the amount of care provided by the male is highly variable and male removal has no average effect on offspring fitness under laboratory conditions (Smiseth et al. 2005). For this reason, I removed males from all treatments after eggs were laid but before the larvae had hatched. In treatments (*ii*) and (*iv*), I also removed females at the same time, whereas females were left to care for their brood until dispersal in treatments (*i*) and (*iii*).

When all larvae had dispersed from the carcass, I recorded the date, the number of surviving larvae, and the total mass of the brood. These data were used to calculate time to dispersal and average larval mass for each brood. All larvae, up to a maximum of 15 per brood, were placed into large transparent boxes filled with moist soil. At eclosion, I recorded the proportion of individuals that eclosed successfully from each brood and placed up to six beetles into individual containers. I tracked the mortality of these beetles (*n* = 449) by checking them twice a week until death.

7.2.2 Data analysis

All data were analysed using R version 3.1.1. I used general linear models for traits that had a normal error structure (average larval mass and time to dispersal). For survival to eclosion, I used a generalized linear model fitted with a quasibinomial error distribution and for post-eclosion lifespan, I used a generalized linear model fitted with a negative binomial error distribution. Because of a high proportion of zeros in the larval survival data, I ran a zero-adjusted Poisson (ZAP) regression, using the hurdle function in the pscl package (Jackman 2014). A Poisson structure was assumed for the count model and a binomial structure for the zero-hurdle model. Significant values on the count model indicate that a given variable had an effect on the number of larvae surviving to dispersal, whereas significant values on the zero-hurdle model indicate that a given variable had an effect on the probability of having zero versus non-zero larvae at dispersal.

All models included parental care (presence or absence) and inbreeding status (inbred or outbred offspring), as well as an interaction between these two factors. Carcass size was included as a covariate in the models for time to dispersal, survival to dispersal, and average larval mass, because the amount of resources available may influence offspring growth and survival. I also added female age to the models for time to dispersal, survival to dispersal, and average larval mass, because the amount of care a female provides may depend on her age. Lastly, I added sex as a factor in the lifespan model because of the possibility of sex-specific mortality (Fox et al. 2006). Decisions as to which variables to include in the final model were based on AIC model selection criteria. To compare inbreeding depression in offspring fitness traits between the care and no care treatments, I calculated inbreeding depression as a proportional change in the mean fitness of outbred (w_o) and inbred offspring (w_i) based on the equation $\delta = (w_o - w_i)/w_o$ (Hedrick and Kalinowski 2000).

7.3 Results

Maternal care shortened the time to dispersal from the carcass, but there was no difference in time to dispersal between inbred and outbred broods, and there was no effect of the interaction between maternal care and the offspring's inbreeding status (Tables 7.1 and 7.2). Time to dispersal was significantly shorter when the mother was younger (Estimate = 0.038 days, SE = 0.008, $t_{84} = 4.87$, $P < 0.0001$). Finally, there was no effect of carcass size ($t_{84} = 1.21$, $P = 0.23$) or the number of larvae dispersing ($t_{84} = -0.127$, $P = 0.90$) on time to dispersal.

Table 7.1: Effects of parental care and offspring inbreeding status on fitness-related offspring traits. The data were analysed using general linear models for time to dispersal and larval mass, and generalized linear models for survival to eclosion (fitted with a quasibinomial error structure) and lifespan (fitted with a negative binomial error structure). I used a ZAP regression to analyse the zero-inflated data on survival to dispersal, and here I present the results for the count model (see text for zero-hurdle model results).

	Parental care		Inbreeding		Interaction	
Offspring trait	*z/t*	*P*	*z/t*	*P*	*z/t*	*P*
Time to dispersal	-4.0	<0.001	0.64	0.52	-1.3	0.21
Survival to dispersal	6.8	<0.0001	7.6	<0.0001	-4.2	<0.0001
Larval mass	3.5	<0.001	0.85	0.40	1.0	0.31
Survival to eclosion	3.0	0.004	3.7	<0.001	-0.15	0.88
Lifespan	5.8	<0.0001	4.2	<0.0001	-4.8	<0.0001

Maternal care significantly increased the probability that at least one larva in the brood survived to dispersal (zero hurdle model: Estimate = 0.964, SE = 0.241, $z_{84} = 4.00$, $P < 0.0001$). The offspring's inbreeding status had a nonsignificant effect on this component of larval survival (zero hurdle model: $z_{84} = 1.91$, $P = 0.056$), and there was no effect of the interaction between maternal care and inbreeding status (zero hurdle model: $z_{84} = 1.09$, $P = 0.28$). For broods in which at least one larva was present at dispersal, both maternal care and offspring inbreeding status had an effect on larval survival (Tables 7.1 and 7.2). There was also a significant effect of the interaction between these two factors (Table 7.1), as maternal presence improved the survival of inbred larvae more than that of outbred larvae (Table 7.2). There were no detectable effects of carcass size ($z_{84} = -0.62$, $P = 0.54$) or female age ($z_{84} = 1.77$, $P = 0.076$) on larval survival to dispersal.

Table 7.2: Means (± SE) and estimates of inbreeding depression (δ) for offspring that did or did not receive maternal care during the larval stage. For each of these fitness-related traits, I used the equation $\delta = (w_o - w_i)/w_o$ to calculate inbreeding depression as a proportional change in mean fitness of outbred and inbred offspring.

	Means ± SE		Δ	
Trait	Care	No care	Care	No care
Time to dispersal				
Outbred	9.57 ± 0.23	10.90 ± 0.26	-0.028	0.054
Inbred	9.84 ± 0.24	10.31 ± 0.43		
Survival to dispersal				
Outbred	23.28 ± 2.56	12.67 ± 2.74	0.36	0.69
Inbred	14.97 ± 2.20	3.94 ± 1.27		
Larval mass at dispersal				
Outbred	0.180 ± 0.006	0.147 ± 0.005	0.072	0.048
Inbred	0.167 ± 0.004	0.140 ± 0.006		
Survival to eclosion				
Outbred	96.54 ± 1.44	90.33 ± 3.09	0.14	0.35
Inbred	82.72 ± 3.98	58.7 ± 10.7		
Post-eclosion lifespan				
Outbred	32.93 ± 1.34	39.15 ± 1.92	-0.006	0.41
Inbred	33.12 ± 1.19	23.11 ± 2.29		

Maternal care significantly increased larval mass at dispersal, but there was no significant effect of inbreeding status on larval mass and no significant interaction between maternal care and inbreeding status (Tables 7.1 and 7.2). Furthermore, larval mass did not depend on carcass size ($t_{84} = -0.15$, $P = 0.88$) or female age ($t_{84} = -1.08$, $P = 0.28$). Both maternal care and inbreeding status had significant effects on the offspring's survival to eclosion. Maternal care increased survival to eclosion, and outbred larvae had higher survival than inbred ones (Tables 7.1 and

7.2). Survival to eclosion was not influenced by the interaction between parental care and inbreeding status (Table 7.1). Lastly, maternal care increased the lifespan of offspring after eclosion, and outbred offspring had longer lifespan than inbred ones (Tables 7.1 and 7.2). The statistically significant interaction between inbreeding status and maternal care reflected that receiving care improved the lifespan of inbred adults but not of outbred adults (Tables 7.1 and 7.2). There was no evidence for a difference in lifespan after eclosion between males and females ($z_{448} = -0.33$, $P = 0.74$).

Overall, I found evidence for inbreeding depression in survival to dispersal, survival from dispersal to eclosion, and lifespan after eclosion (Table 7.1). Moreover, I found evidence for a significant interaction between maternal care and inbreeding status for survival to dispersal and lifespan, which indicates a buffering effect of maternal care (Table 7.1). This conclusion is supported by the inbreeding depression estimates (δ), which show that maternal care moderates the severity of inbreeding depression in these two traits (Table 7.2, Figure 7.1).

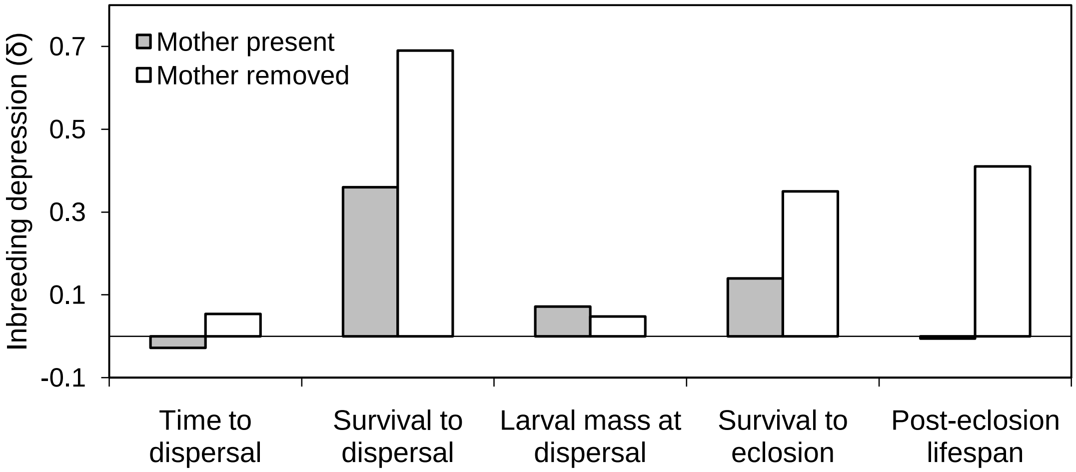

Figure 7.1: Inbreeding depression (δ) in offspring when the female parent was present (grey) or absent (white) during the larval stage. Inbreeding depression was calculated as a proportional change in mean fitness of outbred (w_o) and inbred (w_i) offspring, using the equation $\delta = (w_o - w_i)/w_o$.

7.4 Discussion

This work provides experimental evidence supporting the suggestion that parental care can buffer against the deleterious effects of inbreeding on offspring fitness (Avilés and Bukowski 2006). Firstly, I found that maternal care had a greater positive effect on larval survival to dispersal in inbred broods than in outbred broods. Secondly, I found that maternal care increased the lifespan of inbred offspring but not of outbred offspring. Thus, my results show that the buffering effects of parental care are detectable not only during the period when offspring depend on parental care (i.e., from egg laying to dispersal from the carcass) but also later in life when offspring have become independent. To my knowledge, this is the first study to show a causal effect of parental care on the severity of inbreeding depression. I provide a more detailed discussion of my results below.

My first key finding was that maternal care had a stronger effect on survival to dispersal in inbred than in outbred larvae. This finding provides clear evidence that maternal care buffers against inbreeding depression during the period when larvae depend on maternal care. Before independence, larvae benefit directly from various components of maternal care, such as food provisioning (Mattey et al. 2013, Eggert et al. 1998), defense against conspecific intruders (Trumbo 2007), and defense against bacterial and fungal competitors through antimicrobial secretions (Arce et al. 2012). Thus, during this period, caring parents are in a position to neutralize directly the negative effects of the environmental stresses that are otherwise expected to exacerbate the fitness costs of inbreeding depression (Fox and Reed 2011, Reed et al. 2012).

My second key finding was that maternal care increased the adult lifespan of inbred offspring but not of outbred offspring. This finding shows that parental care can buffer against inbreeding depression in offspring long after they have become independent of their parents. The extended adult lifespan of inbred offspring due to maternal care may reflect that maternal care increases the general condition of inbred offspring, thereby enhancing their survival prospects after the end of the parental care period. My results highlight that the buffering effects of parental care against inbreeding depression can occur across different life stages, reinforcing the importance of measuring fitness consequences across an individual's whole life span (Charlesworth and Charlesworth 1987).

My finding that maternal care in *N. vespilloides* buffers against inbreeding depression in larval survival and adult lifespan contrasts with a recent study that found no evidence for a buffering effect on larval survival in the European earwig (Meunier and Kölliker 2013). One potential explanation for these opposing results is that no inbreeding depression in larval survival was observed in European earwigs. In contrast, I found evidence for substantial inbreeding depression in this trait, which is consistent with previous work on *N. vespilloides* (Mattey et al. 2013). I suggest that parental care can buffer against inbreeding depression only when the following two conditions are met: (*i*) offspring suffer from inbreeding depression in a particular trait and (*ii*) parental care can improve offspring performance with regard to that trait. My results confirm that both conditions were met in *N. vespilloides*, while only the second condition was met in European earwigs (Meunier and Kölliker 2013).

Given that matings between close relatives are relatively uncommon in most natural populations of animals (Bulmer 1973, Walling et al. 2011), it seems unlikely that parental care evolved specifically to buffer against inbreeding depression. Instead, it is generally accepted that parental care evolves as a mechanism for neutralizing the effects of environmental stresses, such as starvation, predation, parasitism, and competition, on the offspring's fitness (Royle et al. 2012, Tallamy and Wood 1986). However, once parental care has evolved, it may inadvertently moderate the severity of inbreeding depression because it alleviates many of the same stresses that are predicted to exacerbate inbreeding depression. I therefore expect similar buffering effects against inbreeding to be widespread across species with parental care, regardless of whether they have a history of inbreeding or not. Furthermore, I suggest that whenever a previously outbred population becomes subject to inbreeding (e.g., due to habitat fragmentation or a population bottleneck), the severity of inbreeding depression may depend on the pre-existing form or level of parental care. Thus, the buffering effect of parental care is likely to be non-adaptive in the context of coping with inbreeding but adaptive in the context of neutralizing environmental stresses.

Although it seems unlikely that parental care originated to provide a buffer against inbreeding, the form or level of parental care may subsequently be modified due to its capacity to buffer against the fitness costs of inbreeding depression should the population remain inbred over many subsequent generations. Such evolutionary changes in parental care might occur in animal taxa with inbred mating systems (Avilés and Bukowski 2006, Avilés and Purcell 2012). Avilés and Bukowski (2006) proposed that parental care or other forms of sociality that buffer against inbreeding depression could facilitate the transition from an ancestral outbred mating system towards an inbred mating system as found in social spiders and other inbred social systems (Avilés and Purcell 2012). My finding that parental care buffers against inbreeding depression provides experimental evidence that parental care may facilitate the evolution of inbred social systems by reducing the fitness costs of inbreeding depression associated with such a transition. Nevertheless, the argument by Avilés and Bukowski (2006) implicitly assumes that parental care itself is not subject to inbreeding depression. Theoretical considerations suggest that this assumption might be violated (Falconer and Mackay 1996, Linksvayer and Wade 2009), in which case persistent inbreeding might affect the parents' ability to buffer against inbreeding depression in their offspring. Given these theoretical predictions and some mixed evidence from empirical studies on the effect of inbreeding on parental care (Margulis 1998, Pooley et al. 2014, Mattey and Smiseth 2015), I encourage further work in this area.

Based on my findings, I expect selection for inbreeding avoidance to be relaxed when parental care can moderate the deleterious effects of inbreeding in the offspring (provided that parental care itself is not subject to inbreeding depression). Under these conditions, the buffering effects of parental care may favour inbreeding tolerance or even inbreeding preference, a possibility that so far has been overlooked in the literature. Theoretical models emphasize the importance of the costs of dispersal, mating system, mate encounter rate, and kin recognition, as important factors shaping the balance between inbreeding tolerance and avoidance (Waser et al. 1986, Lehmann and Perrin 2003, Kokko and Ots 2006). To my knowledge, the only theoretical study to specifically consider the role of parental care concludes that biparental care should lead to lower inbreeding tolerance, because both parents have to put in a substantial amount of parental care for a relatively small return in the form of inbred offspring (Kokko and Ots 2006). However, existing theory has not considered that parental care might moderate the severity of inbreeding depression in the offspring, in which case it could have the opposite effect of leading to higher inbreeding tolerance. For example, in an African cichlid with biparental care (*Pelvicachromis taeniatus*), both sexes preferentially mate with a close relative (Thünken et al. 2007). There is no evidence for inbreeding depression in this species, and this has been suggested to be due to the occurrence of parental care (Thünken et al. 2007). The buffering effects of parental care on offspring fitness may interact with life-history traits and mating dynamics to determine an organism's inbreeding strategy, which can in turn have profound implications for the maintenance of genetic variation within a population (Charlesworth and Charlesworth 1987, Szulkin et al. 2013). Considering the wider implications of the potential effects of parental care on the severity of inbreeding depression may help us better understand and predict when animals should avoid, tolerate, or prefer inbreeding, which has so far been challenging (Szulkin et al. 2013).

In summary, my findings have important implications for our understanding of inbreeding, a central topic in ecology and evolutionary biology. Firstly, I show that the buffering effects of parental care were detectable not only during the period when larvae depend on parental care (i.e., from egg laying to dispersal from the carcass) but also long after independence. I expect such buffering to be widespread in species with parental care, even in populations with no history of inbreeding, as long as parental care can alleviate environmental stress and kin matings lead to considerable inbreeding depression in the offspring. Secondly, the buffering effects of parental care may favour the evolution of inbred mating systems or inbreeding tolerance by reducing the fitness costs to inbred offspring (Avilés and Bukowski 2006). Therefore, a better understanding of how parental care and other forms of sociality can influence the expression of inbreeding depression may help us explain the observed variation in animal inbreeding strategies.

Chapter 8: Inbreeding and maternal effects due to body size

This chapter has been published as referenced below, and this publication appears as Appendix G in this thesis:

> Pilakouta N, Smiseth PT (2016) Maternal effects alter the severity of inbreeding depression in the offspring. *Proceedings of the Royal Society B: Biological Sciences* 283:20161023.

Abstract

A maternal effect is a causal influence of the maternal phenotype on the offspring phenotype over and above any direct effects of genes. There is abundant evidence that maternal effects can have a major impact on offspring fitness. Yet, no previous study has investigated the potential role of maternal effects in influencing the severity of inbreeding depression in the offspring. Inbreeding depression is a reduction in the fitness of inbred offspring relative to outbred offspring. Here, I tested whether maternal effects due to body size alter the magnitude of inbreeding depression in the burying beetle *Nicrophorus vespilloides*. I found that inbreeding depression in larval survival was more severe for offspring of large females than offspring of small females. This might be due to differences in how small and large females invest in an inbred brood because of their different prospects for future breeding opportunities. To my knowledge, this is the first evidence for a causal

effect of the maternal phenotype on the severity of inbreeding depression in the offspring. In natural populations that are subject to inbreeding, maternal effects may drive variation in inbreeding depression and therefore contribute to variation in the strength and direction of selection for inbreeding avoidance.

8.1 Introduction

Inbreeding results from matings between relatives and can lead to a general loss of heterozygosity, which increases the likelihood that recessive, deleterious alleles are expressed (Charlesworth and Charlesworth 1987). As a result, inbreeding is commonly associated with a reduction in the fitness of any resulting offspring, known as inbreeding depression. Inbreeding depression is an important issue in evolutionary biology and ecology because it can exert strong selection pressures on dispersal strategies, mating systems, reproductive strategies, and social behaviours (Szulkin et al. 2013). Furthermore, inbreeding depression is a growing conservation concern, given that increasing rates of habitat loss and habitat fragmentation can increase the likelihood of inbreeding (Keller and Largiadèr 2003, Andersen et al. 2004), thereby contributing to higher local extinction rates (Keller and Waller 2002). Even though there is abundant evidence for inbreeding depression across a wide range of animal and plant taxa, the severity of inbreeding depression can vary dramatically both among and within species (Crnokrak and Roff 1999, Keller and Waller 2002). Understanding the factors that underlie this variation could have implications for the conservation of many endangered populations, yet these dynamics are still not well understood. Earlier work has proposed that this variation may be partly attributed to differences in the physical or social environment (Armbruster and Reed 2005, Fox and Reed 2010, Reed et al. 2012). Environmental stresses, such as starvation and competition, tend to exacerbate inbreeding depression (Fox and Reed 2010, Reed et al. 2012), whereas benign conditions may moderate inbreeding depression (Avilés and Bukowski 2006, Meunier and Kölliker 2013).

In Chapter 7, I showed that inbreeding depression is more severe in the absence of maternal care, suggesting that the presence of the mother during offspring development can buffer against inbreeding depression. However, it is unclear whether such a buffering effect depends on the mother's phenotype. Maternal condition may affect the quantity or quality of care provided to the offspring (e.g., Andersen et al. 2000, Bales et al. 2002, Wong and Kölliker 2012, Steiger 2013), so we might expect the severity of inbreeding depression to be influenced by maternal traits such as body size, age, nutritional condition, and health status. This type of a causal influence of the maternal phenotype on the offspring phenotype would represent a maternal effect (Wolf and Wade 2009). Even though the mechanisms and consequences of maternal effects have been studied extensively (Mousseau and Fox 1998, Wolf and Wade 2009), previous work has overlooked the potential role of maternal effects in the context of inbreeding depression.

In this experiment, I used the burying beetle *Nicrophorus vespilloides* to examine whether maternal body size, an important component of the maternal phenotype, affects the severity of inbreeding depression in the offspring. Burying beetles are a highly suitable study system for addressing this question. They have facultative biparental care, and male removal has no effect on offspring fitness under laboratory conditions (Smiseth et al. 2005), allowing me to focus on maternal effects. Moreover, I have previously shown that there is severe inbreeding depression in this species, with respect to survival at the larval and pupal stages, as well as adult lifespan (Mattey et al. 2013). I have also shown that inbreeding depression in the offspring is less severe when the mother is present than when she is removed (Chapter 7). Given that small females provide less post-hatching care than large females (Steiger 2013), I hypothesized that inbred offspring would suffer a greater reduction in fitness (compared to outbred offspring) if they had a small mother than if they had a large mother. To test this hypothesis, I used a 2×2 factorial design with the following treatment groups: (*i*) a large female with outbred offspring, (*ii*) a small female with outbred offspring, (*iii*) a large female with inbred offspring, and (*iv*) a small female with inbred offspring. Because inbreeding depression affects traits across the entire life cycle in this species (Mattey et al. 2013), I measured several offspring fitness traits at different life stages: (*i*) hatching success, (*ii*) larval mass at dispersal, (*iii*) survival rate from hatching to dispersal, (*iv*) survival rate from dispersal to eclosion, and (*v*) lifespan after eclosion.

8.2 Methods

I used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. The beetles used in this study comprised of second-generation beetles from lines originally collected in Edinburgh, UK. They were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20 °C and constant light. All non-breeding adults were fed small pieces of raw organic beef twice a week.

8.2.1 Experimental design

In the first part of my experiment, I generated small and large individuals. Because adult body size is determined by larval mass at the dispersal stage (Bartlett and Ashworth 1988, Lock et al. 2004), it is possible to generate different-sized beetles by removing larvae from the carcass at different times after hatching. This methodology was originally established by Steiger (2013) and was also used in Chapters 2, 3, and 4 of this thesis. For each of 89 broods, I removed third-instar larvae weighing 100–150 mg and 200–250 mg to generate small and large adults, respectively. The main advantage of this method was that it allowed me to generate small and large females that were full siblings. I was thus able to remove any potential confounding genetic effects that might have arisen if I had selected small and large individuals from the stock population. After each small or large larva was removed from the carcass, it was placed in an individual container (12 × 8 × 2 cm) filled with moist soil. At eclosion, I measured the pronotum width of all female beetles. As intended, there was a substantial difference in the mean (± SD) pronotum width (mm) of females from the two groups: 4.04 (± 0.24) for small females and 5.33 (± 0.24) for large females. There was also no overlap in the range of pronotum widths for small (3.50–4.59 mm) and large females (4.99–6.00 mm). Steiger (2013) used similar size classes: 3.97 (±0.21) for small females and 5.54 (±0.23) for large females. These categories were based on the size range observed in both the laboratory population and beetles collected in the field (Steiger 2013).

In the second part of my experiment, I used a 2 × 2 factorial design with the following treatment groups: (*i*) a large female with outbred offspring, (*ii*) a small female with outbred offspring, (*iii*) a large female with inbred offspring, and (*iv*) a small female with inbred offspring. To produce outbred offspring for treatments (*i*) and (*ii*), I paired outbred virgin beetles that had no common ancestors for at least two generations. To produce inbred offspring for treatments (*iii*) and (*iv*), I paired outbred virgin beetles that were full siblings. All male and female parents were mated within 15 days after reaching sexual maturity (i.e., 10–25 days after eclosion). On the day of mating, I measured each female's prebreeding mass, which was later used to estimate the female's mass change over the breeding attempt (see below). Each experimental pair (n = 276) was placed in a transparent plastic container (17 × 12 × 6 cm) filled with 1 cm of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (24–26 g). After mating, I checked the containers twice a day for the presence of eggs. As soon as the first eggs were laid, I removed the male from the container. In this species, the amount of care provided by the male is highly variable and male removal has no effect on offspring fitness under laboratory conditions (Smiseth et al. 2005). Right before larvae started hatching, I recorded the number of eggs laid (clutch size) by counting the number of eggs visible at the bottom of the transparent breeding box (Monteith et al. 2012). Because each box contained a very thin layer of soil, the number of eggs at the bottom of the box was very close to the actual clutch size (Monteith et al. 2012).

When all larvae had dispersed from the carcass, I weighed the female again. By subtracting each female's prebreeding mass from her postbreeding mass, I calculated her mass change over the breeding attempt, as a measure of somatic investment and thus allocation to future reproduction (Billman et al. 2014). Females were then transferred to individual containers (12 × 8 × 2 cm) filled with moist soil. They were checked twice a week until death to measure their post-breeding lifespan, as a measure of their residual reproductive value.

At the dispersal stage, I also recorded the number of unhatched eggs visible at the bottom of the box, the number of surviving larvae, and the total mass of the brood. By subtracting the number of unhatched eggs from the clutch size recorded earlier, I estimated the number of eggs that hatched. I then divided the number of eggs that hatched successfully by clutch size to calculate hatching success. I also calculated the average larval mass in each brood by dividing total brood mass by the number of larvae. I placed all larvae from each brood into large transparent containers (17 × 12 × 6 cm) filled with moist soil. Approximately 20 days later, I recorded the number of individuals that eclosed successfully from each brood to calculate the survival rate from dispersal to eclosion. At this stage, up to six beetles from each brood were placed into individual containers (12 × 8 × 2 cm). I tracked the adult lifespan of these offspring ($n = 872$) by checking them twice a week until death. The sample sizes for matings with at least one offspring surviving until eclosion were as follows: $n = 46$ for large females with outbred broods, $n = 54$ for small females with outbred broods, $n = 40$ for large females with inbred broods, and $n = 43$ for small females with inbred broods.

8.2.2 Data analysis

All analyses were done using R version 3.2.3. I used linear models for continuous traits with normally distributed random errors (average larval mass, adult offspring lifespan, female mass change, and female post-breeding lifespan). For discrete traits, I used generalized linear models fitted with a Poisson error distribution (clutch size). For proportion data, I used generalized linear models fitted with a binomial distribution (survival to dispersal and survival to eclosion) or a binomial distribution corrected for overdispersion (hatching success). Proportion data were entered into the models using the 'cbind' function. In all of these models, I analysed absolute differences rather than log-transformed measures (Johnston and Schoen 1994) as relative measures of inbreeding depression are potentially biased (Moorad and Wade 2005).

All models included the following factors: offspring inbreeding status (outbred or inbred), maternal body size (large or small), and the interaction between the two. A statistically significant interaction would suggest that maternal body size affects the severity of inbreeding depression in the offspring (i.e., the extent to which inbred offspring perform less well compared to outbred offspring).

In addition to these factors, carcass size was included as a covariate in all models, because the amount of resources available may influence female reproductive decisions and offspring performance. Indeed, I found that females laid more eggs on larger carcasses (LR χ_1^2 = 8.87, $P < 0.01$), and larvae had a higher survival rate on larger carcasses (LR χ_1^2 = 6.47, $P = 0.01$). There was also a nonsignificant trend for females to gain more mass on larger carcasses ($F = 3.20$, $P = 0.08$). I also added maternal age as a covariate in all models, because age can influence female reproductive decisions and offspring performance. Accordingly, I found that younger females laid fewer eggs (LR χ_1^2 = 8.56, $P < 0.01$) and that their offspring had a higher survival rate from hatching to dispersal (LR $\chi_1^2 = 28.8$, $P < 0.0001$). Lastly, I added sex as a covariate in the model for adult lifespan of the offspring and found that male offspring had a shorter lifespan after eclosion than female offspring ($F = 9.16$, $P < 0.001$). Decisions on which covariates to include in the final models were based on AIC scores.

8.3 Results

Large females laid more eggs, gained relatively more mass over the breeding attempt, and had a longer post-breeding lifespan than small females (Table 8.1). Large females also produced heavier larvae than small females (Table 8.2). However, female body size had no effect on hatching success, survival to eclosion, or adult lifespan of the offspring (Table 8.2).

Table 8.1: Effects of inbreeding (outbred or inbred offspring), maternal body size (large or small female), and their interaction on female reproductive decisions (clutch size, female mass change) and residual reproductive value (postbreeding lifespan).

	Offspring inbreeding status		Female size		Interaction	
	F / LR χ_1^2	P	F / LR χ_1^2	P	F / LR χ_1^2	P
Clutch size	1.90	0.17	263	<0.0001	0.03	0.86
Female mass change (%)	0.11	0.74	11.1	<0.01	0.20	0.65
Female postbreeding lifespan (days)	0.09	0.77	9.7	<0.01	2.21	0.14

Table 8.2: Effects of inbreeding (outbred or inbred offspring), maternal body size (large or small female), and their interaction on the following offspring fitness traits: hatching success, average larval mass at dispersal, survival rate from hatching to dispersal, survival rate from dispersal to eclosion, and lifespan after eclosion.

	Offspring inbreeding status		Female size		Interaction	
	F / LR χ_1^2	P	F / LR χ_1^2	P	F / LR χ_1^2	P
Hatching success (%)	1.83	0.18	1.09	0.30	3.01	0.08
Average larval mass (mg)	0.11	0.74	30.3	<0.001	1.05	0.31
Survival to dispersal (%)	17.8	<0.0001	0.05	0.82	9.49	<0.01
Survival to eclosion (%)	21.5	<0.0001	2.24	0.13	1.01	0.32
Offspring adult lifespan (days)	24.9	<0.001	1.40	0.24	0.32	0.57

Even though there was no difference in clutch size, mass change, or post-breeding lifespan between females that were mated to their brothers and females that were mated to unrelated males (Table 8.1), inbreeding had significant effects on the offspring's fitness (Table 8.2). Inbred larvae suffered reduced survival from dispersal to eclosion and had a shorter lifespan as adults (Figure 8.1). In addition, there was a significant interaction between offspring inbreeding status and female size on survival to dispersal (Table 8.2). This interaction reflected that offspring of large females suffered a greater reduction in fitness due to inbreeding than offspring of small females (Figure 8.2). In other words, inbreeding depression in survival to dispersal was more severe for offspring of large mothers than those of small mothers (Figure 8.1). There was no interaction between offspring inbreeding status and female size on hatching success, larval mass, survival to eclosion, or offspring lifespan after eclosion (Table 8.2). Similarly, there was no interaction on female reproductive decisions or residual reproductive value (Table 8.1).

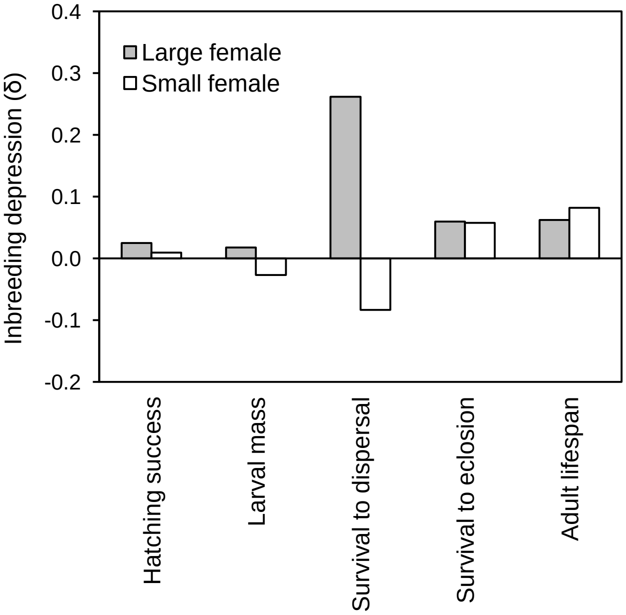

Figure 8.1: Inbreeding depression (δ) in offspring with large mothers (grey bars) or small mothers (white bars).

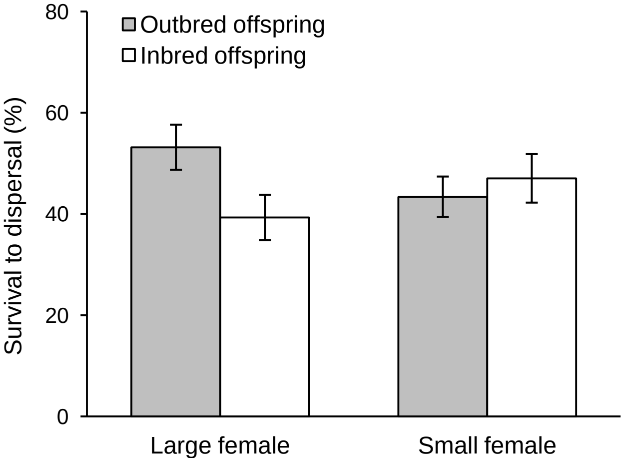

Figure 8.2: Mean (± SE) survival rate from hatching to dispersal for outbred (grey) or inbred (white) offspring of large or small females.

8.4 Discussion

In this study, I tested whether the mother's phenotype influences the severity of inbreeding depression in her offspring. I found evidence for inbreeding depression in three of the five traits I measured: survival from hatching to dispersal, survival from dispersal to eclosion, and post-eclosion lifespan (Table 8.1). In addition, I found a significant interaction between inbreeding and maternal body size on survival to dispersal. This interaction reflected that inbreeding depression in this trait was more severe for offspring of large females than offspring of small females (Figure 8.2). There was no such interaction on survival to eclosion or post-eclosion lifespan. Although I found an interaction in only one of these fitness traits, this trait was also the one with the highest level of inbreeding depression (Figure 8.1). In summary, my key finding was that offspring of large females suffered a lower survival rate from hatching to dispersal if they were inbred than if they were outbred, whereas inbred and outbred offspring of small females had a similar survival rate (Figure 8.2). To my knowledge, this is the first evidence for a causal effect of the maternal phenotype on the severity of inbreeding depression in the offspring.

Until now, very few studies have considered maternal effects in the context of inbreeding depression, and all of these were conducted on plants (Wolfe 1993, Montalvo 1994, Helenurm and Schaal 1996, Picó et al. 2003). Moreover, none of these studies established a causal link between maternal effects and the magnitude of inbreeding depression. For example, Wolfe (1993) found that maternal effects influenced early-life fitness traits in *Hydrophyllum appendiculatum*, while inbreeding depression affected late-life fitness traits. He proposed two plausible explanations for this pattern: (*i*) maternal effects substantially reduce the severity of inbreeding depression in early-life fitness traits, so no inbreeding depression is detected during this life stage, or (*ii*) inbreeding depression is more severe in later life stages because of the cumulative effect of smaller fitness reductions in earlier life stages (Wolfe 1993). Since it was not possible to distinguish between these two explanations, it was unclear whether there was an effect of the maternal phenotype on the severity of inbreeding depression in this species (Wolfe 1993).

Here, I demonstrate that maternal body size can alter the severity of inbreeding depression in larval survival in the burying beetle *N. vespilloides*. Inbred offspring of large females suffered lower survival from hatching to dispersal than outbred ones, whereas offspring of small females had the same survival rate regardless of their inbreeding status. The fact that there was no detectable inbreeding depression in this trait for offspring of small females suggests that maternal effects completely masked the deleterious effects of inbreeding on early-life offspring performance. In a population where the majority of females are small, such a masking effect could effectively hide the inbred genotype from natural selection, with potential consequences for the level of genetic load in the population (Wolfe 1993).

I expected that inbreeding depression would be less severe for offspring of large females than those of small females, because females that are in better condition might have the capacity to provide more care. Yet, I found evidence for the opposite pattern. One plausible explanation is that large females have larger clutches (Table 8.1), resulting in more intense sibling competition, which might in turn exacerbate the severity of inbreeding depression. I think this is unlikely given my findings in Chapter 6, showing that sibling competition does not exacerbate inbreeding depression in this species. Additionally, the mean brood size was relatively small, potentially due to seasonal variation in reproductive success as reported in Andrews et al. (2016), which would suggest a low level of sibling competition in this study. An alternative explanation is that small and large females differ in how they invest in an inbred brood because of their prospects for future breeding opportunities. Large females have a longer lifespan (J Moorad, unpublished data) and are more successful at acquiring and defending a carcass against conspecific competitors (Bartlett and Ashworth 1988). Thus, large females have a higher residual reproductive value than small females, who may only breed once due to their shorter lifespan and lower competitive ability. If this is the case, a small female might maximize her reproductive effort during a breeding attempt regardless of her offspring's inbreeding status. On the other hand, when a large female mates with a relative and produces a brood of inbred, low-quality offspring, she might reduce her investment in current reproduction in order to take advantage of additional breeding opportunities in the future. Such adjustments in maternal investment could be mediated through changes in prehatching effort (e.g., egg size, nutrients deposited into the eggs) and/or posthatching effort (e.g., provisioning rate), leading to a reduction in the survival of inbred offspring reared by large mothers. Nevertheless, this interpretation assumes that *N. vespilloides* females have the ability to recognise their relatives and/or the inbreeding status or overall quality of their offspring. Further work is needed to determine whether females behave differently towards inbred and outbred offspring.

I expect inbreeding to be relatively rare in natural populations of *N. vespilloides*, which makes this species a good model for understanding how the fitness costs of inbreeding are influenced by maternal effects in species that do not regularly inbreed. There are two important reasons it is useful to focus on species where inbreeding is relatively rare: (*i*) inbreeding depression is a greater concern for species with no prior history of inbreeding because deleterious, recessive alleles have not yet been purged from the population, and (*ii*) once a species has a significant history of inbreeding, parental behaviours and other family interactions might be modified by selection due to inbreeding. Thus, species with a history of inbreeding might not be appropriate as models for endangered species that have only recently become subject to inbreeding. In principle, all populations are potentially at risk of inbreeding in the future, given increasing habitat loss and other human-induced disturbances that increase the chances of inbreeding (Andersen et al. 2004). It is therefore important to improve our understanding of how populations that become subject to inbreeding may cope with inbreeding depression.

My study shows that maternal effects have the potential to influence the magnitude of inbreeding depression in the offspring. I encourage future research to investigate this previously unexplored issue in other taxa, since this pattern may be widespread in natural populations that suffer from inbreeding depression. If that is the case, it could have important implications for conservation efforts. Maternal effects are inextricably linked to maternal condition, which may vary over time within a population due to seasonal changes in weather and food availability (Toïgo et al. 2006, Rodriguez-Hidalgo et al. 2010, Mason et al. 2014). Maternal effects may therefore contribute to temporal variation in inbreeding depression in natural populations that are subject to environmental variability. In species where estimates of inbreeding depression (δ) are sensitive to maternal condition, a better understanding of the role of maternal effects may be important for the conservation and management of endangered populations. The presence of maternal effects might cause researchers to overestimate or underestimate inbreeding depression as a threat to population viability depending on the state of females at the time of data collection and on the particular subsample of females used in the study.

These findings also have general implications for evolutionary biology, because if maternal condition influences inbreeding depression in the offspring, we might expect selection for inbreeding avoidance to depend on the average maternal condition in the population. Depending on the parents' capacity to moderate the deleterious effects of inbreeding in the offspring, there might be selection for inbreeding avoidance, tolerance, or even preference. Existing theory has overlooked the possibility that maternal effects might influence animal inbreeding strategies. Until now, theoretical models have focused on how the costs of dispersal, mating system, mate encounter rate, and kin recognition might shape the balance between inbreeding tolerance and avoidance (Waser et al. 1986, Lehmann and Perrin 2003, Kokko and Ots 2006). I propose that incorporating maternal effects into such models could help us better understand and predict when animals should avoid, tolerate, or prefer inbreeding, which has so far been challenging (Szulkin et al. 2013).

In summary, my study provides novel insights into the role of maternal effects in altering the expression of inbreeding depression. I show that inbreeding depression in larval survival was less severe for offspring of small females than for offspring of large females. This pattern might be driven by differences in how small and large females invest in an inbred (low-quality) brood because of their different prospects for future reproduction. I recommend that future research investigates how other maternal traits, such as age, nutritional condition, and health status, might affect the severity of inbreeding depression within or among populations. In natural populations that are subject to inbreeding, maternal effects may contribute to variation in the magnitude of inbreeding depression, as well as variation in the strength and direction of selection for inbreeding avoidance.

Chapter 9: General Discussion

In this thesis, I first presented evidence that parental body size is important in shaping family interactions in *Nicrophorus vespilloides* (Chapters 2–4). In Chapter 2, I showed that males and females adjust their contributions to parental care based on both their own and their partner's body size. This suggests that the body size of the two parents can influence the resolution of sexual conflict over parental care. In Chapter 3, I showed that body size affects the resolution of sexual conflict over the consumption of a shared breeding resource, which in this case refers to the carcass from which the parents and the offspring feed over the course of the breeding attempt. Previous work has established that body size also influences a burying beetle's chances of winning a conspecific contest over a carcass (Otronen 1988, Safryn and Scott 2000). Here, I have shown that both winners and losers (of the same size) increase their investment to current reproduction relative to burying beetles with no fighting experience (Chapter 4). This suggests that reproductive investment decisions are based on experience with a contest (which is independent of body size) rather than the outcome of that contest (which is dependent on body size).

I then showed that inbreeding and inbreeding depresion can also influence and be influenced by family interactions in this species (Chapters 5–8). For example, I found that a female's mating preference for an outbred versus an inbred male was conditional on her own inbreeding status: inbred females preferentially mated with outbred males, whereas outbred females were equally likely to mate with an outbred or an inbred male (Chapter 5). I found no evidence that sibling competition had an effect on the offspring's inbreeding depression, since inbred offspring suffered a similar reduction in fitness when reared in small, medium-sized, and large broods (Chapter 6). Nevertheless, I found that the presence of the mother during larval development buffered against inbreeding depression (Chapter 7) and that this buffering effect depended on the mother's phenotype (Chapter 8).

9.1 Body size and parent-parent interactions

In Chapters 2 and 3, I investigated the mechanisms underlying the resolution of sexual conflict over parental care and over consumption of a shared breeding resource, respectively. Sexual conflict over contributions to parental care occurs because the benefits of care are shared between the two parents while the costs of care are personal (Lessells 2012), whereas sexual conflict over consumption from a shared resource occurs because the costs of consumption are shared between the parents while the benefits of consumption are personal. The three main mechanisms that may mediate the resolution of these two types of conflict are sealed bids, negotiation, and matching (Houston and Davies 1985, McNamara et al. 1999, Johnstone and Hinde 2006). These mechanisms were developed in the context of biparental care but may also apply to consumption from a shared resource given the clear analogy between these two types of conflict mentioned above. In addition to these three mechanisms, I also proposed coercion as an another mechanism that may apply only in the context of conflict over consumption of shared resources (Chapter 3).

In Chapter 2, I showed that small females provided less care than large females, and males and females provided less direct care when paired with a small partner. Thus, parents adjusted their contribution towards care based on both their own state and that of their partner (sealed bids). Both males and females also adjusted their contributions based on the amount of care provided by their partner (negotiation). These effects of the partner's body size and the partner's behaviour were independent. In Chapter 3, I showed that parents adjusted their carrion consumption based on matching and sealed-bid decisions. Female parents gained more mass when their partner gained more mass (matching), and large parents of both sexes gained more mass than small parents (sealed bids).

9.1.1 Implications for the study of sexual conflict

The findings in Chapters 2 and 3 make important contributions to our understanding of sexual conflict. Firstly, different mechanisms seem to be involved in mediating the resolution of different forms of sexual conflict within a species. In *N. vespilloides*, small and large parents resolve conflict over care through negotiation and sealed bids (Chapter 2), but they resolve conflict over carrion consumption through matching and sealed bids (Chapter 3). Interestingly, a recent study by Mattey and Smiseth (2015) in the same species shows that outbred and inbred parents resolve conflict over parental care using the same mechanisms as small and large parents (i.e., negotiation and sealed bids).

Secondly, these findings suggest that the resolution models developed in the context of sexual conflict over biparental care (negotiation, matching, and sealed bids) may apply more generally than previously thought. In fact, given the limited empirical evidence for matching in the context of biparental care (Hinde 2006), I suggest that the matching model (Johnstone and Hinde 2006) might be better suited for the resolution of sexual conflict over foraging from a shared resource. Although this type of conflict has so far been neglected, it could be very common in species with biparental care. For example, in many birds, the two parents share a breeding territory, within which each parent searches for food, both for its own consumption and to provision its nestlings. Also, in many insects with biparental care, the two parents share resources in the form of dung, carrion or wood that serve as food for the parents as well as the developing larvae (Tallamy and Wood, 1986). I therefore encourage future work to examine sexual conflict over consumption from shared resources in other taxa.

Thirdly, these results raise interesting questions as to whether sexual conflict over consumption from shared resources could influence sexual conflict over contributions to parental care. For example, if a parent is prevented from feeding by a physically superior partner, it may retaliate by providing less care. Conversely, if a parent is providing a disproportionate amount of care, its partner may be more tolerant of that parent feeding more from the resource. There are currently no studies investigating how the resolution of one type of sexual conflict may interact with the resolution of a different type of conflict in the same system. Such interactions are potentially widespread; they may occur whenever there are multiple types of sexual conflict occurring either simultaneously or sequentially over the reproductive bout. Further work is thus needed to investigate this issue.

9.1.2 Differential allocation as an alternative explanation

In Chapters 2 and 3, the observed adjustments in the amount of care provided by the two parents were interpreted in the context of sexual conflict resolution. Nevertheless, it is also possible that effects of the partner's size on the focal parent's behaviour were mediated through differential allocation. This is because parents respond to their partner's state not only to adjust for variation in the expected amount of care provided by their partner but also to adjust for their partner’s attractiveness or parental ability (Houston et al. 2005). For example, there is evidence that small parents are less capable of defending their brood against infanticidal intruders (Trumbo 2007). If so, parents mated to a small partner might be more at risk from takeovers by intruders, in which case they might reduce their investment in the current brood due to its lower reproductive value. Since I only allowed parents to breed once, further work would be needed to examine whether parents mated to small partners reduce their investment in the current brood in order invest more in future reproductive attempts.

9.2 Body size and parent-offspring interactions

I have shown that parental effort and allocation to current reproduction may depend on the focal parent's body size, the partner's body size, and the partner's behaviour (Chapters 2 and 3). A parent's body size might also indirectly influence its parental effort and allocation to current reproduction by influencing whether it wins or loses contests with conspecifics (Chapter 4). In burying beetles, body size is an indicator of competitive ability and is the strongest determinant of contest outcome: larger individuals are more likely to win a conspecific fighting contest over a carcass (Otronen 1988, Safryn and Scott 2000). Whenever an individual participates in a fighting contest, it gains information about its size and condition relative to other individuals in the population, which might in turn provide information about its prospects for breeding in the future. Thus, following a fighting contest, a small beetle may increase its investment in current reproduction (as a result of losing the contest), whereas a large beetle may reduce its investment in current reproduction (as a result of winning the contest).

To avoid the potentially confounding effects of body size (Steiger 2013), I used medium-sized females as the focal individuals in my experiment, and I paired them with either small or large females, so that they would win or lose the contest, respectively (Chapter 4). Contrary to my hypothesis, medium-sized parents increased their investment in current reproduction regardless of whether they won or lost a contest over a carcass (Chapter 4). This finding suggests that contest experience (which is independent of size) is more important than contest outcome (which is dependent on size) in influencing reproductive investment decisions. A possible explanation for this is that fighting experience (or lack thereof) may act as a cue for population density and therefore the overall intensity of competition in the population. If competition for carcasses is very high, a previous winner may not win again in the future, as it might have to compete with an even larger individual. Therefore, the best strategy for both winners and losers might be to increase their allocation to current reproduction after being involved in a fighting contest.

9.3 Inbreeding and family interactions

The evolutionary and ecological consequences of inbreeding and inbreeding depression have been a central topic in evolutionary biology, ecology and conservation biology (Szulkin et al. 2013). Yet, there is still a lot of unexplained variation in animal inbreeding strategies and in the severity of inbreeding depression within and between species (Crnokrak and Roff 1999, Keller and Waller 2002, Moorad and Wade 2005). One factor potentially contributing to this variation is that inbreeding depression has traditionally been studied without considering the social context within which inbred and outbred individuals reproduce and survive. Estimates of inbreeding depression are based on comparisons of inbred and outbred individuals with respect to components of their survival and reproduction, which are often determined by how they perform during social interactions with other individuals in the population. Estimates of inbreeding depression measured outside the organism's social context may therefore be biased. This suggestion is supported by the fact that studies conducted in the wild tend to report more severe inbreeding depression than those conducted in zoos or laboratories (Keller and Waller 2002, Armbruster and Reed 2005). This difference has been attributed to the fact that individuals in the wild are exposed to a more stressful physical environment than individuals kept in captivity (Keller and Waller 2002, Armbruster and Reed 2005).

However, an alternative explanation is that studies conducted in zoos and laboratories do not take into account the organisms' social environment. For example, competition between outbred and inbred conspecifics is expected to exacerbate inbreeding depression (Yun and Agrawal 2014), but most laboratory studies use individuals kept under solitary conditions. By excluding competition, these studies may underestimate the magnitude of inbreeding depression.

Chapters 5–8 highlight the importance of studying the effects of inbreeding in a social or family context and provide evidence that inbreeding depression can influence and be influenced by family interactions. Firstly, inbreeding in the parents seems to influence their choice of partner: females are less likely to mate with an inbred male if they are inbred themselves (Chapter 5). This suggests that when there is female choice, breeding pairs where both parents are inbred will be rare. Secondly, the presence of a caring parent during larval development tends to moderate the severity of inbreeding depression in the offspring (Chapter 7), but this effect also depends on the mother's phenotype (Chapter 8). Although I found no evidence that sibling competition has an effect on inbreeding depression in broods of inbred larvae (Chapter 6), this issue needs to be investigated further in taxa where broods with both outbred and inbred offspring are common (see discussion below).

9.3.1 Inbreeding and parent-parent interactions

Earlier mate choice studies have shown that inbred males often suffer reduced mating success (Joron and Brakefield 2003, van Oosterhout et al. 2003, Mariette et al. 2006, Ala-Honkola et al. 2009, Enders and Nunney 2010, Ketola and Kotiaho 2010). In Chapter 5, I showed that inbred females avoid mating with inbred males, but outbred females do not. This suggests that inbred males suffer reduced mating success only when interacting with inbred females. Therefore, in species where female inbreeding status influences mate choice for outbred versus inbred males, the fitness costs of inbreeding with respect to male mating success may be frequency-dependent. In populations with high rates of inbreeding, a larger proportion of breeding females will be inbred, so I would expect inbred males to experience lower mating success than in populations with low rates of inbreeding. Such social effects of inbreeding depression on male mating success may be widespread, but their occurrence is still largely unexplored. Future research should further investigate this issue, as it could have important implications for the rate and direction of sexual selection in populations that are subject to inbreeding. For example, under a scenario where inbred females are choosier than outbred females, directional selection on male sexual traits will be stronger when inbreeding rates are high than when they are low.

9.3.2 Inbreeding and parent-offspring interactions

The effects of social interactions on inbreeding depression may also influence selection for inbreeding avoidance. Given that the occurrence of parental care and the parent's actual phenotype can alter the fitness costs of inbreeding in the offspring, these factors could influence an animal's inbreeding strategy (Chapters 7 & 8). For example, selection for inbreeding avoidance may be relaxed in a species where parental care can moderate the deleterious effects of inbreeding in the offspring. Under these conditions, the buffering effects of parental care may favour inbreeding tolerance or even inbreeding preference. Existing theory has overlooked the possibility that parental care and parental effects might influence animal inbreeding strategies. Theoretical models emphasize the importance of the costs of dispersal, mating system, mate encounter rate, and kin recognition, as important factors shaping the balance between inbreeding tolerance and avoidance (Waser et al. 1986, Lehmann and Perin 2003, Kokko and Ots 2006). Yet, the buffering effects of parental care on offspring fitness could interact with life-history traits and mating dynamics to determine an organism's inbreeding strategy, which can in turn have profound implications for the maintenance of genetic variation within a population (Charlesworth and Charlesworth 1987, Szulkin et al. 2013). Incorporating the parental care system and/or parental effects into theoretical models may help us better understand and predict when animals should avoid, tolerate, or prefer inbreeding, which has so far been challenging (Szulkin et al. 2013).

9.3.3 Inbreeding and offspring-offspring interactions

My results show that the intensity of sibling competition does not influence the magnitude of inbreeding depression in homogeneous broods of inbred offspring (Chapter 6). However, I cannot rule out possible effects of sibling competition on the severity of inbreeding depression in mixed paternity broods comprising of both inbred and outbred offspring. Under these conditions, higher-quality (i.e., outbred) offspring may outcompete their lower-quality (i.e., inbred) half-siblings, thus magnifying differences in fitness between them (Yun and Agrawal 2014). Given that only one previous study has investigated the effect of sibling rivalry on inbreeding depression (Meunier and Kolliker 2013), I think this is an important topic that needs to be investigated further in other taxa.

This issue may be particularly relevant for socially monogamous bird species where females engage in extra-pair copulations. Extra-pair copulations might have evolved as a mechanism for inbreeding avoidance when a female is forced to mate with a related social partner (Reid et al. 2015). In support of this suggestion, earlier work has shown that nestlings sired by the social partner tend to be more inbred, whereas nestlings resulting from extra-pair matings tend to be more outbred (e.g., Blomqvist et al. 2002, Foerster et al. 2003, Brouwer et al. 2011, Reid et al. 2015). Thus, in these species, there is a potential for competition between outbred and inbred nestlings, and outbred nestlings may be competitively superior to their inbred half-siblings. Further work is now needed on the effects of sibling competition among offspring sired by males that vary in their degree of relatedness to the mother.

A better understanding of these interactions is especially important given the substantial temporal and spatial variation in the frequency of extra-pair copulations between populations (Petrie and Kempenaers 1998). If competition between outbred and inbred half-siblings indeed exacerbates inbreeding depression, this could lead to variation in the severity of inbreeding depression within and among populations depending on the frequency of extra-pair copulations and thus the composition of each clutch (i.e., the proportion of inbred versus outbred offspring). Furthermore, it is generally assumed that, by engaging in extra-pair copulations, females can change the mean inbreeding level of their offspring and thus the average fitness of the offspring in a given clutch (Szulkin et al. 2013, Reid et al. 2015). However, if the intensity of sibling competition indeed exacerbates inbreeding depression through competition between outbred and inbred half-siblings, engaging in extra-pair copulations may incur significant costs to females by further reducing the fitness of their inbred nestlings. An interesting question for future studies to address is whether the gain in average fitness through the production of outbred offspring is greater than the fitness reduction experienced by inbred offspring due to competition.

9.3.4 Broader implications for inbred populations

Given the extent of anthropogenic environmental change and habitat loss, many populations with no prior history of inbreeding are potentially at risk of inbreeding in the future. It is therefore important to gain a better understanding of how these populations may cope with inbreeding depression. Whenever inbreeding occurs in a population with no prior history of inbreeding, the fitness costs of inbreeding may be mediated through pre-exisiting mechanisms and behaviours (e.g., mate choice, parental care) that have evolved outside the specific context of inbreeding (Mattey and Smiseth 2015). For example, the avoidance of inbred males by inbred females in *N. vespilloides* (Chapter 5) is unlikely to have evolved in direct response to inbreeding. Instead, these mating preferences were likely mediated through pre-existing mechanisms that evolved to serve an adaptive function in a different context; females might have evolved general mating preferences for high-quality males, which may be conditional upon the females' own quality. Another example is parental care, which is unlikely to have evolved specifically to buffer against inbreeding depression in this species (Chapters 7 & 8). However, once parental care has evolved, it may inadvertently moderate the severity of inbreeding depression because it alleviates many of the same stresses that are predicted to exacerbate inbreeding depression (Fox and Reed 2011). Thus, the buffering effect of parental care is likely to be nonadaptive in the context of coping with inbreeding but adaptive in the context of neutralizing environmental stresses. Overall, my results demonstrate that whenever a population becomes subject to inbreeding, the associated fitness costs may be mediated through pre-existing mechanisms that evolved to serve an adaptive function in a different context (Mattey and Smiseth 2015).

9.4 Interpreting interactions and main effects

A statistically significant interaction between two explanatory variables can either indicate that (*i*) one of the variables only has an effect on the response variable within a certain range or category of the other variable (that is either continuous or categorical, respectively) or that (*ii*) one of those two variables always has an effect on the response variable, but the magnitude of this effect varies depending on the value or category of the other variable. When there is a statistically significant interaction and no statistically significant main effects, this indicates scenario (*i*). However, when both the interaction and at least one of the main effects appear to be statistically significant, this may not necessarily indicate scenario (*ii*), where there is a true main effect in addition to the interaction. This is because when an interaction between explanatory variables is statistically significant, the *P*-values obtained for its component variables can no longer be used to draw conclusions about their independent effects on the response variable.

In Chapters 2, 3, and 7, I made some inferences about main effects where interactions where significant, so I have re-examined these results to assess their validity. After visually inspecting the data in Chapter 7, it seems that inbred offspring tend to perform less well than outbred offspring in both treatments (parental care and no parental care). This suggests that there is an overall effect of inbreeding on offspring fitness in addition to the effect of the interaction between inbreeding and parental care (Table 7.2). In this particular case, the interaction effect indicates that inbred offspring always suffer reduced fitness, but this reduction in fitness due to inbreeding is smaller when the offspring are receiving care than when they are not (Table 7.2).

However, in Chapters 2 and 3 some of the main effects may have been misinterpreted due to the presence of interaction effects. For example, in Chapter 2, there was a significant effect of the interaction between female and male body size on the amount of direct care provided by the female (Table 2.1). After visually inspecting the data (Figure 2.1), it does not appear that the two parents' body sizes had additional independent effects on female direct care. Similarly, in Chapter 3, there was a significant effect of the interaction between female and male body size on the male's feeding rate and mass change (Tables 3.1 and 3.2). With respect to male feeding rate, there are no obvious independent effects of male and female body size in addition to the interaction (Figure 3.1). With respect to male mass change, there might be an independent effect of the male's own size (in addition to the interaction between male and female size), but this is difficult to determine conclusively. I thus urge caution in interpreting these effects as sealed-bid responses.

9.5 Concluding remarks

Here, I present evidence that inbreeding and parental body size are important in shaping family interactions in *N. vespilloides*. For example, the body size of the two parents influences the resolution of sexual conflict over parental care and over the consumption of the carcass on which they breed. Nevertheless, I found no evidence that body size influences reproductive investment decisions indirectly through the outcome of a fighting contest. Furthermore, there was no effect of sibling competition on the severity of inbreeding depression, but there were important interactions between inbreeding and two other family interactions: mate choice and parental care. In sum, the evidence reported in this thesis may help us better understand the resolution of sexual conflict and the wider implications of fighting contests, and it could also inform conservation efforts for endangered populations that are subject to inbreeding.

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State-dependent cooperation in burying beetles: parents adjust their contribution towards care based on both their own and their partner's size

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Abstract

Handicapping experiments on species with biparental care show that a focal parent increases its contribution when its partner is handicapped. Such results are interpreted as evidence for negotiation, whereby each parent adjusts its amount of care to that of its partner. However, it is currently unclear whether the focal parent responds to a change in its handicapped partner's behaviour or state. To address this gap, we conducted an experiment on the burying beetle *Nicrophorus vespilloides* where we first generated different-sized males and females by varying the duration of larval development. We then used a 2 × 2 factorial design in which a small or large male was paired with a small or large female. Small females provided less direct care (food provisioning and interactions with larvae) than large females, and both males and females provided less direct care when paired with a small partner. Thus, the focal parent adjusted its contribution towards care based on both its own state and that of its partner. There was also evidence for negotiation between the two parents as the focal parent adjusted its contribution based on the amount of care by its partner. However, there was no evidence that negotiation accounted for how the focal parent responded to its partner's size. Our results have important implications for our understanding of biparental cooperation as they show that each parent adjusts its contribution not only based on the amount of care provided by its partner but also based on its own state and its partner's state.

Introduction

Parents of many animals, including the majority of birds (Cockburn, 2006) as well as some mammals, fishes, and insects (Balshine, 2012; Trumbo, 2012), cooperate to provide care for their joint offspring. Until now, most work on biparental cooperation has focused on how a focal parent adjusts its contribution based on the amount of care provided by its partner (Lessells, 2012). This focus is motivated by theoretical models for the evolutionary resolution of sexual conflict between caring parents (Houston *et al.*, 2005; Lessells, 2012). Sexual conflict arises because the benefit of care to each parent depends on the parents' combined effort, whereas the cost depends only on the parent's personal effort (Lessells, 2012). Thus, each parent will be under selection to reduce its personal cost by shifting as much of the workload as possible over to its partner. Theoretical models suggest that this conflict can be resolved through three behavioural mechanisms: negotiation, matching, and sealed-bid decisions. Negotiation and matching occur when each parent adjusts its level of care in direct response to its partner's contribution. When there is negotiation, the focal parent responds to a reduction in amount of care provided by its partner by increasing its contribution, though only such that it compensates incompletely for the partner's reduction (McNamara *et al.*, 1999). In contrast, when there is matching, the focal parent responds by matching any increase or reduction in its partner's contribution

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(Johnstone & Hinde, 2006). Finally, sealed-bid decisions occur when each parent makes an initial fixed decision about how much care to provide and that decision is independent of that of its partner (Houston & Davies, 1985). Experimental studies on birds and other taxa provide evidence in support of all three mechanisms (e.g. negotiation: Wright & Cuthill, 1989; matching: Hinde, 2006; sealed bid: Schwagmeyer *et al.*, 2002), although a meta-analysis of studies on birds found overall support for negotiation (Harrison *et al.*, 2009).

Much of the evidence showing that the focal parent adjusts its contribution based on the amount of care provided by its partner comes from handicapping experiments (Wright & Cuthill, 1989; Harrison *et al.*, 2009; Lessells, 2012). The rationale of such experiments is to reduce the contribution of one parent, typically by adding weights to the back of the handicapped parent (birds and insects: e.g. Wright & Cuthill, 1989; Suzuki & Nagano, 2009) or clipping some of its flight feathers (birds only: e.g. Sanz *et al.*, 2000), and then monitor any subsequent adjustments in the amounts of care provided by the two parents. In general, such experiments show that the handicapped parent provides less care, presumably as a consequence of the increased costs of providing care, whereas the other parent provides more care (Wright & Cuthill, 1989; Harrison *et al.*, 2009). Traditionally, the increased amount of care by the other parent is interpreted as a response to the change in the handicapped parent's behaviour. However, an alternative interpretation is that this increase is a direct response to the change in the handicapped parent's state. Currently, we have insufficient evidence to determine whether the increase in care by the focal parent is mediated through a response to the change in the handicapped parent's behaviour or state. Here, we extend previous work in this field by investigating whether cooperating parents adjust their contribution based on variation in their own state as well as the state of their partner, and by investigating whether any responses to the partner's state are in direct response to the partner's state itself or whether they are mediated through the partner's behaviour. We also extend the specific focus on handicapping to the wider issue of how the dynamics of biparental cooperation are influenced by variation in components of the parents' state, such as their body size, age, nutritional condition, and health.

Nicrophorus burying beetles are well suited as a system for investigating these issues because parental care by both parents is very flexible (Eggert *et al.*, 1998; Smiseth & Moore, 2004). Burying beetles breed on carcasses of small vertebrates, which provide the sole source of food for the developing larvae (Scott, 1998). Both parents help prepare the carcass, protect it and the brood from predators and conspecifics, apply antimicrobials to the carcass, and provision the larvae with predigested carrion (Eggert *et al.*, 1998; Rozen *et al.*, 2008; Walling *et al.*, 2008; Arce *et al.*, 2012). Females often spend more time provisioning food for the larvae and stay on the carcass for longer than males, whereas males spend more time maintaining the carcass (Fetherston *et al.*, 1994; Eggert *et al.*, 1998; Smiseth & Moore, 2002; Rauter & Moore, 2004; Smiseth *et al.*, 2005; Walling *et al.*, 2008). Previous studies based on mate removal, handicapping, or random-pairing designs provide mixed evidence with some support for both negotiation (Fetherston *et al.*, 1994; Rauter & Moore, 2004; Smiseth & Moore, 2004; Smiseth *et al.*, 2005; Suzuki & Nagano, 2009; Creighton *et al.*, 2015) and sealed-bid models (Jenkins *et al.*, 2000; Rauter & Moore, 2004; Smiseth *et al.*, 2005; Suzuki & Nagano, 2009). A recent study on the effects of inbreeding on biparental care found evidence for both negotiation and sealed-bid models, suggesting that these two mechanisms are not mutually exclusive (Mattey & Smiseth, 2015).

The state of an individual can refer to a number of different parameters, including its body size, age, nutritional condition, health, and whether it is subjected to handicapping or not. A focal parent may adjust its level of care to variation in its own state. The reason for this is that parental care incurs costs in terms of energy and time expenditure (Alonso-Alvarez & Velando, 2012) and reflects the trade-off between investment in current and future reproduction (Trivers, 1972), both of which are likely to be conditional on the parent's own state. Furthermore, a focal parent may adjust its contribution based on the state of its partner if the amount of care provided by the partner is determined by the partner's state. Here, we focus specifically on body size as the state component of interest because a previous study on the same species found that large females had higher reproductive success than smaller ones (Steiger, 2013). Thus, smaller females might be less capable of providing care, potentially as a consequence of physiological and/or anatomical differences between small and large females. To address whether male and female parents adjust their parental behaviour based on their own body size and that of their partner, we used a 2 × 2 factorial design where a large or small male was paired with a large or small female. To this end, we experimentally generated different-sized males and females by varying the duration of their larval development (Steiger, 2013). We predicted that small parents would provide less care than large ones given that small females have reduced reproductive success (Steiger, 2013). We also expected that a focal parent would provide more care when mated to a small than to a large partner. We then tested whether any adjustments in the level of care by a focal parent to its partner's size were mediated through negotiation, matching, or sealed-bid decisions. If such adjustments were mediated through negotiation or matching, we predicted that they would be dependent on the amount of care by the partner. In contrast, if such adjustments were mediated through sealed-bid decisions, we predicted they would occur in direct response to the partner's state and thus be independent of the amount of care by the partner.

Materials and methods

General methodology

We used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. We maintained a large population and only mated unrelated individuals (no common ancestors for at least two generations) to avoid inbreeding in the stock population. The beetles used in this study comprised of sixth-, seventh-, and eighth-generation beetles from lines originally collected in Edinburgh, UK, and Warmond, the Netherlands. They were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20 °C and constant light. Non-breeding adults were fed raw organic beef twice a week.

Experimental design

In the first part of this experiment, we generated beetles of different sizes using a full-sib design based on previously established methodology for this species (Steiger, 2013). This design allowed us to exclude potential confounding effects due to genetic differences between individuals of different body sizes (Steiger, 2013). To this end, we paired up unrelated virgin males and females, provided them with a previously frozen mouse carcass and allowed them to produce a brood. For each of these 90 broods, we removed half of the brood from the carcass once the larvae reached the third instar and achieved a mass of 80–120 mg (approximately 2 days after hatching). We recorded the mass of each of these larvae and kept them in individual containers until they reached adulthood, when they were used as the small parents in our experiment. We left the remaining larvae on the carcass until almost the entire carcass was consumed, removing them right before dispersal (4–5 days after hatching). We again measured their individual mass and put each larva in a separate container until they reached adulthood, when they were used as the large parents in our experiment. The larvae do not feed after dispersal and before eclosion, and the size of a larva at dispersal therefore determines its adult body size (Lock *et al.*, 2004).

When these small and large individuals reached adulthood, they were bred to collect data on their own and their partner's parental care behaviour. All beetles were virgins and were bred within 2 weeks after sexual maturity to avoid behavioural variation due to differences in age. To investigate the effects of male and female state on the dynamics of biparental care, we used a 2 × 2 factorial design with the following treatment groups: a large male paired to a large female ($n = 25$), a large male paired to a small female ($n = 25$), a small male paired to a large female ($n = 25$) and a small male paired to a small female ($n = 25$). The larval mass of our experimental beetles ranged from approximately 80 to 230 mg, and the beetles that weighed <150 mg when removed from the carcass were classified as small (mean ± SD = 111 ± 14 mg), whereas beetles that weighed more than 150 mg were classified as large (mean ± SD = 203 ± 24 mg).

The experimental pairs ($n = 100$) were transferred to transparent plastic containers (17 cm × 12 cm × 6 cm) with 1 cm of moist soil and provided with a previously frozen mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (22–25 g). Immediately after the eggs were laid, we moved the parents and the carcass to a new container with fresh, moist soil. When the eggs started hatching, we used the newly hatched larvae to generate experimental broods of 15 larvae by pooling larvae from eggs laid by different females across all treatments (Mattey & Smiseth, 2015). This cross-fostering design ensures that any effects of variation on the focal parent's behaviour due to its own or its partner's body size can be attributed to interactions between the two parents rather than effects mediated through maternal effects or the number of larvae in the brood (Mattey & Smiseth, 2015). Due to temporal kin discrimination in this species, parents cannot distinguish between manipulated foster broods and their own broods, as long as the larvae are at the same developmental stage (Oldekop *et al.*, 2007). As parents kill any larvae that arrive on the carcass before their eggs are expected to hatch (Müller & Eggert, 1990), we only provided experimental pairs with a brood once their own eggs had hatched. Before placing the larvae on the carcass, we weighed the brood, which allowed us to calculate offspring growth from hatching to later stages of larval development.

We conducted behavioural observations 24 h after the parents were provided with a brood, given that this stage in larval development corresponds to a peak in parental food provisioning in this species (Smiseth *et al.*, 2003, 2007). We used instantaneous sampling every 1 min for 30 min in accordance with established protocols (Smiseth & Moore, 2002; Mattey & Smiseth, 2015). We recorded the number of scans each parent spent providing (i) direct care, defined as food provisioning to the larvae (i.e. mouth-to-mouth contact with at least one larva) or interacting with the larvae (i.e. inside or around the crater and allowing larvae to beg), and (ii) indirect care, defined as carcass maintenance (i.e. deposition of secretions to the surface of the carcass or excavation of the crypt) or guarding (i.e. standing still in a position where it could defend the brood from predators or interspecific competitors).

At the end of the 30-min observation, we measured the total mass of the brood and counted the number of larvae on the carcass. The larvae were then returned to the carcass, and the parents were allowed to care for the brood undisturbed until the larvae dispersed from the carcass about 3–4 days later. At dispersal from the carcass, we recorded the date, number of larvae, and total brood mass.

Statistical analyses

All data were analysed using R version 3.1.1. We used general linear models for traits that had a normal error structure (number of larvae at dispersal, average larval mass at dispersal, and early larval growth rate from hatching until the observation) and generalized linear models for traits that had a Poisson error distribution (female direct care, female indirect care, total direct care, and total indirect care) or a negative binomial error distribution (time to dispersal). Because of the high proportion of zeros in the data on male care, we ran a zero-adjusted negative binomial (ZANB) regression (male direct care) and a zero-adjusted Poisson (ZAP) regression (male indirect care), using the 'hurdle' function in the 'pscl' package (Jackman, 2014). A binomial structure was assumed for the zero-hurdle model, and a negative binomial and a Poisson structure for the count model on male direct and indirect care, respectively. Significant values on the count model indicate that a given variable has an effect on the amount of care provided, whereas significant values on the zero-hurdle model indicate that a given variable has an effect on the probability of providing no care vs. some care. For all of these models, decisions on whether to include the interaction term and any additional effects were based on the lowest AIC score. When the difference in the AIC score was <2, we used the simpler model.

We conducted separate analyses for the amount of direct and indirect care provided by small and large parents of each sex. All such models included the main effects of male and female size (small or large) and the interaction between male and female body size. Note that for male behaviours, male size represents the focal parent's size and female size represents the partner's size, whereas for female behaviours, female size represents the focal parent's size and male size represents the partner's size. We also tested for an effect of the partner's behaviour on the amount of care provided by the focal parent. Carcass size was added as a covariate to all models on parental care because resource availability can influence parental behaviour (Mattey & Smiseth, 2015). Indeed, males provided more direct care on larger carcasses ($z = 2.0$, $P = 0.047$), whereas female provided more direct care on smaller carcasses ($z = -2.4$, $P = 0.014$). Carcass size had no effect on indirect care provided by males ($z = 0.24$, $P = 0.81$) or females ($z = -1.6$, $P = 0.11$). We also added brood size at the time of the observation to all parental care models, because, although we provided all parents with a brood of 15 larvae, there was some variation in the number of larvae that were alive at the time of the observation. Both males ($z = 1.99$, $P = 0.047$) and females ($z = 4.86$, $P < 0.0001$) spent more time providing direct care to larger broods, but brood size had no significant effect on the amount of male indirect care ($z = 1.66$, $P = 0.098$) or female indirect care ($z = 1.9$, $P = 0.054$).

To assess whether partner responses were mediated through a negotiation or matching process, we compared models in which the amount of time that the partner spent providing care was either added or removed as an additional effect. If such responses are mediated through a negotiation or matching process, we predicted that including the partner's behaviour would remove or reduce the effect of the partner's body size on the amount of care by the focal parent. To examine the level of compensation, we conducted separate analyses for the total amount of direct and indirect care by the two parents. These models included male and female body size as main effects, the interaction between these two factors, as well as carcass size and brood size.

Lastly, we tested whether parent size had an effect on early larval growth rate, time to dispersal, number of larvae surviving to dispersal, and average larval mass at dispersal. Total direct care was added as a factor in these models, because the amount of care provided by the parents is expected to have an effect on offspring fitness. Furthermore, we included the number of larvae dispersing as a covariate in the model for average larval mass at dispersal, as previous studies have shown a relationship between number and size of larvae at dispersal (Smiseth *et al.*, 2014).

Results

Do parents adjust their parental behaviour based on their own size?

As expected, we found that small females spent less time providing direct care to their offspring than large females (Table 1; Fig. 1) and that small males were less likely to provide direct care than large males (zero-hurdle model: Estimate = -0.60, SE = 0.31, $z = -2.0$, $P = 0.048$). However, there was no difference in the amount of direct care provided by small and large males for those males that provided at least some direct care (count model; Table 1). Likewise, there were no differences in the amount of indirect care provided by small and large females or by small and large males (Table 1; Fig. 2).

Table 1 Effects of parental body size on biparental cooperation. We provide information on the parameter estimates (Est), standard errors (SE), test statistics (*z*- and *t*-values), and *P*-values for the effects of the focal parent's size, the partner's size, and the interaction between the two parents' sizes. The reference category for the focal parent's size and the partner's size was 'large'. For simplicity, we present the results for the count model for the ZANB and ZAP regressions used to analyse male direct and indirect care, respectively (see text for zero-hurdle model results). Data on female care were analysed using a GLM fitted with a Poisson error structure. Statistically significant *P*-values are indicated in bold.

	Focal parent's size				Partner's size				Interaction			
Type of care	Est	SE	*z*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*
M direct care	−1.06	0.63	−1.7	0.093	−1.10	0.49	−2.2	**0.026**	1.52	0.83	1.4	0.066
F direct care	−0.51	0.14	−3.5	**< 0.001**	−0.32	0.14	−2.3	**0.022**	0.75	0.20	3.8	**< 0.001**
M indirect care	−0.23	0.32	−0.7	0.48	0.40	0.21	1.9	0.059	0.68	0.41	1.6	0.10
F indirect care	−0.19	0.10	−1.9	0.059	−0.01	0.1	−0.09	0.93	−0.04	0.20	−0.2	0.85

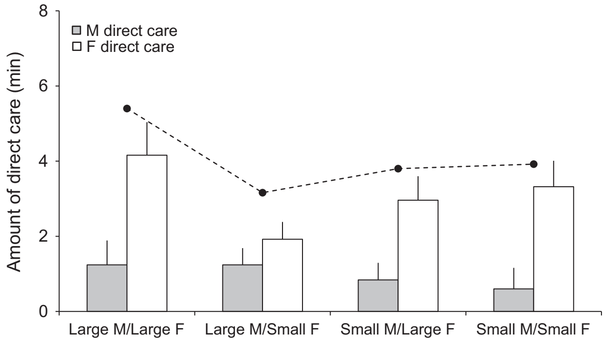

Fig. 1 Amount of time spent providing direct care (mean ± SE) by small or large males (grey bars) and small or large females (white bars) during a 30-min observation conducted 24 h after providing the parents with an experimental brood. Direct care behaviours comprise food provisioning and interactions with larvae. The filled circles indicate mean total direct care provided by the two parents in each treatment group. The line connecting the filled circles illustrates the level of compensation. In this case, the line declines from the treatment where both parents are large to the other three treatments, indicating that the total amount of care is reduced when at least one of the parents is small.

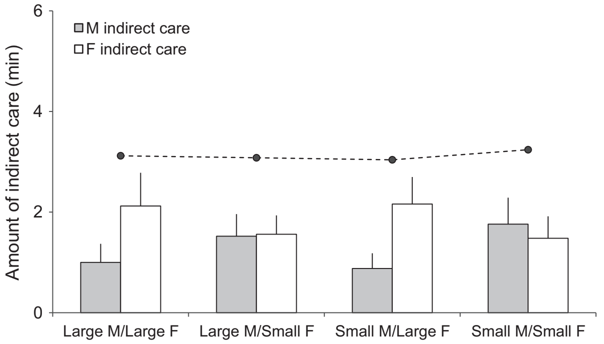

Fig. 2 Amount of time spent providing indirect care (mean ± SE) by small or large males (grey bars) and small or large females (white bars) during a 30-min observation conducted 24 h after providing the parents with an experimental brood. Indirect care behaviours comprise guarding and carcass maintenance. The filled circles indicate mean total indirect care provided by the two parents in each treatment group. The line connecting the filled circles illustrates the level of compensation. In this case, the line is straight across the four treatments, indicating that the total amount of care is similar regardless of male and female body size.

Do parents adjust their parental behaviour based on their partner's size?

As expected, there was a significant effect of the partner's size on the amount of direct care provided by both males and females (Table 1; Fig. 1). However, in contrast to what we expected, both males and females spent significantly less time providing direct care when they were mated to a small partner than when they were mated to a large one. As a result, the total amount of direct care provided by the two parents was significantly lower when at least one of the parents was small (Table 2; Fig. 1). There was no difference in the amount of indirect care provided by males or females paired to a small or large partner (Table 1; Fig. 2), and the total amount of indirect care provided by the two parents was not affected by the parents' size (Table 2; Fig. 2).

Are responses to the partner's size mediated through the partner's behaviour?

To determine whether the adjustment in the amount of direct care by the focal parent based on its partner's body size was mediated through a response to the partner's behaviour, we compared models in which we included or excluded the amount of direct care provided by the partner as an additional effect in our models. We first tested for evidence for negotiation by testing whether the focal parent adjusted its contribution based on the amount of care provided by its partner. As expected if the two parents negotiate how much care each should provide, we found that females spent more time providing direct care when the male

Table 2 Effects of parental body size on total care provided by the two parents. Each row represents the total amount of time spent providing direct and indirect care during a 30-min observation period. These data were analysed using a GLM fitted with a Poisson error structure. We provide information on the parameter estimates (Est), standard errors (SE), test statistics (*z*-values), and *P*-values for the effects of the male's size, the female's size, and the interaction between the two. The reference category for male size and female size was 'large'. Statistically significant *P*-values are indicated in bold.

	Male size				Female size				Interaction			
Type of care	Est	SE	*z*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*
Total direct care	−0.42	0.14	−3.0	**0.003**	−0.54	0.14	−3.7	**< 0.001**	0.64	0.20	3.2	**0.001**
Total indirect care	−0.02	0.47	−0.04	0.97	0.33	0.48	0.69	0.49	0.23	0.67	0.35	0.73

Table 3 Effects of parental body size on offspring fitness. Data on early larval growth, larval mass at dispersal, and number of larvae were analysed using general linear models. Data on time to dispersal were analysed using a GLM fitted with a negative binomial distribution. We provide information on the parameter estimates (Est), standard errors (SE), test statistics (*t*- and *z*-values), and *P*-values for the effects of the male's size, the female's size, and the interaction between the two. The reference category for male size and female size was 'large'. Statistically significant *P*-values are indicated in bold.

	Male size				Female size				Interaction			
Offspring trait	Est	SE	*t/z*	*P*	Est	SE	*t/z*	*P*	Est	SE	*t/z*	*P*
Early larval growth	0.02	0.06	0.41	0.69	−0.09	0.04	−2.1	**0.039**	−0.06	0.08	−0.67	0.51
Time to dispersal	−0.01	0.15	−0.08	0.94	0.05	0.15	0.32	0.75	0.08	0.22	0.38	0.71
Larval mass at dispersal	0.008	0.004	1.94	0.056	0.002	0.006	0.35	0.73	−0.01	0.01	−0.64	0.52
Number of larvae at dispersal	0.66	1.2	0.55	0.58	−0.81	1.2	−0.65	0.52	0.88	1.7	0.51	0.61

provided less direct care (Estimate = −0.084, SE = 0.020, $z = -4.3$, $P < 0.0001$). Furthermore, males were more likely to provide direct care when their partner was providing less direct care (zero-hurdle model: Estimate = −0.12, SE = 0.06, $z = -2.1$, $P = 0.037$), although there was no evidence that the amount of direct care provided by the male was influenced by the amount of direct care provided by the female (count model: $z = 0.19$, $P = 0.85$). However, we found no evidence that negotiation accounted for the focal parent's adjustment to its partner's size, as focal parents mated to small partners still spent significantly less time providing care compared with parents mated to large partners when the amount of direct care provided by the partner was included in the model (male direct care: $z = -2.2$, $P = 0.028$; female direct care: $z = -2.4$, $P = 0.018$). Thus, the adjustment by the focal parent to its partner's size was independent of the partner's behaviour, as expected if this adjustment was mediated through a sealed-bid decision.

Does the interaction between own size and partner's size influence parental behaviour?

Our experimental design also allowed us to test for an effect of the interaction between the focal parent's size and the size of its partner. We found a significant interaction effect on the amount of direct care provided by females, which reflected that small females spent more time providing direct care when they were mated to a small male, whereas large females provided a similar amount of care regardless of whether they were mated to a small or large male (Table 1; Fig. 1). There was no evidence for such an interaction effect on male direct or indirect care and female indirect care (Table 1).

Does the parents' size affect offspring fitness?

We finally tested for effects of the parents' size on components of the offspring's fitness. We found that larval growth during the first 24 h on the carcass was higher when the female was large, whereas there was no effect of male size (Table 3). We also found that larval growth rate during the first 24 h on the carcass was higher in larger broods (Estimate = 0.046, SE = 0.008, $t = 6.41$, $P < 0.0001$). Similarly, average larval mass at dispersal was higher in large broods (Estimate = 0.0014, SE = 0.0007, $t = 2.15$, $P = 0.035$), and there was a non-significant effect of total direct care on larval mass at dispersal ($t = 1.95$, $P = 0.055$). There were no effects of male or female size on either time to dispersal, larval mass at dispersal, or number of larvae surviving to dispersal (Table 3).

Discussion

Here, we report evidence from a burying beetle with biparental care showing that individual parents adjust their contribution towards parental care based on both their own body size and that of their partner. Specifically, we found that small females provided less direct care than large ones and that both males and females provided less direct care when paired with a small partner than when paired with a large one. As a consequence, the amount of total direct care provided by the two parents was lower when at least one of the parents was small. The difference in the amount of care between parents mated to different-sized partners was not related to variation in the amount of care provided by the partner. This suggests that the adjustment in care made by parents mated to a small partner was independent of the amount of care provided by the partner, as predicted by sealed-bid models for the resolution of sexual conflict (Houston & Davies, 1985). There was also an effect of the interaction between the size of the focal parent and its partner, as small females provided more care when paired with a small male, whereas large females provided the same amount of care regardless of whether they were paired with a small or large male. Below we provide a detailed discussion of the wider implications of our results for our understanding of biparental cooperation.

Our first main finding was that small females provided less direct care than large ones, whereas there was a nonsignificant trend in the same direction for males. This finding confirms that female parents adjust their contribution towards parental care based on variation in their own body size. Previous work on the same species shows that small females have lower reproductive success than large females (Steiger, 2013). Taken together, the results from our study and this previous study show that small female parents provide less parental care, presumably reflecting some kind of physiological or anatomical constraint on small females. For example, small females might provide less care and have lower reproductive success because they have a reduced capacity to predigest carrion for the larvae and/or produce antimicrobials than large females. There is mounting evidence showing that cooperating parents adjust their contributions towards offspring care based on variation in components of their own state. In addition to evidence showing that handicapped parents provide less care than control parents (Wright & Cuthill, 1989; Harrison *et al.*, 2009; Suzuki & Nagano, 2009), there is evidence that the amount of care that a parent provides is dependent on its age (Benowitz *et al.*, 2013), testosterone level (Saino & Møller, 1995), and inbreeding status (Pooley *et al.*, 2014; Mattey & Smiseth, 2015). Given that parents vary with respect to multiple state components, such as nutritional condition and health, there is now a need for further work to explore how male and female parents adjust their level of parental care based on variation in different state components.

Our second main finding was that both males and females provided less care when they were mated to small partners than when they were mated to large ones. This result confirms that parents of both sexes adjust their contribution based on the body size of their partner. However, in contrast to what we predicted, parents reduced the amount of care they provided when mated to a small partner. This finding is surprising given that small parents provided less care than large ones and that theoretical models for the evolution of biparental cooperation predict that parents should either compensate (incompletely) or not alter the amount of care that they provide in response to a reduction in the amount of care provided by its partner (Houston & Davies, 1985; McNamara *et al.*, 1999). Indeed, previous empirical work on *Nicrophorus vespilloides* and other species in the genus *Nicrophorus* provides good evidence that parents respond to mate removal or mate handicapping by either increasing or not altering the amount of care that they provide (Smiseth & Moore, 2004; Smiseth *et al.*, 2005; Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Mattey & Smiseth, 2015). Similar results have been reported in birds (Wright & Cuthill, 1989; Harrison *et al.*, 2009). One potential explanation for our result is that parents respond to their partner's state not only to adjust for variation in the expected amount of care provided by their partner but also to adjust for their partner's attractiveness or parental ability (Houston *et al.*, 2005). For example, there is evidence that small parents are less capable of defending their brood against infanticidal intruders (Trumbo, 2007). If so, parents mated to a small partner might be more at risk from takeovers by intruders, in which case they might reduce their investment in the current brood due to its lower reproductive value. Further work is needed to examine whether parents mated to small partners reduce their investment in the current brood in order to invest more in future reproductive attempts.

A key aim of our study was to identify the potential mechanisms whereby the focal parent adjusted its contribution based on its partner's size. We predicted that such responses would be mediated through negotiation, matching, or sealed-bid responses. We found evidence for negotiation as both males and females provided more direct care when their partner provided less direct care (see also Smiseth & Moore, 2004; Mattey & Smiseth, 2015). Nevertheless, including the partner's behaviour in the models did not remove or reduce the initial effect of the partner's size on the amount of care provided by the focal parent. This suggests that the way in which parents responded to their partner's size was not mediated through a response to the amount of care provided by the partner as predicted by negotiation or matching models (McNamara *et al.*, 1999; Johnstone & Hinde, 2006) but rather that it was independent of the partner's behaviour as predicted by sealed-bid models (Houston & Davies, 1985). This finding has important implications for our understanding of the behavioural mechanisms mediating the resolution of sexual conflict over parental care. Negotiation, matching, and sealed-bid responses have been traditionally considered as mutually exclusive mechanisms. However, our study provides evidence for both negotiation, as parents adjusted the amount of care that they provided based on the amount provided by their partners, and sealed-bid responses, as the focal parent's adjustment based on its partner's state was independent of the partner's behaviour. These results are consistent with those of a previous study investigating the effects of inbreeding on biparental cooperation in the same species (Mattey & Smiseth, 2015).

We suggest a simple graphical model based on behavioural reaction norms to illustrate the difference between sealed-bid responses and negotiation and how these two mechanisms might coexist (Fig. 3). In this model, the intercept depicts a sealed-bid decision, whereas the slope depicts negotiation between the two parents. Sealed-bid decisions represent a parent's initial decision about how much care to provide to the current brood, which may or may not depend on its own state or its partner's state (Fig. 3a). In contrast, negotiation represents subsequent changes in the parent's decision on how much care to provide based on information on the actual amount of care provided by the partner (Fig. 3b). This simple model suggests that these two mechanisms can coexist and that variation in the amount of care provided by a focal parent might reflect variation in its initial decision about how much care to provide (i.e. the intercept), and its subsequent responses to variation in the amount of care provided by its partner (i.e. the slope; Fig. 3c). We also argue that we now need to recognize different types of sealed-bid decisions. In Houston & Davies's (1985) classic sealed-bid model, the levels of male and female care were allowed to change over evolutionary time, whereas there was no scope for facultative adjustments in parental care based on either the parent's own state or its partner's state. Our results provide evidence for facultative sealed-bid responses adjusted to both the parent's own state and its partner's state. We therefore distinguish between three types of sealed-bid responses: (i) classic nonfacultative sealed-bid responses, as modelled by Houston & Davies (1985); (ii) facultative sealed-bid responses, where the focal parent adjusts its level of care to its own state; and (iii) facultative sealed bid responses, where the focal parent adjusts its level of care to both its own state and that of its partner. We encourage further theoretical and empirical work to consider different types of sealed-bid responses and the coexistence of sealed-bid responses and negotiation.

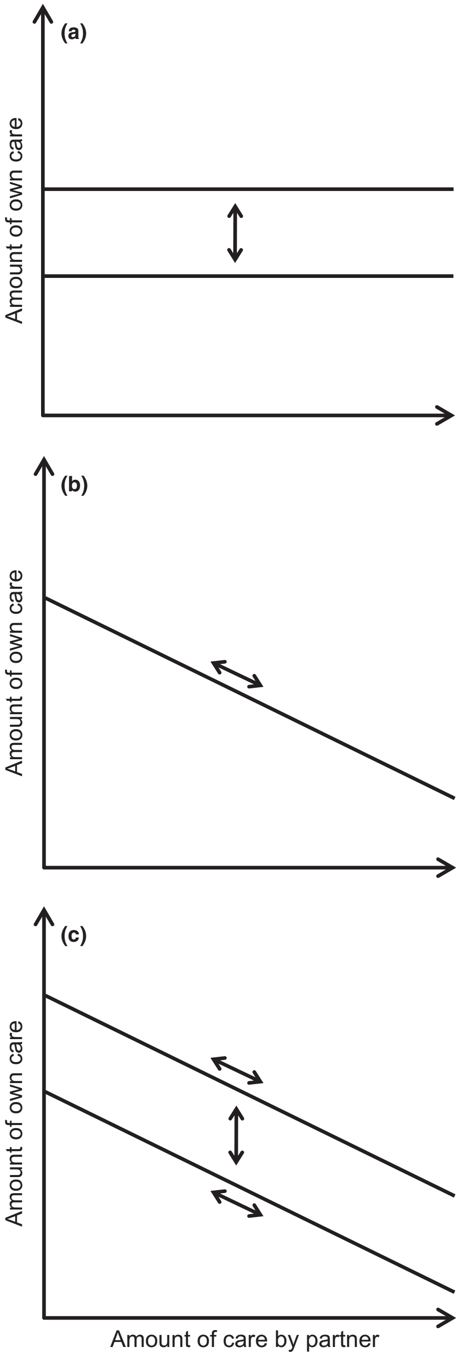

Fig. 3 Graphical model illustrating sealed-bid decisions (a), negotiation (b), and a combination of sealed-bid decisions and negotiation (c). In all cases, the intercept represents a fixed initial decision that is independent of the amount of care provided by the partner as assumed by sealed-bid models, whereas the slope represents a flexible adjustment in care based on the amount of care provided by the partner as assumed by negotiation models.

We also found evidence for an effect of the interaction between the parent's own state and the state of its partner on the amount of direct care provided by females. Such an interaction effect might reflect that the focal parent's ability to adjust its contribution to its partner's state is dependent on its own state. For example, if small parents are working closer to their maximum capacity, their ability to adjust their contribution when mated to a small partner might be constrained by their own state. We found no support for this suggestion as small females provided more care when mated to a small male than when mated to a large one, whereas large females provided the same amount of care regardless of whether they were mated to a small or large male. Thus, there is no evidence that the observed interaction effect is due to constraints on the focal parent's ability to adjust their contribution towards care. Instead, visual inspection of our results suggests that small females reduce their contribution when mated to a large male, whereas they provide as much as large females when they are mated to a small male (Fig. 1). Although we urge caution in interpreting this pattern, one potential explanation is that small females increase their contribution to care when mated to a small male in order to prevent detrimental effects on the offspring that otherwise might occur when both parents are small. We encourage further work to investigate whether an increase in the workload of small females mated to a small male has a greater beneficial effect on the offspring's fitness as compared to an increase in the workload of small females mated to a large male.

Finally, we found little evidence that variation in the state of the parents had any consequences for the offspring's fitness. Small females had larvae that grew more slowly early on (i.e. until 24 h after hatching) than large females, but this difference did not persist until the time of larval dispersal from the carcass. Thus, our results suggest that the lower amount of care provided by small females is associated with reduced larval growth in the early stages of development, but that parents and/or larvae are capable of compensating for this during the later stages of development. In *N. vespilloides*, larval size at dispersal determines adult body size (Lock *et al.*, 2004), which is an important determinant of the reproductive success of adults during fights for possession of carcasses (Otronen, 1988). Thus, there would be strong selection on any mechanism that would compensate for reduced early growth, including an extended period of food provisioning by parents and an extended period of self-feeding by larvae. Further work should now examine these potential mechanisms for compensatory growth in this system.

In conclusion, we report evidence for a species with biparental cooperation showing that each parent adjusts its contribution towards parental care based not only on the amount of care provided by its partner but also on its own state and that of its partner. Our results highlight the need to incorporate information on variation in the parents' state and its implications on the amount of care provided by parents in future theoretical and empirical work on biparental cooperation.

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If you eat, I eat: resolution of sexual conflict over consumption from a shared resource

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Sexual conflict arises whenever males and females have divergent reproductive interests. The mechanisms mediating the resolution of sexual conflict have been studied extensively in the context of parental care, where each parent adjusts its decision about how much care to provide based on its partner's workload. However, there is currently no information on the mechanisms mediating the resolution of sexual conflict over personal consumption from a shared resource. We address this gap in the burying beetle *Nicrophorus vespilloides*, which breeds on small vertebrate carcasses. The carcass serves as a source of food for both the developing larvae and the caring parents, and parents feed from the carcass for self-maintenance. To study the mechanisms mediating conflict resolution, we experimentally varied the two parents' body size to create variation in carcass consumption. We then assessed whether each parent adjusted its consumption based on its own size, its partner's size and its partner's consumption. As expected, large parents gained more mass than small parents. Furthermore, males paired to large females gained more mass than males paired to small females, and females responded to their partner's mass change, gaining more mass when their partner did. Our study provides insights into the resolution of a new form of sexual conflict, showing that it is mediated through both matching and sealed-bid responses. Our findings also suggest that the resolution models developed in the context of sexual conflict over biparental care may apply more generally than previously thought.

Sexual conflict arises whenever males and females have divergent reproductive interests and can occur in various contexts before mating (e.g. male harassment and female resistance; Arnqvist & Rowe, 2005), during mating (e.g. duration of copulation; Schneider, Gilberg, Fromhage, & Uhl, 2006) or after mating (e.g. contribution to parental care; Houston, Székely, & McNamara, 2005). Even though previous research has examined many types of sexual conflict (Arnqvist & Rowe, 2005; Houston et al., 2005; Parker, 2006), one type of conflict that so far has been neglected is that over the consumption of a food resource that is shared by the two parents and their offspring.

Sexual conflict over the consumption of a shared food resource might be common in species with biparental care. For example, in many birds, the two parents share a breeding territory, within which each parent searches for food, both for its own consumption and to provision its nestlings. Also, in many insects with biparental care, the two parents share resources in the form of dung, carrion or wood that serve as food for the parents as well as the developing larvae (Tallamy & Wood, 1986). Each parent benefits personally by consuming from the shared resource, as it allows that parent to invest in self-maintenance and thereby enhance its future reproductive potential (Billman, Creighton, & Belk, 2014; Creighton, Heflin, & Belk, 2009). However, given that resources are finite, increased consumption by one parent leaves less of the resource for the offspring and the partner. A study on the burying beetle *Nicrophorus vespilloides* suggested that sexual conflict over shared resources during the breeding attempt may negatively affect female longevity (Boncoraglio & Kilner, 2012). Thus, there is evidence for a conflict battleground between the two sexes over personal consumption from the shared resource, with each parent preferring to consume more resources than would be optimal from its partner's perspective. Nevertheless, the mechanisms underlying the resolution of this form of conflict are still unexplored.

We suggest four mechanisms that might be involved in the resolution of sexual conflict over consumption from a shared resource. The first potential mechanism is coercion based on physical interference between the two parents. If coercion is mediating the resolution of this conflict, consumption of the

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resource should depend on asymmetries in fighting ability between the two parents, as the stronger parent might be in a position to control the feeding behaviour of its partner. The other three possible mechanisms (negotiation, matching and sealed-bid decisions) derive from theoretical models for the resolution of sexual conflict over contribution towards parental care. Negotiation and matching occur when each parent adjusts its own contribution in direct response to its partner's contribution (Johnstone & Hinde, 2006; McNamara, Gasson, & Houston, 1999). When there is negotiation, the focal parent responds to a reduction in the amount of care provided by its partner by increasing its contribution (McNamara et al., 1999), while when there is matching, the focal parent matches any increase or reduction in its partner's contribution (Johnstone & Hinde, 2006). Sealed-bid decisions occur when each parent makes an initial fixed decision about how much to contribute that is independent of its partner's contribution (Houston & Davies, 1985). We suggest that these mechanisms might also apply to the resolution of sexual conflict over consumption from a shared resource because there are clear analogies between these two forms of conflict. Sexual conflict over contributions to parental care occurs because the benefits of care are shared between the two parents while the costs of care are personal (Lessells, 2012), whereas sexual conflict over consumption from a shared resource occurs because the costs of consumption are shared between the parents while the benefits of consumption are personal.

In this study, we investigated the mechanisms underlying the resolution of sexual conflict over carrion consumption in the burying beetle *N. vespilloides*, an insect that breeds on carcasses of small vertebrates (Eggert, Reinking, & Müller, 1998). The carcass serves as a source of food for the two parents and their developing offspring, so the more each parent consumes from the resource, the less will be left for its partner and the offspring (Boncoraglio & Kilner, 2012; Scott, 1989). Previous work in the burying beetle *Nicrophorus orbicollis* has shown that there is substantial variation in the parents' mass change over the breeding attempt and that this mass change serves as a proxy for investment in future reproduction (Billman et al., 2014; Creighton et al., 2009). Because we were interested in whether each parent adjusts its carrion consumption in response to that of its partner, we experimentally varied the body size of the two parents on the assumption that larger individuals consume more carrion. This asymmetry in body size inadvertently introduced asymmetry in the physical strength of the two parents (Otronen, 1988), allowing the possibility that the larger parent might enforce their feeding optimum by eating more while interfering with its partner's access to the carcass. Evidence for physical interference between partners has been observed in the closely related *Nicrophorus defodiens*. In this species, females behave aggressively towards their male partner to prevent him from attracting additional females (Eggert & Sakaluk, 1995).

To study the mechanisms mediating conflict resolution and assess whether each parent adjusts its consumption based on its own size, its partner's size and its partner's consumption, we recorded (1) the amount of time spent feeding on the carcass by each parent during a 30 min observation and (2) the change in the mass of each parent over the reproductive attempt (Billman et al., 2014; Creighton et al., 2009). If sexual conflict over carrion consumption is resolved through negotiation, we predicted that the focal parent would reduce its consumption in response to an increase in consumption by its partner. If it is resolved through matching, we predicted that the focal parent would increase its consumption in response to an increase in consumption by its partner. If the conflict is resolved through sealed-bid decisions, each parent's decisions about how much to consume should be independent of its partner's consumption. Lastly, if the conflict is resolved through coercion, we predicted that the larger parent would prevent its smaller partner from consuming from the carcass.

METHODS

General Methodology

We used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. The beetles used in this study comprised sixth-, seventh- and eighth-generation beetles from lines originally collected in Edinburgh, U.K. and Warmond, The Netherlands. They were housed individually in transparent plastic containers (12 × 8 cm and 2 cm deep) filled with moist soil and kept at 20 °C and constant light. Nonbreeding adults were fed raw organic beef twice a week.

Experimental Design

To induce variation in carcass consumption by the parents, we first generated small and large beetles using a full-sib design based on previously established methodology (Pilakouta, Richardson, & Smiseth, 2015; Steiger, 2013). For each of these 90 broods, we removed half of the brood from the carcass once the larvae reached the third instar, leaving the remaining larvae on the carcass until right before dispersal. We recorded the mass of each larva and kept the larvae in individual containers with moist soil. Larvae weighing less than 150 mg were categorized as small (mean ± SD: 111 ± 14 mg), while larvae weighing more than 150 mg were categorized as large (203 ± 24 mg). Larval mass at dispersal determines adult size, as larvae do not feed in the period between dispersal from the carcass and eclosion (Bartlett & Ashworth, 1988; Lock, Smiseth, & Moore, 2004).

All beetles were bred within 2 weeks after sexual maturity (10–24 days after eclosion) using a 2 × 2 factorial design: a large male paired with a large female ($N = 25$), a large male paired with a small female ($N = 25$), a small male paired with a large female ($N = 25$) and a small male paired with a small female ($N = 25$). Paired beetles were virgins and did not share common ancestors for at least two generations. The pairs were transferred to transparent plastic containers (17 × 12 cm and 6 cm deep) with moist soil and were provided with freshly thawed mouse carcasses (Livefoods Direct Ltd, Sheffield, U.K.) of a standardized size (22–25 g). For each of these matings, we recorded the mass of the carcass and the prebreeding mass of each parent. Immediately after eggs were laid, we moved the parents and the carcass to a new container. When the eggs started hatching, we generated experimental broods of 15 larvae by pooling larvae from eggs across all treatments (Mattey & Smiseth, 2015). This design ensured that there were no effects due to parent-offspring coadaptation (Lock et al., 2004) and that any differences in the parents' consumption of the carcass were not mediated through differences in brood size.

Twenty-four hours after providing the parents with a brood, we conducted behavioural observations using instantaneous sampling every 1 min for 30 min (Martin & Bateson, 1986; Smiseth & Moore, 2002; Smiseth, Darwell, & Moore, 2003). During this time, we recorded the number of scans that each parent spent feeding on the carcass. Parents were then allowed to care for the brood undisturbed until the larvae dispersed from the carcass about 4 days later. At dispersal, which corresponds to the end of the parental care period, we recorded the postbreeding mass of each parent. We calculated each parent's change in mass during the breeding period, by subtracting its prebreeding mass from its postbreeding mass.

Statistical Analyses

Data were analysed using R version 3.2.0. In all analyses for mass change, we used absolute rather than relative changes in mass, because we were specifically interested in examining differences in the amount of carrion consumed. Mass change data had a normal error structure, so we used general linear models for those analyses. Because the behavioural data (time spent feeding on carcass) were zero-inflated, we ran zero-adjusted negative binomial (ZANB) regressions, using the hurdle function in the pscl package (Jackman, 2014), which splits the data into two components. Significant values on the zero-hurdle model indicate that a given variable influenced the probability of consuming carrion, whereas significant values on the count model indicate that a given variable influenced how much time was spent consuming carrion. All models included male size, female size, the interaction between male and female size, as well as time spent feeding or mass change by the partner. Previous studies on the same species investigating the resolution of sexual conflict over biparental care found that the focal parent's response to the partner's behaviour and the partner's state were independent (Mattey & Smiseth, 2015; Pilakouta, Richardson, & Smiseth, 2015), so we included both variables in our starting models. Note that in all models, the reference category for male and female size was ‘large’.

Carcass size was included as a covariate in all models because resource availability may influence the parents' consumption. Males but not females spent more time feeding on larger carcasses (male: $z = 2.03$, $P = 0.042$; female: $z = 1.54$, $P = 0.12$), and carcass size had no effect on mass change in either sex (male: $t = -0.70$, $P = 0.48$; female: $t = -0.91$, $P = 0.36$). We also added brood size at the time of the observation as a factor, because although we provided all parents with a brood of 15 newly hatched larvae, there was some variation in the number of larvae alive at the time of the observation. Nevertheless, brood size did not have a significant effect on the amount of time parents spent feeding on the carcass (male: $z = -1.89$, $P = 0.059$; female: $z = -1.61$, $P = 0.11$) or the parents' change in mass (male: $t = -0.78$, $P = 0.44$; female: $t = -1.40$, $P = 0.17$).

Decisions about which variables to include in the final models were based on AIC model selection criteria. We also tested for multicollinearity in all models by estimating variance inflation factors using the vif function in the car package (Fox & Weisberg, 2011). The largest variance inflation factors were ≤ 3, indicating absence of multicollinearity.

Ethical Note

Our study adheres to the ASAB/ABS Guidelines for the Use of Animals in Research, the legal requirements of the U.K., as well as all institutional guidelines at The University of Edinburgh. None of the procedures used in this study had the potential to cause pain or distress.

RESULTS

Do Parents Base Consumption on Their Own Size?

Large parents spent more time feeding from the carcass (Table 1, Fig. 1) and also gained more mass over the reproductive attempt (Table 2, Fig. 2) than small parents.

Do Parents Base Consumption on Their Partner's Size?

Males spent more time feeding from the carcass and gained more mass when they were paired to a large female than when paired to a small female (Tables 1, 2, Figs. 1, 2). However, there was no significant difference in the time spent feeding or mass change by females paired to large and small males (Tables 1, 2, Figs. 1, 2).

Does the Interaction Affect Consumption?

There was a significant effect of the interaction between male and female size on male feeding behaviour and mass change (Tables 1, 2). This interaction effect reflected that large males spent more time feeding and gained more mass when paired to a large female, whereas small males spent a similar amount of time feeding and gained the same mass regardless of the size of their partner (Figs. 1, 2). There was no significant effect of the interaction between male and female size on female mass change or feeding behaviour (Tables 1, 2).

Do Parents Base Own Consumption on That of Their Partner's?

Males were more likely to feed from the carcass when their partner was feeding less (zero-hurdle model: estimate $= -0.12$, SE $= 0.05$, $z = -2.2$, $P = 0.027$), but males that fed from the carcass did not adjust the amount of time they spent feeding based on their partner's feeding behaviour (Table 1). Females did not adjust their feeding behaviour to that of their partner (zero-hurdle model: estimate $= -0.34$, SE $= 0.19$, $z = -1.8$, $P = 0.070$; count model: Table 1). Lastly, females responded to their partner's mass change, gaining more mass when their partner gained more mass (Table 2). Males, on the other hand, did not adjust their mass change in response to that of their partner (Table 2).

DISCUSSION

In this study, we examined the mechanisms that mediate the resolution of sexual conflict in a previously neglected context: conflict over personal consumption from a resource that is shared by the two parents and their dependent offspring. We found some evidence for sealed-bid decisions (i.e. decisions that are independent of the partner's behaviour) as parents of both sexes adjusted their consumption of carrion based on their own size, and males adjusted their consumption based on the size of their partner. We also found some evidence for matching as females gained more mass when their partner gained more mass. We found no evidence for size-dependent coercion, as parents did not feed less when paired to large partners. Our results therefore suggest that the resolution models developed in the context of sexual conflict over biparental care may apply more generally than previously thought.

The main aim of our experimental design was to induce variation in the parents' consumption of carrion by experimentally varying the body size of the focal parent and its partner. As intended, large parents of both sexes consumed more carrion than small parents. This result confirms our initial assumption that large individuals need more food to replenish their energy reserves and also provides some evidence for sealed-bid decisions, whereby a parent's decision about how much to consume is independent of its partner's behaviour. An inadvertent consequence of this size manipulation was that we introduced asymmetry in the physical strength of the two parents (Otronen, 1988). However, we found no evidence that large parents used their physical strength to prevent a small partner from feeding on the carcass. Our results thus do not support the hypothesis that size asymmetry between parents can influence the resolution of sexual conflict through coercion or punishment. The absence of coercion in this context might be due to its potential costs; attacking a partner that contributes towards providing care for the offspring may incur costs to the focal parent in terms of receiving less assistance from the partner in the future.

Table 1
Effects on the amount of time spent feeding from the carcass by male (M) and female (F) parents during a 30 min observation

	M Size				F Size				Interaction				Partner's feeding rate			
	Est	SE	*z*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*
M feeding	−1.2	0.5	−2.2	**0.028**	−1.6	0.6	−2.7	**0.006**	2.0	0.8	2.5	**0.011**	−0.001	0.1	−0.01	0.99
F feeding	−0.21	0.26	−0.8	0.43	−0.5	0.27	−1.9	0.058	−0.4	0.5	−0.7	0.47	−0.36	0.19	−1.9	0.054

Data were analysed using ZANB regressions. For simplicity, we present the results for the count model (see text for zero-hurdle model results). We provide information on the parameter estimates (Est), standard errors (SE), test statistics (*z* values) and *P* values. Statistically significant *P* values are indicated in bold.

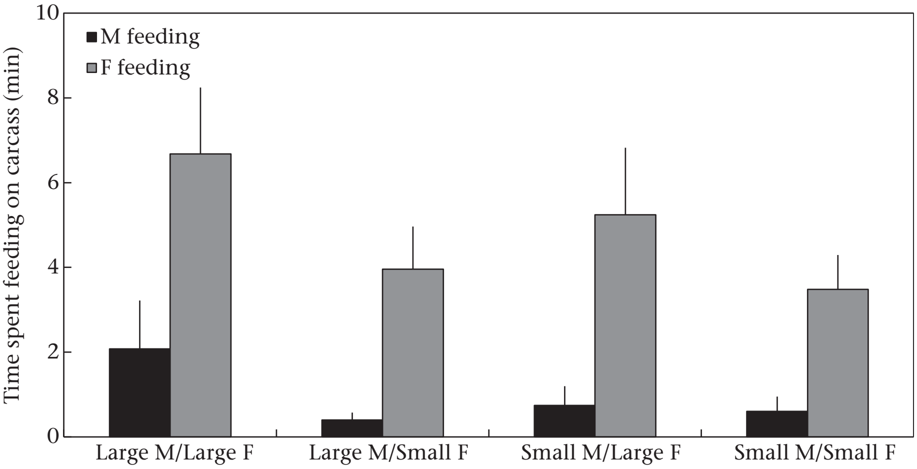

Figure 1. Means ± SE for amount of time spent feeding on the carcass (min) by small or large males (M: black bars) and small or large females (F: grey bars) during a 30-min observation.

Table 2
Effects on male (M) and female (F) mass change during breeding

	M Size				F Size				Interaction				Partner's mass change			
	Est	SE	*t*	*P*	Est	SE	*t*	*P*	Est	SE	*t*	*P*	Est	SE	*t*	*P*
M mass change	−29	5.6	−5.2	**<0.0001**	−18	5.7	−3.2	**0.002**	21	7.9	2.7	**0.009**	0.13	0.10	1.4	0.17
F mass change	−5.1	5.1	−1.0	0.32	−28	4.6	−6.1	**<0.0001**	−3.1	9.5	−0.3	0.74	0.22	0.11	2.0	**0.047**

Data were analysed using general linear models. We provide information on the parameter estimates (Est), standard errors (SE), test statistics (*t* values) and *P* values. Statistically significant *P* values are indicated in bold.

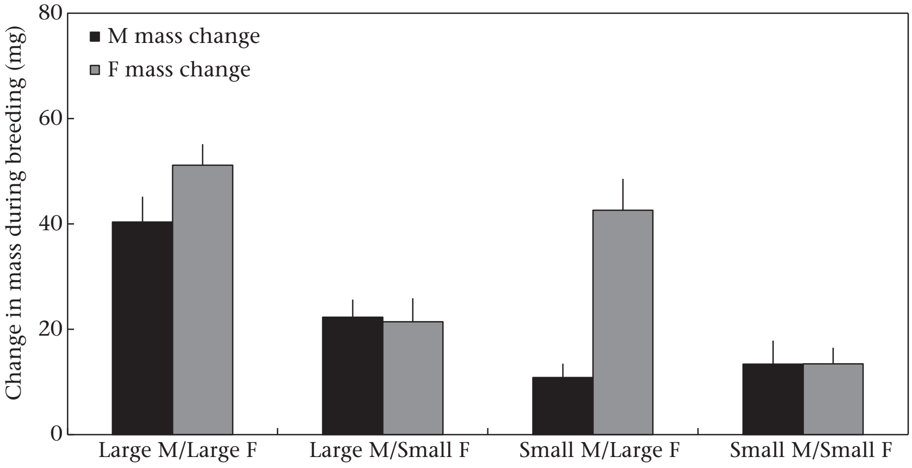

Figure 2. Means ± SE for mass change (mg) over the reproductive attempt for small or large males (M: black bars) and small or large females (F: grey bars).

Another key finding in our study was that each parent adjusted its consumption of carrion based on attributes of its partner. Females gained more mass when their partner gained more mass, while males adjusted their mass gain based on their partner's body size rather than its consumption of carrion. These results suggest that there is a sex difference in how parents respond to attributes of their partner: females match their consumption to that of their partner as predicted by matching models (Johnstone & Hinde, 2006), while males make decisions that are independent of the behaviour of their partner as predicted by sealed-bid models (Houston & Davies, 1985). Previous work on the same species has reported sex differences in how caring parents respond to mate removal: males provide more care following the removal of the female, while females provide a similar amount of care regardless of whether the male is present or absent (Smiseth, Dawson, Varley, & Moore, 2005). The sex difference in personal consumption reported here may reflect that females spend more time on the carcass than males (Smiseth & Moore, 2004; Smiseth et al., 2005) and that females therefore have better access to information about their partner's feeding rate. In contrast, males typically spend more time away from the carcass and may adjust their mass change to the expected feeding rate of their partner based on their partner's size. Indeed, we found that males spent more time feeding on the carcass and gained more mass over the reproductive attempt when they were paired to a large female, which consumed more carrion than a small female. The finding that females match their consumption to that of their male partner is interesting given that there is limited empirical evidence for matching in the context of biparental care (Hinde, 2006). Thus, we suggest that the matching model (Johnstone & Hinde, 2006) might be better suited for the resolution of sexual conflict over foraging from a shared resource.

For males, the observed pattern for feeding behaviour (Table 1) closely matched the pattern for mass change over the reproductive attempt (Table 2); both male feeding behaviour and male mass change were influenced by the male's own size, his partner's size and the interaction between the two (Tables 1, 2). In contrast, female mass change was influenced by her own size and her partner's mass change (Table 2), but this pattern was not reflected in the female's feeding behaviour (Table 1). One potential explanation for this sex difference is that, when both parents provide care, females are typically much more involved in provisioning food to the larvae than are males (Smiseth & Moore, 2004; Walling, Stamper, Smiseth, & Moore, 2008). Thus, females may regurgitate most of the carrion they consume to the larvae, whereas males may consume carrion primarily to replenish their own energy reserves. This interpretation is supported by visual inspection of our data, which suggest that the overall mass change was very similar for males and females in most treatments (Fig. 2) even though females spent significantly more time feeding on the carcass (Fig. 1). An alternative explanation is that females appear to be spending more time feeding but instead they are making the carcass more accessible to the larvae. In this species, the larvae obtain some of their food by self-feeding from the day of hatching (Smiseth et al., 2003), and parents may enhance the larvae's ability to self-feed by cutting it open. It is not possible to discriminate between feeding and cutting the carcass open during behavioural observations. Another plausible explanation for the difference between the results for female feeding behaviour and mass change is that females incurred high energetic costs during egg production and laying, and they were consuming carrion to compensate for this initial energy cost. We cannot differentiate between these explanations based on the results from our experiment.

Overall, we found some evidence for sealed-bid decisions, as parents adjusted their consumption of carrion based on cues that were independent of their partner's behaviour: their own size (males and females) and their partner's size (males). However, we also found some evidence for matching, as females gained more mass when their partner gained more mass. The resolution of conflict over feeding from a shared resource is thus mediated through both matching and sealed-bid responses in this species. Interestingly, two recent papers on *N. vespilloides* showed that parents resolve conflict over parental care contributions using negotiation and sealed bids (Mattey & Smiseth, 2015; Pilakouta, Richardson, & Smiseth, 2015). Our findings suggest that, even within the same species, different mechanisms may be involved in mediating the resolution of different forms of sexual conflict. We encourage researchers to explore whether this might also be the case in other taxa.

Lastly, our results raise interesting questions as to whether sexual conflict over consumption from a shared resource could influence sexual conflict over contributions to parental care. For example, if a parent is prevented from feeding by a physically superior partner, it may retaliate by providing less care. On the other hand, if a parent is providing a disproportionate amount of care, its partner may be more tolerant of that parent feeding more from the resource. We are not aware of any studies investigating how the resolution of one type of sexual conflict may interact with the resolution of a different type of conflict in the same system. Such interactions might be expected whenever there are multiple types of sexual conflict occurring either simultaneously or sequentially over the reproductive bout, and we encourage future research to address this gap.

In summary, this study provides novel insights into the resolution of a previously ignored form of sexual conflict by showing that parents use information on their partner to decide how much food to consume from a shared resource. These adjustments in feeding are directly related to the parents' future reproductive potential, as consumption of the breeding resource serves as a proxy for investment in future reproduction (Billman et al., 2014; Creighton et al., 2009). Our findings also suggest that parents use different mechanisms for the resolution of different forms of sexual conflict, and they raise the possibility of interactions between different sources of conflict occurring over the breeding attempt.

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Effects of Prior Contest Experience and Contest Outcome on Female Reproductive Decisions and Offspring Fitness

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ABSTRACT: Winning or losing a prior contest can influence the outcome of future contests, but it might also alter subsequent reproductive decisions. For example, losers may increase their investment in the current breeding attempt if losing a contest indicates limited prospects for future breeding. Using the burying beetle *Nicrophorus vespilloides*, we tested whether females adjust their prehatching and posthatching reproductive effort after winning or losing a contest with a same-sex conspecific. Burying beetles breed on carcasses of small vertebrates for which there is fierce intrasexual competition. We found no evidence that winning or losing a contest influenced reproductive investment decisions in this species. Instead, we show that a female's prior contest experience (regardless of its outcome) influenced the amount of posthatching care provided, with downstream consequences for the female's reproductive output; both winners and losers spent more time provisioning food to their offspring and produced larger broods than females with no contest experience. We discuss the wider implications of our findings and present a conceptual model linking contest-mediated adjustments in parental investment to population-level processes. We propose that the frequency of intraspecific contests could both influence and be influenced by population dynamics in species where contest experience influences the size and/or number of offspring produced.

Keywords: fighting contest, *Nicrophorus vespilloides*, parental care, population density, reproductive investment, winner-loser effects.

Introduction

Animals commonly fight over mates, territories, food, and other resources. Winner-loser effects occur when an individual's prior experience with a fighting contest influences the outcome of its subsequent contests. Previous work in a wide range of taxa has shown that the winner of a contest is more likely to win a future fight, whereas the loser is more likely to lose again (Chase et al. 1994; Safryn and Scott 2000; Hsu et al. 2006; Rutte et al. 2006; Fawcett and Johnstone 2010; Kasumovic et al. 2010). Two recent studies have challenged this idea by showing that both winners and losers have increased fighting success in future contests (Benelli et al. 2015*a*, 2015*b*). This suggests that prior experience with a fight, regardless of its outcome, may give individuals an advantage over inexperienced individuals.

Despite an extensive literature on winner-loser effects, little is known about the wider implications of winning or losing a fight beyond an effect on success in future fights. For example, the outcome of a contest may influence an individual's subsequent reproductive decisions by providing information about its size and condition relative to its competitors (Hsu and Wolf 2001; Walling et al. 2008; Okada et al. 2010). We are aware of only one study that has investigated the effects of contest outcome in the context of reproduction. Okada et al. (2010) compared male flour beetles (*Gnatocerus cornutus*) that lost or won a prior contest and found that losers transferred more sperm during a subsequent copulation compared to winners. Although this study shows that losing a contest can affect sperm allocation in males, there is no information as to whether contest outcome may also influence decisions over parental effort. If losing indicates limited prospects for success in future breeding attempts, a loser might increase its parental effort in the current breeding attempt, thereby altering both its own fitness and the fitness of its offspring. Alternatively, individuals might base their reproductive investment decisions on whether they have participated in a fighting contest rather than whether they won or lost such a contest. This could occur when encounters with conspecific competitors serve as a cue for the intensity of competition in the population and therefore the likelihood of future breeding opportunities.

Our study addresses this gap in our knowledge using the burying beetle *Nicrophorus vespilloides*, which breeds on carcasses of small vertebrates. A number of attributes make

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this species particularly well suited for studying how contest outcome and contest experience might affect parental care decisions. First, there is fierce intrasexual competition over the possession of a carcass, which is an ephemeral, high-value resource (Safryn and Scott 2000). Body size is the strongest determinant of the outcome of these contests, with larger beetles being more successful at both acquiring and defending a carcass (Bartlett and Ashworth 1988). A study on the related *Nicrophorus humator* reported evidence for winner-loser effects, as the outcome of a prior contest affected the likelihood of success in subsequent contests (Otronen 1990). Second, *N. vespilloides* females (sometimes assisted by a male) provide elaborate parental care that enhances larval growth and survival (Eggert et al. 1998; Smiseth and Moore 2002; Smiseth et al. 2003; Pilakouta et al. 2015*a*). Prehatching care includes preparation of the carcass and investment of nutrients in eggs (Rozen et al. 2008; Monteith et al. 2012), while posthatching care includes brood defense, secretion of antimicrobials, and food provisioning (Eggert et al. 1998; Smiseth et al. 2003; Rozen et al. 2008). Last, there is evidence for a trade-off between investment in current and future reproduction in *N. vespilloides* and the related *Nicrophorus orbicollis*: females that overproduce offspring in the first breeding attempt suffer a reduction in fecundity in future breeding attempts (Creighton et al. 2009; Ward et al. 2009; Billman et al. 2014).

The aim of our study was to investigate whether females adjust their parental effort in the current brood depending on whether they won or lost a prior contest. We focused on female reproductive decisions because females are more involved in care and stay on the carcass longer than males (Fetherston et al. 1994; Eggert et al. 1998; Smiseth and Moore 2002; Rauter and Moore 2004; Smiseth et al. 2005). Furthermore, there is no evidence that the additional presence of a male has a positive effect on larval growth or survival under laboratory conditions (Smiseth et al. 2005). To avoid a possible confounding effect of body size on the reproductive decisions of females (Steiger 2013), we compared medium-sized females with no fighting experience (controls) with medium-sized females that either won or lost a prior contest to small or large females, respectively. Although we hypothesized that the outcome of a prior contest would influence subsequent reproductive decisions, females might also adjust their reproductive investment simply due to their experience with a contest, regardless of its outcome. We thus used an experimental design that would allow us to disentangle the potential effects due to experience with a prior contest and the outcome of that contest (see “Methods”).

We first tested whether females adjusted their prehatching investment (egg number and egg size) and posthatching investment (amount of direct care and amount of indirect care) based on contest outcome or contest experience. We then tested whether any adjustments in investment had fitness consequences for the mother by measuring brood size (a measure of reproductive output) and maternal postbreeding longevity (a measure of residual reproductive value). Last, to determine whether contest outcome or contest experience had consequences for offspring fitness, we measured larval begging rate during early development and average larval mass at the dispersal stage, which corresponds to the end of the parental care period. We predicted that losers would increase their investment in current reproduction and consequently suffer higher mortality after breeding. As a result of such an increase in parental investment, we also expected that losers would have more and/or larger offspring at the end of the parental care period.

Methods

We used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. The beetles used in this study comprised fourth-, fifth-, and sixth-generation beetles from lines originally collected in Edinburgh, United Kingdom, and Warmond, the Netherlands. All adult beetles were housed individually in transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil and kept at 20°C and constant light (Mattey and Smiseth 2015*a*). All nonbreeding adults were fed small pieces of raw organic beef twice a week.

Experimental Design

Given that the outcome of contests over the possession of a carcass is largely determined by body size (Bartlett and Ashworth 1988) and that larger females provide better care to their offspring (Steiger 2013), it was essential to use an experimental design that would allow us to separate effects due to winning or losing a contest from effects due to body size. To this end, we compared medium-sized females that had different experiences from a prior contest; these medium-sized females competed with either larger or smaller females and thus lost or won the contest, respectively. This design excludes confounding effects of focal female size but not of competitor size, and it does not separate the effects of contest experience from effects of merely encountering a conspecific.

Because adult body size is determined by larval mass at the dispersal stage (Bartlett and Ashworth 1988; Lock et al. 2004), it is possible to generate different-sized beetles by removing larvae from the carcass at various times after hatching (Steiger 2013; Pilakouta et al. 2015*b*, 2016*b*). Thus, for each of 100 broods, we removed third-instar larvae weighing 80–100, 140–160, and 200–220 mg to generate small, medium-sized, and large adults, respectively. We kept each of these larvae in individual containers (12 cm × 8 cm × 2 cm) filled with moist soil until sexual maturity. After eclosion, we measured the pronotum length of all females to confirm that adults from the three groups differed in body size. As intended, there were clear differences in the mean (± SD) pronotum length (mm) for the three groups: 2.89 (± 0.14) for small females, 3.65 (± 0.14) for medium-sized females, and 4.30 (± 0.11) for large females.

We then set up contests over the possession of a carcass by pairing medium-sized females with either a small or large female competitor, which provided us with winners and losers of the same size. We expected the medium-sized female to win the contest if she was paired with a small female, whereas we expected her to lose the contest if she was paired with a large female. We only used females that had been sexually mature for up to 2 weeks (i.e., 10–24 days after eclosion), because female age has been shown to influence contest outcome in the closely related *Nicrophorus orbicollis* (Trumbo 2012). At the start of the experiment, we transferred pairs of females to transparent plastic containers (17 cm × 12 cm × 6 cm) with 1 cm of moist soil and a freshly thawed mouse carcass of a standardized size (20–22 g). We left the pairs undisturbed for 3 days, at which point we determined the winner by checking which beetle was present on the carcass (Safryn and Scott 2000; Trumbo 2012). In the vast majority of cases, the outcome of these contests was consistent with what we expected. However, when a medium-sized female won the contest to a large female or a medium-sized female lost the contest to a small female, she was excluded from the rest of the experiment. For the next part of the experiment, we allowed winners to breed on the mouse they had successfully competed for to mimic the outcome of winning a contest in the wild. In contrast, we prevented losers from breeding on the same mouse that was used for the contest, because losers would be driven away by the winner under natural conditions. Allowing losers to breed on the same mouse would have introduced uncertainty as to whether the female perceived herself to be the winner or loser of the contest. We therefore transferred losers to a new container (17 cm × 12 cm × 6 cm) with 1 cm of moist soil and a new mouse of the same size (20–22 g). Winners and losers were mated with an unrelated virgin male immediately following the contest—that is, 3 days after they were paired up with a small or a large female, respectively.

In addition to the winner and loser treatments, we added two control treatments of medium-sized females that had no prior experience with a contest. Because losers had been exposed to two carcasses and winners had been exposed to only one, one of the control treatments matched the winner treatment, while the other control treatment matched the loser treatment. To this end, winner-control females were exposed to only one carcass, and a male was added to the box 3 days later. Meanwhile, loser-control females were exposed to two carcasses; they stayed on the first carcass for 3 days, at which time they were transferred to a different box with a new carcass of the same size and an unrelated virgin male. We also collected data on the females' prebreeding mass to be added as a covariate in our statistical models. For females exposed to only one carcass (winners and winner-controls), we measured prebreeding mass before placing the females on the carcass. For females exposed to two carcasses (losers and loser-controls), we measured prebreeding mass before placing the females on the second carcass, which was the one they bred on. The total sample size for this experiment was $n = 224$ females, and the sample size for each treatment was as follows: $n = 56$ for loser females, $n = 57$ for loser-control females, $n = 58$ for winner females, and $n = 53$ for winner-control females.

To test for effects of contest outcome on prehatching reproductive effort, we recorded the number of eggs laid by each female and measured average egg size in each clutch. To do this, we checked the containers twice a day after mating for the presence of eggs. When the first eggs were laid, we removed the male to exclude any effects of male presence on the female's posthatching reproductive decisions. Immediately before hatching, we scanned the bottom of each container using a CanoScan 9000F Mark II scanner (Canon, Tokyo) to calculate egg size (Ford and Smiseth 2016). For each image, we measured the length and width of six randomly selected eggs in pixels using ImageJ (Abramoff et al. 2004; Monteith et al. 2012). We then converted these measurements to metric length (mm) and calculated a prolate spheroid volume V for each egg using the equation $V = (1/6)\pi w^2 l$, where w is the width and l is the length of the egg (Berrigan 1991).

To test for effects of contest outcome on posthatching reproductive effort, we conducted behavioral observations for a random subset of broods ($n = 15$ for loser females, $n = 15$ for loser-control females, $n = 17$ for winner females, and $n = 18$ for winner-control females). This was done 24 h after the first larva hatched in each brood, as this stage in larval development corresponds to the peak in posthatching care in this species (Smiseth et al. 2003). We used instantaneous sampling every 1 min for 30 min in accordance with established protocols (Smiseth and Moore 2002; Mattey and Smiseth 2015*b*; Pilakouta et al. 2015*b*, 2016*b*). We recorded the number of scans that a female spent providing (i) direct care, defined as food provisioning to the larvae (i.e., mouth-to-mouth contact with at least one larva) or interacting with the larvae (i.e., inside or around the crater and allowing larvae to beg), and (ii) indirect care, defined as carcass maintenance (i.e., deposition of secretions to the surface of the carcass or excavation of the crypt) or guarding (i.e., standing still in a position where it could defend the brood from predators or interspecific competitors). We also measured the larvae's begging rate as a proxy for offspring condition during early development (Smiseth and Moore 2004). To this end, we recorded the number of larvae begging at each scan, defined as larvae raising their head toward the parent while waving their legs or touching the parent with their legs (Smiseth and Moore 2002). From these data, we calculated an average begging rate for each brood, adjusting for brood size and the amount of time the parent spent in proximity to the larvae, given that larvae only beg in the presence of a parent (Rauter and Moore 1999; Smiseth and Moore 2002). This rate was based on the equation $b_i = \sum b/L \times 100/p$, where b_i is the percentage of time spent begging by each larva in the brood, $\sum b$ is the total number of larval begging events counted during the 30 scans of an observation session, L is the brood size, and p is the number of scans where the parent was in close proximity to the larvae (Smiseth and Moore 2002, 2004). At the end of the 30-min observation, we counted the number of larvae and weighed the whole brood. The larvae were returned to the carcass, and the female was allowed to care for the brood undisturbed until the larvae dispersed from the carcass about 4 days later.

At dispersal from the carcass, we recorded the size of the brood and total brood mass. We calculated average larval mass by dividing the total mass of the brood by the number of larvae in that brood. Brood size was used as a measure of the female's reproductive output. Last, females were transferred to individual containers filled with moist soil and were checked twice a week until death to record their postbreeding longevity, which is a measure of residual reproductive value (Boncoraglio and Kilner 2012).

Data Analysis

Raw data are available from the Dryad Digital Depository: http://dx.doi.org/10.5061/dryad.fq22f (Pilakouta et al. 2016*a*). All analyses were performed using R, version 3.2.0. We used general linear models for traits with a normal distribution (egg size, larval begging rate, average larval mass at dispersal, and postbreeding longevity) and generalized linear models for traits with a Poisson distribution (amount of direct and indirect care) or a negative binomial distribution (egg number and brood size at dispersal). Correlations between all of our response variables are provided in table A1.

Our initial hypothesis was that the outcome of a prior contest would influence female reproductive decisions. Nevertheless, it was also possible that breeding females would adjust their reproductive investment simply due to the experience of a prior contest, regardless of its outcome. To disentangle potential effects due to experience with a contest and the outcome of that contest, all starting models included the following three factors: (i) prior contest experience, which compares the winners and losers to the controls (contest experience vs. no contest experience); (ii) number of mice encountered, which compares the winners and winner-controls to the losers and loser-controls (one mouse vs. two mice); and (iii) the interaction between these two factors, which reflects the effect of contest outcome (winning vs. losing). We also added female prebreeding mass and age at the time of mating as covariates in the models for egg number, egg size, brood size at dispersal, and larval mass at dispersal. The models for the behavioral data (direct care, indirect care, and larval begging rate) included carcass size, brood size, and average larval mass at the time of the observation as covariates, because parents might adjust the amount of care they provide based on the size of the carcass, the brood size, and the developmental stage of the larvae (Smiseth et al. 2003). Last, we included female age at the dispersal stage in the model for female postbreeding longevity. Decisions on whether to include any of the covariates in the final models were based on the lowest Akaike information criterion score.

Results

Does Contest Experience or Outcome Influence Prehatching or Posthatching Effort?

We found no evidence that prior experience with a contest or the outcome of that contest influenced prehatching in-

Table 1: Effects of contest experience (yes or no), the number of mice encountered by the female (one or two), and the interaction between these two factors (reflecting the effect of contest outcome) on female prehatching and posthatching investment

	Contest experience		No. mice		Interaction	
	LR χ^2_1	*P*	LR χ^2_1	*P*	LR χ^2_1	*P*
Prehatching investment:						
Egg number	.05	.82	.41	.52	.47	.50
Egg size	1.06	.30	.01	.92	.28	.60
Posthatching investment:						
Amount direct care	52.77	**<.0001**	.55	.46	.48	.49
Amount indirect care	.42	.52	3.21	.07	.29	.59

Note: LR = likelihood ratio. Statistically significant *P* value (<.05) is shown in boldface.

vestment, as measured by egg number and egg size (tables 1, A2). However, prior experience with a contest had a significant effect on posthatching investment, with winners and losers providing more direct care (food provisioning and interactions with the larvae) than control females (tables 1, A2; fig. 1*A*). There was no additional effect of the outcome of the contest on posthatching investment (see "Interaction" in table 1); winners and losers spent a similar amount of time providing direct care to the developing larvae (table A2; fig. 1*A*). There were no effects of either contest experience or contest outcome on the amount of time females spent providing indirect care (tables 1, A2). However, females provided more indirect care when breeding on smaller carcasses (likelihood ratio [LR] $\chi^2_1 = 6.25$, $P = .01$) and when the larvae were larger at the time of observation (LR $\chi^2_1 = 21.0$, $P < .0001$). There were no effects of the number of mice encountered on either prehatching or posthatching investment (table 1).

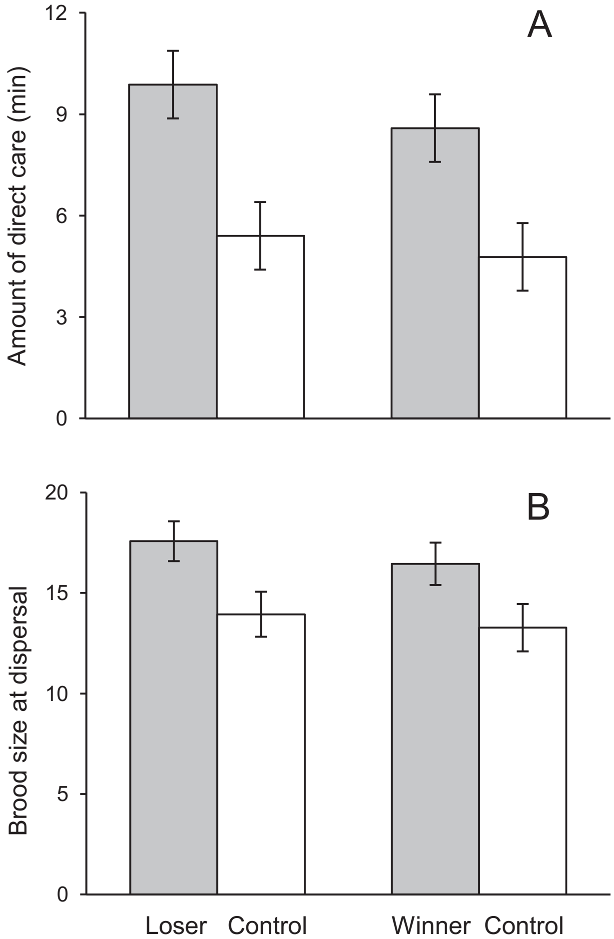

Figure 1: Amount of direct care (*A*) and brood size at dispersal (*B*) for females that either lost or won a prior contest and their corresponding controls. Error bars indicate SE. Losers and winners provided more direct care to their offspring (*A*) and had larger broods (*B*) than control females, which had no prior contest experience. There was no significant difference between losers and winners with respect to direct care (*A*) or brood size at dispersal (*B*).

Does Contest Experience or Outcome Have Fitness Consequences for Females or Their Offspring?

Prior experience with a contest had a significant effect on the reproductive output of females, as winners and losers had larger broods at the dispersal stage than control females (table 2; fig. 1*B*). However, brood size was not influenced by the number of mice encountered by a female or the outcome of the contest (i.e., the interaction between number of mice and contest experience; table 2; fig. 1*B*). Female postbreeding longevity was not influenced by prior experience with a contest, the outcome of that contest, or the number of mice encountered by the female (tables 2, A2).

With respect to offspring fitness, none of the main factors had a significant effect on larval begging rate, but larger larvae spent more time begging than smaller larvae (LR $\chi^2_1 = 11.2$, $P < .001$), and there was a nonsignificant trend for larvae to beg more on larger carcasses (LR $\chi^2_1 = 3.62$, $P = .06$). Similarly, neither contest experience nor contest outcome had a detectable effect on average larval mass at dispersal (tables 2, A2; fig. 2*A*). However, females that had encountered two mice (losers and loser-controls) produced heavier offspring than females that encountered one mouse (winners and winner-controls). This pattern may have been driven by differences in prebreeding mass between these females (fig. 2*B*), given the correlation between the mother's prebreeding mass and the offspring's average larval mass (Pearson correlation: $r = 0.31$, $P < .0001$). Female prebreeding mass (LR $\chi^2_1 = 4.65$, $P = .03$) as well as female age (LR $\chi^2_1 = 4.88$, $P = .03$) were included in the final model for average larval mass. Because of the correlation between female prebreeding mass and number of mice encountered (fig. 2*B*), we tested for multicollinearity in this model. We estimated variance inflation factors using the vif function in the car package (Fox and Weisberg 2011). The largest variance inflation factors were <2, indicating absence of multicollinearity.

Discussion

Here, we tested the hypothesis that females increase their investment in current reproduction after losing a contest with a larger competitor and that such adjustments in investment alter the female's own fitness and the fitness of her offspring. We found no evidence to support this hypothesis, as contest outcome had no effect on either prehatching or posthatching investment (fig. 1*A*) and had no fitness consequences for either the female or her offspring (figs. 1*B*, 2*A*). Instead, our key finding was that experience

Table 2: Effects of contest experience (yes or no), the number of mice encountered by the female (one or two), and the interaction between these two factors (reflecting the effect of contest outcome) on the mother's reproductive output (brood size) and residual reproductive value (postbreeding longevity), offspring condition during early development (larval begging rate), and offspring size at the end of the parental care period (larval mass at dispersal)

	Contest experience		No. mice		Interaction	
	LR χ^2_1	*P*	LR χ^2_1	*P*	LR χ^2_1	*P*
Consequences for mother:						
Brood size at dispersal	8.01	**<.01**	<.01	>.99	.08	.78
Postbreeding longevity	1.34	.25	.49	.48	.01	.91
Consequences for offspring:						
Larval begging rate	.92	.34	1.36	.24	.02	.89
Larval mass at dispersal	.83	.36	10.88	**<.01**	2.27	.13

Note: LR = likelihood ratio. Statistically significant *P* values (<.05) are shown in boldface.

with a prior contest, regardless of its outcome, influenced the subsequent reproductive decisions of females, thus altering their reproductive output. Both winners and losers spent almost twice as much time providing direct care (food provisioning and interactions with larvae) than females with no contest experience, and they had larger broods at the dispersal stage. Our findings are in line with two recent studies on winner-loser effects showing that contest experience might be more important than contest outcome in determining fighting success in future contests (Benelli et al. 2015*a*, 2015*b*).

One potential explanation for why both winners and losers increased their investment in the current brood is that females use the presence or absence of conspecific competitors as an indicator of the intensity of competition over limited breeding resources in the population. For example, if the absence of conspecific competitors indicates a low population density, control females may have invested less in their current brood to take advantage of additional breeding opportunities in the future (McNamara et al. 2009). An alternative explanation is that involvement in a contest might have resulted in injuries given the fierce competition over carcasses in *Nicrophorus* (Trumbo 1991; Cotter et al. 2011), and injured females might have increased their investment in the current brood due to a higher risk of infection. A previous study on *Nicrophorus vespilloides* found that immunologically challenged females produced heavier broods than control females (Cotter et al. 2011). Nevertheless, we observed visible injuries in the form of missing antennae or legs only in one loser, and we never observed such injuries in any of the winners. Thus, the most likely explanation for the observation that winners and losers increased their investment in current reproduction is that they responded to the presence of a competitor, which served as a cue about the intensity of competition in the population.

As a result of this increased investment, winners and losers produced more offspring at the dispersal stage than control females. To our knowledge, this is the first evidence that a female's prior experience with a contest influences her reproductive output by altering her subsequent parental investment decisions. On the other hand, we found no evidence for an effect of either contest experience or contest outcome on the offspring's fitness. This result was somewhat surprising given that larval mass determines adult size, which in turn determines the likelihood of acquiring a carcass for breeding (Bartlett and Ashworth 1988; Safryn and Scott 2000; Lock et al. 2004). Thus, we might have expected an adjustment in offspring size by females that had prior contest experience, due to an anticipatory maternal response to the intense levels of competition at high population density (Creighton 2005). Instead, we found that offspring size depended on the number of mice females had encountered. Losers and loser-controls, which had encountered two mice, produced larger larvae compared to winners and winner-controls, which had encountered only one mouse. This pattern might be driven by the higher mass of losers and loser-controls (fig. 2*B*), which had access to an additional carcass before being placed on the mouse they eventually bred on. Mouse carcasses are a highly nutritional food resource for burying beetles, and parents feed on the carcass before and during a breeding attempt to replenish their energy reserves (Pilakouta et al. 2016*b*). If losers and loser-controls had more energy reserves at the start of their breeding attempt, they might have consumed less of the second carcass, leaving more food for the larvae.

Overall, our results show that females with prior contest experience invest more in current reproduction and produce larger broods, but they do not adjust their offspring's size. This finding is in contrast to studies in other species showing that mothers produce fewer but larger offspring at high densities (Both 2000; Creighton 2005; Goubault et al. 2007; Plaistow et al. 2007; Allen et al. 2008; Leips et al. 2009). Thus, there is mixed empirical evidence with respect to how females adjust the number versus the size of their offspring in response to intense levels of competition. This highlights the

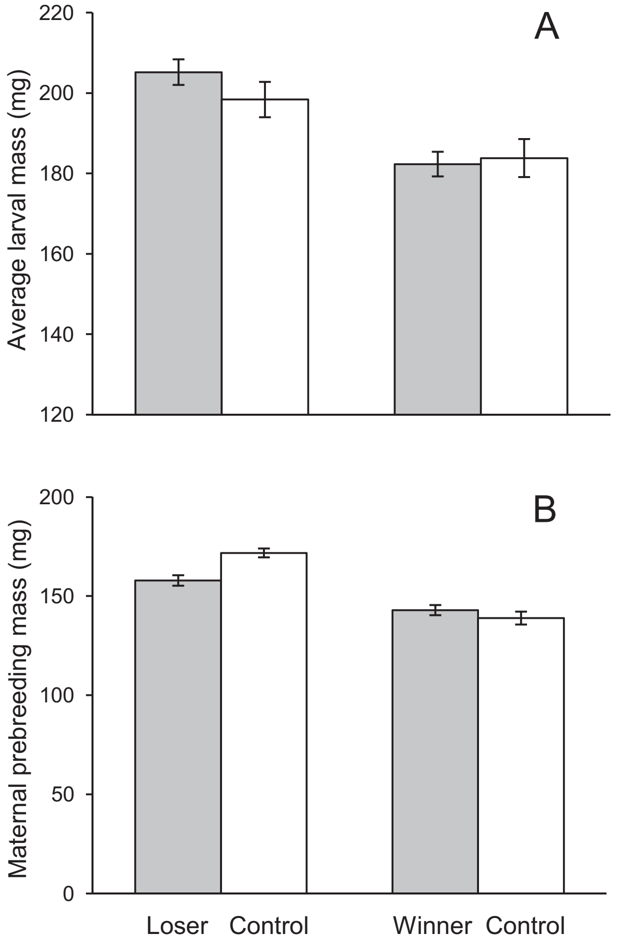

Figure 2: Average larval mass (*A*) and maternal prebreeding mass (*B*) for females that either lost or won a prior contest and their corresponding controls. Error bars indicate SE. For females exposed to only one carcass (winners and winner-controls), we measured prebreeding mass before placing the females on the carcass. For females exposed to two carcasses (losers and loser-controls), we measured prebreeding mass before placing the females on the second carcass, which was the one they bred on.

need for further work on this topic, given that different scenarios for how females respond to high population density could have divergent consequences for population dynamics.

To illustrate this issue, we propose a conceptual model based on the simple assumption that variation in population density determines the intensity of intraspecific competition over resources used for breeding (Creighton 2005). When population density is low, there will be little competition over resources. Under these conditions, most breeding individuals will have no experience with a prior contest, in which case they may show reproductive restraint because they have a relatively high reproductive potential. Conversely, when population density is high, the majority of breeding individuals will encounter competitors, leading to potential adjustments in their subsequent reproductive decisions. If females produce more offspring of the same size by increasing their investment in current reproduction, a greater number of offspring will be recruited into the breeding population at high densities. On the other hand, if females produce fewer but larger offspring without increasing their overall investment in the current brood, a smaller number of offspring will be recruited into the breeding population at high densities. Given that offspring recruitment into the breeding population is inextricably linked to population growth, these interactions could create a feedback loop between population density, intraspecific competition, investment in current reproduction, and offspring recruitment into the population.

Such feedback loops could occur in any species where there is size-dependent competition over resources and parents make reproductive investment decisions based on cues about the population density. Evidence for density-dependent adjustments in the number and/or size of offspring has been documented in a variety of taxa, including birds, fishes, insects, and aquatic invertebrates (Both 2000; Creighton 2005; Goubault et al. 2007; Plaistow et al. 2007; Allen et al. 2008; Leips et al. 2009; Rauter et al. 2010). In most of these studies, parents produced fewer, larger offspring at high densities and more, smaller offspring at low densities. Under this scenario, we would expect a negative feedback loop, where the population density fluctuates around a stable equilibrium. In contrast, if parents produce more offspring (of the same size) at high densities, as we found here, this would lead to a positive feedback loop. These two scenarios focus on how parents might adjust their investment during the current breeding attempt, but these adjustments might also have implications for future reproduction, which will in turn contribute to population dynamics. Parents that increase their overall investment in current reproduction are expected to suffer future costs in the form of a smaller second brood (Creighton et al. 2009; Ward et al. 2009; Billman et al. 2014), whereas there might not be any future costs associated with merely adjusting the trade-off between number and size of offspring. Theoretical modeling and long-term field studies are now needed to better understand these dynamics. A suitable data set would provide multigeneration information on parental investment patterns (e.g., incubation time or nestling provisioning rate for birds), clutch size, offspring size, offspring recruitment into the population, and estimated population size.

In summary, we propose that in species where contest experience mediates parental adjustments in the size and/or number of offspring, the frequency of intraspecific contests could both influence and be influenced by population dynamics. This potential link between individual-level behavioral changes and population-level processes has so far been overlooked in the literature. More generally, previous studies have largely ignored the wider fitness consequences of contest experience and contest outcome beyond an effect on success in subsequent fights. Our finding that contest experience can alter a female's reproductive decisions highlights the need for further empirical work in this area. Even though we did not find evidence for an effect of contest outcome on reproductive investment in this study, such an effect may still exist in other systems, making this an interesting question for future studies to explore. Last, although our study focused on parental investment, participation in a fighting contest might influence a variety of other behaviors and life-history trade-offs, and we encourage future research to consider these effects.

Conclusion

This study provides the first evidence that a female's experience with a prior contest can have consequences for her reproductive output through an adjustment in parental care. Winners and losers spent more time providing care to their offspring than females with no contest experience, resulting in larger broods at the end of the parental care period. In species where parents adjust their reproductive strategies based on their contest experience and where these adjustments affect the number and/or size of their offspring, contests could influence and be influenced by population dynamics, leading to a feedback loop between local-scale social interactions, individual-level behavioral changes, and population-level processes.

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APPENDIX

Supplementary Tables

Table A1: Correlation matrix for all response variables

	Egg number	Egg size	Direct care	Indirect care	Larval begging rate	Brood size	Average larval mass
Egg size	$r = .15$ $P = .12$						
Direct care	$r = .03$ $P = .92$	$r = -.40$ $P = .20$					
Indirect care	$r = .82$ **$P = .001$**	$r = .29$ $P = .36$	$r = .05$ $P = .66$				
Larval begging rate	$r = .60$ $P = .40$	$r = .08$ $P = .93$	$r = -.28$ $P = .07$	$r = .04$ $P = .82$			
Brood size	$r = .75$ **$P < .001$**	$r = .36$ **$P < .001$**	$r = .20$ $P = .11$	$r = .13$ $P = .32$	$r = -.03$ $P = .87$		
Average larval mass	$r = -.24$ $P = .01$	$r = .29$ $P = .05$	$r = .27$ $P = .03$	$r = .19$ $P = .15$	$r = -.05$ $P = .78$	$r = -.14$ $P = .06$	
Postbreeding longevity	$r = .212$ $P = .03$	$r = .24$ $P = .01$	$r = -.09$ $P = .53$	$r = .22$ $P = .45$	$r = .27$ $P = .16$	$r = .25$ **$P = .001$**	$r = .02$ $P = .81$

Note: Statistically significant P values are shown in boldface (based on $\alpha = 0.002$ due to Bonferroni correction).

Table A2: Means (± SE) for female prehatching investment (egg number and egg size), posthatching investment (amount of direct and indirect care), maternal reproductive output (brood size), maternal residual reproductive value (postbreeding longevity), and fitness-related offspring traits (larval begging rate and larval mass at dispersal)

	Loser	Loser-control	Winner	Winner-control
Prehatching investment:				
Egg number	17.3 ± 1.7	18.3 ± 1.5	19.1 ± 1.7	17.6 ± 1.9
Egg size (mm^3)	1.47 ± .03	1.42 ± .03	1.44 ± .03	1.43 ± .03
Posthatching investment:				
Amount direct care (min)	9.88 ± 1.90	5.40 ± 1.99	8.59 ± 1.79	4.78 ± 1.21

Table A2 (*Continued*)

	Loser	Loser-control	Winner	Winner-control
Amount indirect care (min)	4.27 ± 1.01	4.67 ± 1.08	5.41 ± 1.05	4.78 ± .93
Consequences for mother:				
Brood size at dispersal	17.6 ± 1.0	13.9 ± 1.1	16.4 ± 1.1	13.3 ± 1.2
Postbreeding longevity (days)	44.6 ± 2.0	42.1 ± 2.0	42.8 ± 2.1	40.8 ± 1.9
Consequences for offspring:				
Larval begging rate (%)	15.7 ± 5.1	22.7 ± 9.3	9.7 ± 3.0	14.7 ± 4.3
Larval mass at dispersal (mg)	205 ± 3	198 ± 4	182 ± 3	184 ± 5

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A female burying beetle (*Nicrophorus vespilloides*) feeding predigested carrion to a begging larva. Photo credit: Per T. Smiseth.

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Female mating preferences for outbred versus inbred males are conditional upon the female's own inbreeding status

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Nicrophorus vespilloides
sexual selection

Inbreeding occurs when relatives mate with each other, and it often has detrimental effects for the fitness of any resulting offspring. It is an important issue in ecology and evolutionary biology with profound implications for genetic variation and the evolution of mating systems and reproductive strategies. For example, inbreeding may shape mate choice through the avoidance of outbred, related individuals to prevent inbreeding, or through the avoidance of inbred, unrelated individuals that have been produced through inbreeding. Although the former has been studied extensively, little is known about mating preferences based on the inbreeding status of potential partners. It is also unclear whether these mating preferences are influenced by the inbreeding status of the choosing sex. Here, we examined female mating preferences for outbred versus inbred males using dichotomous choice tests in the burying beetle *Nicrophorus vespilloides*. We found that these mating preferences were conditional upon the female's own inbreeding status: inbred females preferentially mated with outbred males, whereas outbred females did not show such a preference. Our findings suggest that inbred males suffer reduced mating success only when interacting with inbred females. In species where this is the case, the fitness costs of inbreeding with respect to male mating success will therefore depend on the frequency of inbred females relative to outbred females, which depends on the rate of inbreeding in the population.

Inbreeding refers to the mating between close relatives and is often associated with a reduction in the fitness of any resulting offspring, known as inbreeding depression (Charlesworth & Charlesworth, 1987). These fitness costs are due to a general loss of heterozygosity, which increases the likelihood that recessive, deleterious alleles are expressed (Charlesworth & Charlesworth, 1987). Given its detrimental effects for the fitness of inbred offspring, inbreeding may influence mate choice, which is the outcome of interactions between males and females, with females usually being the choosing sex and males the competing sex (Andersson, 1994).

Inbreeding can affect mating patterns at two distinct levels. First, the costs of inbreeding may lead to mating preferences for unrelated over related individuals. Active mate choice is a key mechanism for inbreeding avoidance, whereby individuals avoid mating with relatives to reduce the risk of producing inbred offspring (Frommen & Bakker, 2006; Gerlach & Lysiak, 2006; Hansson et al., 2007). Second, females might avoid mating with inbred, unrelated partners if outbred, unrelated partners are of higher quality (Ilmonen, Stundner, Thoss, & Penn, 2009). Hence, inbreeding may shape mate choice through the avoidance of related individuals to prevent inbreeding and/or through the avoidance of low-quality individuals produced by inbreeding.

Inbreeding avoidance by active mate choice has been studied extensively across a wide range of taxa (Pusey & Wolf, 1996; Szulkin, Stopher, Pemberton, & Reid, 2013; Tregenza & Wedell, 2000). This work has focused on when and why animals may avoid, tolerate or in some cases prefer to mate with their relatives (Kokko & Ots, 2006; Szulkin et al., 2013). Relatively little is known about whether and when inbred individuals might be less preferred as potential mates, although a growing number of empirical studies in mammals, birds, fishes and insects have shown that outbred partners are typically preferred over inbred ones (Ala-Honkola et al., 2015; Bolund, Martin, Kempenaers, & Forstmeier, 2010; Ilmonen et al., 2009; McKee, Newton, & Carter, 2014; Okada, Blount, Sharma, Snook, & Hosken, 2011; Pölkki, Krams, Kangassalo, & Rantala, 2012; Zajitschek & Brooks, 2010; but see also Drayton, Milner, Hunt, & Jennions, 2010; Michalczyk, Martin,

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Millard, Emerson, & Gage, 2010). Theoretical work suggests that these preferences for outbred males are unlikely to be driven by indirect (genetic) benefits, because homozygosity is not heritable (Lehmann, Keller, & Kokko, 2007; Reinhold, 2002; but see Neff & Pitcher, 2008; Nietlisbach, Keller, & Postma, 2016). A more likely explanation is that inbreeding reduces overall male quality and condition such that females gain fewer direct benefits from mating with an inbred male (Fox, Xu, Wallin, & Curtis, 2012). Direct benefits that may be affected by inbreeding include sperm number and quality, nuptial gift size and parental care ability (Fox et al., 2012).

Traditionally, studies on female mate choice have focused only on the inbreeding status of males, giving outbred females a choice between outbred and inbred males. Nevertheless, the females' own inbreeding status might also influence their mating preferences. If low-quality females cannot afford the costs of being choosy (Burley & Foster, 2006; Cotton, Small, & Pomiankowski, 2006; Hunt, Brooks, & Jennions, 2005; Ilmonen et al., 2009; McKee et al., 2014), we might expect a stronger mating bias towards outbred males by outbred (high-quality) females than by inbred (low-quality) females. On the other hand, if the benefits gained from being choosy are inversely related to female quality, inbred females should have a stronger preference for outbred males to compensate for their own shortcomings (Ilmonen et al., 2009). It is important to better understand how inbreeding affects female choosiness since this may have important implications for sexual selection dynamics in inbred populations.

In this study, we used the burying beetle *Nicrophorus vespilloides*, to test whether females preferentially mate with outbred over inbred males and whether female choosiness is influenced by the female's own inbreeding status. Mattey and Smiseth (2015a) found no evidence for inbreeding avoidance in this species despite severe inbreeding depression in the offspring (Mattey, Strutt, & Smiseth, 2013; Pilakouta, Jamieson, Moorad, & Smiseth, 2015; Pilakouta, Sieber, Smiseth, 2016; Pilakouta & Smiseth, 2016) and heavy investment by both sexes in parental care (Pilakouta, Richardson, & Smiseth, 2015; Smiseth, Dawson, Varley, & Moore, 2005; Smiseth & Moore, 2004). Nevertheless, it is possible that females exhibit mating preferences based on the inbreeding status rather than the relatedness of potential partners. To test this, we conducted dichotomous choice tests during which we recorded the copulation rate of an outbred or inbred female presented with two potential mates, one outbred and one inbred. We predicted that females would avoid mating with inbred males, because they are low-quality mates (Mattey et al., 2013). We also expected that outbred and inbred females would differ in their choosiness, but we did not have an a priori prediction about the direction of this effect.

METHODS

Beetle Husbandry

We used virgin beetles from an outbred laboratory population maintained at the University of Edinburgh. The beetles used in this study comprised second- and third-generation beetles from lines originally collected in Edinburgh, U.K. They were housed individually in transparent plastic containers (12 × 8 cm and 2 cm high) filled with moist soil and kept at 22 °C and a 16:8 h light:dark cycle. All nonbreeding adults were fed small pieces of raw organic beef twice a week.

Generating Outbred and Inbred Beetles

In the first part of our experiment, we generated outbred and inbred males and females for use in the mate choice trials. To produce outbred individuals, we paired outbred beetles ($N = 25$) that had no common ancestors for at least two generations. To produce inbred individuals, we paired outbred beetles ($N = 25$) that were full siblings. Each pair ($N = 50$) was placed in a transparent plastic container (17 × 12 cm and 6 cm high) filled with 1 cm of moist soil. Burying beetles use carcasses of small vertebrates as a breeding resource, so we provided each of these pairs with a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, U.K.). We did not disturb them until the larvae started dispersing from the carcass, which occurs approximately 5 days after hatching. At the dispersal stage, we placed five larvae from each brood into individual containers (12 × 8 cm and 2 cm high) filled with moist soil. The inbred and outbred offspring eclosed as adults about 20 days later, at which point they were sexed based on differences in the terminal segments of the abdomen (Trumbo, 1996). We only used one female and two males from each family. We also recorded the body size of all individuals by measuring their pronotum width using a digital calliper with a precision of 0.01 mm (Bartlett & Ashworth, 1988).

Dichotomous Choice Tests

In this species, adult beetles become sexually mature around 10 days after eclosion. For our mate choice trials, we only used virgin beetles aged between 10 and 20 days after eclosion to minimize variation in male and female age and to prevent variation due to previous mating experience. Each trial consisted of a single outbred or inbred female that was given a choice between an outbred and an inbred male. This design simulates a situation where a female encounters multiple males on a carcass in the wild (i.e. simultaneous mate choice). In half of the trials, we used an outbred female ($N = 15$) and in the other half we used an inbred female ($N = 15$). The two males used in each trial were size-matched based on their pronotum width (difference <0.10 mm) to exclude differences in female mating preferences due to male size. We always used unrelated individuals in each trial.

Mate choice trials took place in a transparent container (17 × 12 cm and 6 cm high) filled with 0.5 cm of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, U.K.) of a standardized size (27–30 g). We first tethered each male by tying one end of a piece of dental floss around the male's pronotum and taping the other end to the side of the box. The two males were tethered to opposite sides of the box to prevent competition, which otherwise would restrict the female's ability to choose between them (Otronen, 1988). We tied the dental floss such that there was about 3 cm of give to ensure that we did not limit the males' ability to mount and mate with the female (Mattey & Smiseth, 2015a). Both males could reach the carcass, which was placed in the middle of the box, but they could not come in direct contact with each other. We alternated between trials whether the outbred or inbred male was tethered on the side close to the front versus the back of the carcass (Mattey & Smiseth, 2015a).

At the start of the trial, we placed the female at the centre of the carcass such that she was equidistant from the two males. We recorded the time when the female first came into contact with the outbred and the inbred male and the number of copulations she had with each male over the next 45 min. Successful copulations occurred when the male inserted his aedeagus (intromittent organ) into the female's vagina (House et al., 2008). Given that each copulation typically lasts about 90 s and females do not have a refractory period (House et al., 2008), it was possible for females to mate repeatedly with the same male or both males. All outbred and inbred females mated at least once over the course of the 45 min mate choice trial. All trials ($N = 30$) were included in the analyses described below.

Data Analysis

A female might show a preference for the outbred male either by only mating with that male or by mating with the outbred male more times than she mates with the inbred male. To examine the former scenario, we used a generalized linear model (GLM) where the response variable indicated whether a female mated with only one male or both males during the dichotomous choice test. Our two explanatory variables were female inbreeding status (outbred or inbred) and female pronotum width. This model was fitted using a binomial error distribution with a complementary log-log link function. To examine the latter scenario, we first tested for a negative correlation between the number of times the female copulated with the outbred and inbred male in each trial, which would indicate that mating with one male reduced the likelihood of mating with the other male. After confirming the absence of such a correlation (Spearman rank test: $\rho = 0.063$, $P = 0.74$), we tested whether females copulated more frequently with the outbred or the inbred male, using a generalized linear mixed model (GLMM) with a Poisson error distribution (‘glmer’ function in the ‘lme4’ package). The starting model included the following factors: male inbreeding status (outbred or inbred), female inbreeding status (outbred or inbred), female pronotum width, the male's position relative to the carcass (front or back), and whether that male was the first the female interacted with (yes or no). Female identity was added as a random effect to account for the nonindependence between the observations on the two males in the same trial. Decisions about which variables to include in the final model were based on AIC model selection criteria to obtain the minimal adequate model. After model simplification, our final model included the following factors: male inbreeding status, female inbreeding status and the interaction between male and female inbreeding status. Statistical results for factors dropped from the final model (i.e. female pronotum width, the male's position relative to the carcass and whether he was the first male the female interacted with) are the values from the full model prior to being removed. This model was fitted using maximum likelihood methods. Lastly, we used a Mann-Whitney *U* test to compare the total number of copulations by outbred and inbred females, as a measure of female mating activity or eagerness to mate. We used R version 3.3.1 (R Core Team, 2013) for all analyses.

Ethical Note

Our study adheres to the ASAB/ABS Guidelines for the Use of Animals in Research, the legal requirements of the U.K. and all institutional guidelines at The University of Edinburgh. None of the procedures used in this study had the potential to cause pain or distress to the beetles.

RESULTS

We found that female mating preferences for inbred versus outbred males were conditional upon the female's own inbreeding status (Fig. 1). Inbred females copulated more with outbred males, while outbred females showed no preference between outbred and inbred males (GLMM: male inbreeding status: LR $\chi_1^2 = 5.47$, $P = 0.02$; female inbreeding status: LR $\chi_1^2 = 0.87$, $P = 0.35$; interaction: LR $\chi_1^2 = 9.01$, $P < 0.01$). These mating preferences were not influenced by female pronotum width (GLMM: LR $\chi_1^2 = 0.15$, $P = 0.70$), whether the male was tethered to the side closest to the front or back of the carcass (GLMM: LR $\chi_1^2 = 0.54$, $P = 0.46$) or which male the female interacted with first (GLMM: LR $\chi_1^2 = 0.34$, $P = 0.56$). Inbred females were also less likely to mate with both males during the dichotomous choice test (GLM: $\chi_1^2 = 0.4.32$, $P = 0.038$; Fig. 2). Female pronotum width did not influence the likelihood of mating with both males (GLM: $\chi_1^2 = 0.42$, $P = 0.52$). Lastly, we found that outbred and inbred females were equally eager to mate, as measured by the number of total copulations they had over the course of the 45 min choice test (Mann-Whitney *U* test: $W = 81$, $P = 0.18$).

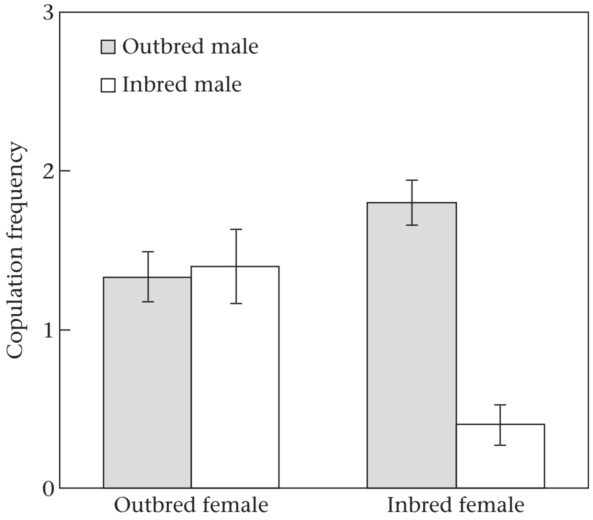

Figure 1. Mean ± SE number of times an outbred or inbred female mated with the outbred male (grey) and the inbred male (white) during a 45 min mate choice trial.

DISCUSSION

We found that female mating preferences for outbred versus inbred males were conditional upon the female's own inbreeding status: inbred females preferred outbred males over inbred males, whereas outbred females did not show a preference between outbred and inbred males. Inbred females not only copulated with the outbred male more often than with the inbred male (Fig. 1) but most inbred females also mated exclusively with the outbred male (Fig. 2). Our results highlight the potential importance of male inbreeding status as a factor influencing female choice and

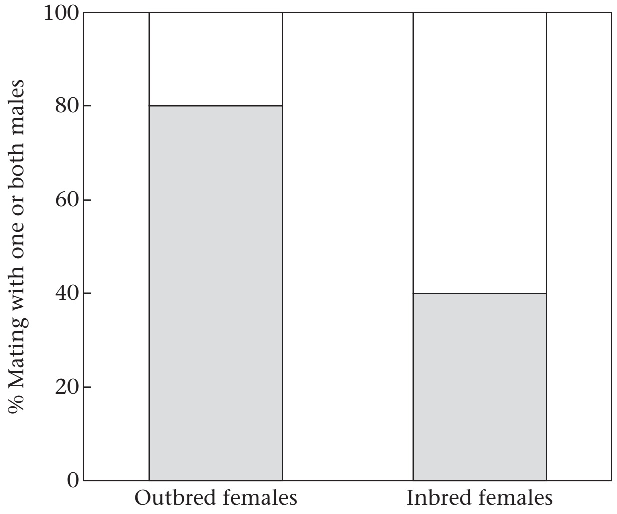

Figure 2. Percentage of outbred and inbred females that mated with only one of the two males (white) or both males (grey) over the course of the 45 min mate choice trials.

demonstrate that female choice may depend on the female's own inbreeding status. Below, we discuss possible explanations for our findings and their wider implications for female mate choice and male mating success in other species.

We found that inbred females showed a mating preference for outbred males over inbred males, whereas outbred females showed no such preference. Given that choosiness is thought to be costly (Pomiankowski, 1987), our results suggest that inbred females may be prepared to pay the costs of being choosy to gain higher marginal benefits (Bolund et al., 2010; Mazzi, Kunzler, Largiader, & Bakker, 2004). Theoretical models predict only small indirect (genetic) benefits to mating with outbred over inbred males (Lehmann et al., 2007; Reinhold, 2002), because mating with an unrelated partner restores offspring heterozygosity regardless of whether that partner is inbred or outbred. Thus, the observed preference of inbred females for outbred males is more likely to be due to direct benefits (Fox et al., 2012).

One direct benefit that plays a role in mate choice in many species is parental care (Johnstone, Reynolds, & Deutsch, 1996; Møller & Jennions, 2001). In burying beetles, males often assist the female in providing care to the offspring, by removing any fur or feathers from the carcass, applying antimicrobials to prevent bacterial and fungal growth, protecting the brood from predators and conspecifics and provisioning the larvae with predigested carrion (Arce, Johnston, Smiseth, & Rozen, 2012; Eggert, Reinking, & Müller, 1998; Pilakouta, Richardson, & Smiseth, 2015; Rozen, Englemoer, & Smiseth, 2008; Walling, Stamper, Salisbury, & Moore, 2008). However, it is unlikely that the observed preference of inbred *N. vespilloides* females for outbred males is driven by a direct benefit of paternal care. This is because the opportunity for females to choose their social partner is restricted by male–male competition over ownership of the carcass. Vertebrate carcasses suitable for breeding are relatively scarce in the wild, so it is common for multiple male and female burying beetles to arrive on a carcass at the same time, resulting in fierce intrasexual competition (Otronen, 1988). Thus, if the female's preferred mate is defeated by another male and driven away from the carcass, he will not provide any care for the resulting offspring.

Instead, it is more likely that the mating preferences we observed were driven by another type of direct benefit, such as sperm number or quality. Inbred males tend to transfer less sperm during copulations and their sperm is less motile and has more abnormalities, leading to lower fertilization success (Ala-Honkola et al., 2013; Fitzpatrick & Evans, 2014; Zajitschek, Lindholm, Evans, & Brooks, 2009). Inbred and outbred females may also produce eggs of different quality, which may be differentially affected by low- versus high-quality sperm produced by inbred and outbred males, respectively. We suggest that the lower sperm quality of inbred males might be more detrimental to fertilization success if the female is also inbred, but to our knowledge, this has not yet been tested. We encourage future research to investigate whether there is an interaction between male and female inbreeding status on fertilization success.

Although the avoidance of inbred males by inbred females might have evolved in direct response to inbreeding, another possibility is that it reflects a general response to an overall decline in condition due to inbreeding depression. Inbreeding is expected to be rare in this species, so it seems unlikely that the mating preferences we observed evolved in the specific context of inbreeding (Mattey & Smiseth, 2015b; Pilakouta, Jamieson et al., 2015). Instead, these mating preferences may be mediated through pre-existing mechanisms that evolved to serve an adaptive function in a different context. For example, females might have evolved general mating preferences for high-quality males, which may be conditional upon their own quality. All populations are potentially at risk of inbreeding in the future, given increasing habitat loss and other human-induced disturbances that increase the chances of inbreeding (Andersen, Fog, & Damgaard, 2004). Whenever species with no prior history of inbreeding depression become subject to inbreeding, the associated fitness costs may be mediated through pre-existing mechanisms that evolved outside this context (Mattey & Smiseth, 2015b; Pilakouta, Jamieson et al., 2015).

The fact that inbred females preferentially mated with outbred males suggests that females responded to a cue that differentiated inbred and outbred males, such as cuticular hydrocarbons (CHCs) or other chemical cues (Howard & Blomquist, 2005). In insects, CHCs are often used to discriminate between relatives and nonrelatives (Howard & Blomquist, 2005; Tsutsui, 2004; Weddle, Hunt, & Sakaluk, 2013). More specifically, in burying beetles, CHCs are used for partner recognition based on information about sex and breeding status (Müller, Eggert, & Elsner, 2003; Steiger, Peschke, & Muller, 2007), as well as for parent–offspring discrimination (Smiseth, Andrews, Brown, & Prentice, 2010). Females might have been under selection to differentiate between males based on their CHC profiles specifically as a mechanism to avoid mating with inbred males, or as a more general mechanism to avoid mating with males that are in poor condition. Our suggestion that female burying beetles use CHCs to discriminate between outbred and inbred males is in line with a recent study in the butterfly *Bicyclus anynana* showing that inbreeding reduces the production of a male sex pheromone, thereby allowing females to discriminate between males based on their inbreeding status (van Bergen, Brakefield, Heuskin, Zwaan, & Nieberding, 2013). Similarly, there is evidence that female discrimination between outbred and inbred males in mealworm beetles is odour-based (Pölkki et al., 2012). Given that there is a genetic basis to CHCs (Dronnet, Lohou, Christides, & Bagnères, 2006; Ferveur, 2005; Foley, Chenoweth, Nuzhdin, & Blows, 2007) and that traits with a genetic basis are prone to inbreeding (van Bergen et al., 2013), CHCs are a plausible mechanism for discrimination between outbred and inbred individuals in *N. vespilloides* and many other insects.

We believe that our findings could have important implications for male mating success in the wild. Earlier work has shown that inbred males often suffer reduced mating success (Ala-Honkola, Uddstrom, Diaz Pauli, & Lindstrom, 2009; Enders & Nunney, 2010; Joron & Brakefield, 2003; Ketola & Kotiaho, 2010; Mariette, Kelley, Brooks, & Evans, 2006; van Oosterhout et al., 2003). Here, we demonstrate that inbred females avoid mating with inbred males but outbred females do not. This suggests that inbred males suffer reduced mating success only when interacting with inbred females. We therefore propose that in species in which female inbreeding status influences mate choice for outbred versus inbred males, the fitness costs of inbreeding with respect to male mating success may be frequency dependent. In populations with high rates of inbreeding, a larger proportion of breeding females will be inbred, and we would expect inbred males to experience lower mating success than in populations with low rates of inbreeding. Such social effects on inbreeding depression in male mating success may be widespread, but their occurrence is still largely unexplored. We encourage future research to further investigate this issue, as it could have important implications for the rate and direction of sexual selection in populations that are subject to inbreeding. For example, under a scenario where inbred females are choosier than outbred females, directional selection on male sexual traits will be stronger when inbreeding rates are high than when they are low.

In summary, we have shown that a female's mating bias for an outbred versus an inbred male depends on her own inbreeding status. This is the first example of a species in which inbred females discriminate against inbred males while outbred females show no preference between inbred and outbred males. Our findings suggest that inbred females may gain more direct benefits from mating with an outbred male than outbred females do. Lastly, in species in which female inbreeding status influences mate choice for outbred versus inbred males, the fitness costs of inbreeding with respect to male mating success may depend on the frequency of inbred females relative to outbred females and thus the rate of inbreeding in the population.

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Sibling competition does not exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*

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Abstract

Inbreeding results from matings between relatives and can cause a reduction in offspring fitness, known as inbreeding depression. Previous work has shown that a wide range of environmental stresses, such as extreme temperatures, starvation and parasitism, can exacerbate inbreeding depression. It has recently been argued that stresses due to intraspecific competition should have a stronger effect on the severity of inbreeding depression than stresses due to harsh physical conditions. Here, we tested whether an increase in the intensity of sibling competition can exacerbate inbreeding depression in the burying beetle *Nicrophorus vespilloides*. We used a 2 × 3 factorial design with offspring inbreeding status (outbred or inbred) and brood size (5, 20, or 40 larvae) as the two factors. We found a main effect of inbreeding status, as inbred larvae had lower survival than outbred larvae, and a main effect of brood size, as larvae in large broods had lower survival and mass than larvae in medium-sized broods. However, there was no effect of the interaction between inbreeding status and brood size, suggesting that sibling competition did not influence the severity of inbreeding depression. Since we focused on sibling competition within homogeneous broods of either inbred or outbred larvae, we cannot rule out possible effects of sibling competition on inbreeding depression in mixed paternity broods comprising of both inbred and outbred offspring. More information on whether and when sibling competition might influence inbreeding depression can help advance our understanding of the causes underlying variation in the severity of inbreeding depression.

Introduction

Inbreeding results from matings between relatives and can cause a reduction in offspring fitness, known as inbreeding depression (Crnokrak & Roff, 1999). These negative fitness effects are due to the higher degree of homozygosity associated with inbreeding, which increases the risk that deleterious recessive alleles are expressed (Charlesworth & Charlesworth, 1987). Although there is widespread evidence for inbreeding depression in a range of taxa (Crnokrak & Roff, 1999), there is substantial variation both among and within species with respect to the severity of inbreeding depression (Crnokrak & Roff, 1999; Keller & Waller, 2002; Moorad & Wade, 2005; Meunier & Kölliker, 2013). This variation may be in part driven by differences in the physical and social environment, which can have profound effects on inbreeding depression (Fox & Reed, 2011; Reed *et al.*, 2012). For example, environmental stresses, such as extreme temperatures, starvation, parasitism and competition, can exacerbate inbreeding depression (Fox & Reed, 2011; Meunier & Kölliker, 2013), whereas parental care, which evolved to neutralize environmental stresses to the offspring, can buffer against inbreeding depression (Avilés & Bukowski, 2006; Pilakouta *et al.*, 2015a). Although there is growing evidence that physical and social stresses can alter the severity of inbreeding depression (Fox & Reed, 2011; Reed *et al.*, 2012), little is known about the mechanisms by which particular environmental stresses influence its expression.

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A recent study suggested that stresses due to intense intraspecific competition over limited resources should have a stronger effect on the severity of inbreeding depression than stresses due to harsh physical conditions, such as extreme temperatures (Yun & Agrawal, 2014). Intraspecific competition over limited resources can take several forms and can occur at different stages of the life cycle, including competition with siblings during development (Mock & Parker, 1997). Sibling competition for resources provided by the parents occurs because parents usually produce an optimistic brood size, thereby creating a mismatch between the supply of resources from the parents and the demand for resources by the offspring (Mock & Parker, 1997). Sibling competition is an important determinant of the offspring's growth and survival in many species and may therefore be a key source of environmental stress to the offspring (Mock & Parker, 1997; Roulin & Dreiss, 2012; Meunier & Kölliker, 2013). Thus, sibling competition should be associated with an increase in this mismatch between supply and demand of resources, which in turn may exacerbate inbreeding depression. To our knowledge, the only experimental study to test this hypothesis was conducted on the European earwig, *Forficula auricularia* (Meunier & Kölliker, 2013). This study found no effect of the interaction between the intensity of sibling competition and the offspring's inbreeding status on offspring fitness, suggesting that sibling competition did not influence the severity of inbreeding depression (Meunier & Kölliker, 2013). However, the absence of such an interaction effect may reflect that there was no evidence for a main effect of inbreeding status on offspring fitness during the early life stages (Meunier & Kölliker, 2013). To advance our understanding of whether sibling competition can exacerbate inbreeding depression, it is now essential to focus on species in which inbred offspring suffer a significant reduction in fitness and sibling competition negatively affects offspring fitness.

Here, we tested whether sibling competition influences the severity of inbreeding depression in the burying beetle *Nicrophorus vespilloides*. This species is well suited for addressing this question, because previous work has shown that inbred offspring suffer significant fitness costs during the larval stage (Mattey *et al.*, 2013; Pilakouta *et al.*, 2015a) and that larvae in experimentally enlarged broods suffer a reduction in fitness due to the increased mismatch between supply of and demand for resources (Smiseth *et al.*, 2007a). In this species, which breeds on carcasses of small vertebrates, larvae compete for resources by begging for predigested carrion from the parents and by self-feeding directly from the carcass (Smiseth *et al.*, 2003). Earlier work has also shown that sibling competition reduces offspring fitness only in the presence of the parents, reflecting interference competition due to successful larvae excluding their siblings from getting access to the parents (Smiseth *et al.*, 2007a,b). Although the independent effects of inbreeding status and sibling competition on offspring fitness are well established, there is no prior information on the effect of their interaction.

To test for such an interaction, we used a 2 × 3 factorial design with offspring inbreeding status and brood size as the two factors. We assessed the joint effects of inbreeding status and sibling competition on fitness traits previously shown to be affected by these two factors (Smiseth *et al.*, 2007a; Mattey *et al.*, 2013; Pilakouta *et al.*, 2015a): (i) average larval mass at dispersal; (ii) survival from hatching to dispersal; (iii) survival from dispersal to eclosion; and (iv) total survival from hatching to eclosion. If sibling competition exacerbates inbreeding depression, we would expect offspring in larger broods to incur higher fitness costs if they are inbred than if they are outbred.

Materials and methods

Study species

Like all burying beetles of the genus *Nicrophorus*, *N. vespilloides* breeds on carcasses of small vertebrates and has highly elaborate forms of parental care (Scott, 1998). Once a carcass is found, parents bury it into the soil, remove any fur or feathers, deposit antimicrobial secretions to its surface, and lay eggs around it 24–48 h after mating (Eggert, 1992; Scott, 1998). When the eggs hatch approximately 60 h later (Smiseth *et al.*, 2006), the larvae crawl to the carcass and start feeding in a crater created by the parents on the top of the carcass. The larvae can self-feed, but the parents also provision larvae with predigested carrion (Smiseth *et al.*, 2003). Although both parents typically provide care, females often stay on the carcass for longer than males and spend more time provisioning food to the larvae (Fetherston *et al.*, 1994; Eggert *et al.*, 1998; Smiseth & Moore, 2002; Rauter & Moore, 2004; Smiseth *et al.*, 2005; Pilakouta *et al.*, 2015b). Larvae disperse from the carcass about 5 days after hatching, which corresponds to the end of the parental care period. They eclose as adults about 20 days later.

Experimental design

We used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. The beetles used in this study comprised of sixth-, seventh- and eighth-generation beetles from lines originally collected in Edinburgh, UK and Warmond, the Netherlands. Beetles were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20 °C and constant light. Nonbreeding adults were fed raw organic beef twice a week.

To test for a causal effect of sibling competition on the severity of inbreeding depression, we used a 2 × 3 factorial design with offspring inbreeding status (outbred or inbred) and brood size (5, 20 or 40 larvae) as the two factors. Inbred larvae were produced by pairing males and females that were full siblings (*n* = 186), whereas outbred larvae were produced by pairing unrelated males and females that shared no common ancestors for at least two generations (*n* = 187). We only used outbred parents in this experiment as inbreeding in the parents has a negative effect on offspring survival (Mattey *et al.*, 2013). These breeding pairs (*n* = 373) were transferred to transparent plastic containers (17 × 12 × 6 cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (22–25 g). Immediately after the eggs were laid, we removed the male and moved the female and the carcass to a new container with fresh, moist soil. We removed the males because the amount of care provided by the male is highly variable and male removal has no effect on offspring fitness under laboratory conditions (Smiseth *et al.*, 2005). We left the females to provide care for the brood because previous work on this species showed that sibling competition reduces offspring fitness only when larvae compete by begging for food from a parent (Smiseth *et al.*, 2007a,b).

When the eggs started hatching, we used the newly hatched larvae to generate inbred and outbred broods comprising of 5, 20 or 40 larvae. All experimental broods included larvae of mixed maternity in accordance with established protocols (Smiseth *et al.*, 2007a, b; Pilakouta *et al.*, 2015b). This brood size manipulation is within the natural variation of brood size in *N. vespilloides* (mean ± SD: 21 ± 10 larvae, range: 2–47 larvae; Smiseth & Moore, 2002) and corresponds to small, average and large broods (i.e. low, medium and high level of sibling competition), respectively. We used a design with more than two levels of stress, because the relationship between stress and inbreeding depression may be nonlinear (Fox & Reed, 2011).

Each experimental brood (outbred or inbred) was randomly assigned to an unrelated female who had been mated either to her full-sib brother or to an unrelated male. To account for potential effects of relatedness between the female and her male partner (who was always removed before the female was provided with a foster brood), we added this information as a factor in all of our models (see below). In this species, parents cannot distinguish between unrelated foster broods and their own broods, as long as the larvae are at the same developmental stage (Müller & Eggert, 1990). As parents kill any larvae that arrive on the carcass before their eggs are expected to hatch (Müller & Eggert, 1990), we only provided females with a brood once their own eggs had hatched.

Females were left to care for their brood until the larvae dispersed from the carcass about 5 days later. At dispersal from the carcass, we recorded the number of larvae and total brood mass to calculate larval survival rate and average larval mass. Lastly, to assess survival after independence (i.e. from dispersal to eclosion), we placed all dispersing larvae from each brood into a large transparent container (17 × 12 × 6 cm) filled with moist soil. About 20 days after dispersal, we recorded the number of individuals that eclosed successfully from each brood and calculated the survival rate from dispersal to eclosion.

The total sample size in the experiment was *n* = 166 broods. The sample sizes for the different treatments were as follows: *n* = 31 for outbred broods with 5 larvae, *n* = 32 for outbred broods with 20 larvae, *n* = 22 for outbred broods with 40 larvae, *n* = 31 for inbred broods with 5 larvae, *n* = 30 for inbred broods with 20 larvae and *n* = 20 for inbred broods with 40 larvae.

Statistical analysis

Data were analysed using R version 3.2.0. Larval mass at dispersal was analysed using a linear model. Proportion data (i.e. survival rates from hatching to dispersal, from dispersal to eclosion and from hatching to eclosion) were analysed using generalized linear models fitted with a binomial distribution corrected for overdispersion. Proportion data were entered into the models using the 'cbind' function.

All models included offspring inbreeding status (outbred or inbred), brood size (small, medium or large) and the interaction between these two factors. As additional factors, we included carcass mass, the relatedness between the foster mother and her removed male partner (i.e. whether the female rearing the brood had been mated to a full sibling or an unrelated male), the interaction between foster parent relatedness and offspring inbreeding status and the interaction between foster parent relatedness and brood size. Decisions on which factors to include in the final models were based on AIC scores. For models where brood size was found to have a statistically significant effect, we used the 'glht' function in the 'multcomp' package (Hothorn *et al.*, 2008) to perform a Tukey test for *post hoc* pairwise comparisons.

Results

We found evidence for a main effect of offspring inbreeding status on survival to dispersal, survival to eclosion and total survival, as inbred larvae suffered lower survival than outbred larvae (Table 1; Fig. 1b–d). There was no significant difference in average larval mass between inbred and outbred larvae (Table 1; Fig. 1a). In addition, we found evidence for a main effect of sibling competition on offspring fitness: larvae in large broods were smaller and had a lower rate of survival to dispersal than larvae in medium-sized broods (Tables 1 and 2; Fig. 1a–b). Sibling competition also had a nonsignificant effect on total survival (Tables 1 and 2; Fig. 1d). In contrast, larvae in small broods had a lower rate of survival to eclosion than larvae in medium-sized or large broods (Table 2; Fig. 1c). We found no evidence that sibling competition exacerbated inbreeding depression, as there was no effect of the interaction between the offspring's inbreeding status and the size of the brood on any component of offspring fitness (Table 1). In addition, there was no effect of foster parent relatedness (i.e. whether the foster mother had been mated to a brother or an unrelated male) or the interaction between foster parent relatedness and offspring inbreeding status on offspring fitness (Table S1). There was an effect of the interaction between foster parent relatedness and brood size on survival to eclosion but not on larval mass, survival to dispersal or total survival (Table S1). Lastly, larvae reared on larger carcasses had a higher larval mass at dispersal and higher overall survival (Table S1).

Discussion

We find no evidence for an effect of the interaction between sibling competition and inbreeding status, suggesting that sibling competition does not exacerbate inbreeding depression in *N. vespilloides*. We show that inbreeding status negatively affected offspring fitness, as inbred larvae suffered lower survival during both the larval and pupal stages. These results demonstrate that there is significant inbreeding depression in this species as reported in previous studies (Mattey *et al.*, 2013; Pilakouta *et al.*, 2015a). Furthermore, we show that sibling competition reduced offspring fitness, as larvae in large broods were smaller and suffered lower survival to dispersal than larvae in medium-sized broods. These results confirm that the intensity of sibling competition has a negative effect on larval fitness as previously reported by Smiseth *et al.* (2007a). Even though there were significant main effects of both inbreeding status and sibling competition, there was no effect of the interaction between these two factors on offspring fitness. To our knowledge, the only other study to investigate this question (conducted on the European earwig) found no evidence for a main effect of inbreeding status on offspring fitness in the early life stages (Meunier & Kölliker, 2013). Given the absence of inbreeding depression, it was not possible to test whether inbreeding depression becomes more severe when sibling competition increases. Thus, our study is the first to show that sibling competition for resources provided by the parents does not exacerbate inbreeding depression in a species where inbred offspring do suffer substantial fitness costs.

There is accumulating evidence that the negative effects of inbreeding can be intensified by a wide range of environmental stresses, such as parasitism, starvation, population density, extreme temperatures and exposure to chemicals (Armbruster & Reed, 2005; Waller *et al.*, 2008; Fox & Reed, 2011; Reed *et al.*, 2012). Nevertheless, we still have a limited understanding of the mechanisms by which particular stresses exacerbate inbreeding depression. Yun & Agrawal (2014) argue that density-dependent stresses caused by intense competition among conspecifics should have a stronger effect on the severity of inbreeding depression than density-independent stresses caused by harsh physical conditions. In their study on *Drosophila melanogaster*, they found a moderate correlation between environmental stress and density dependence, but inbreeding depression was significantly more correlated with density dependence than environmental stress *per se* (Yun & Agrawal, 2014).

We expected that an increase in sibling competition should affect the severity of inbreeding depression because sibling competition is a density-dependent source of environmental stress caused by a mismatch between the supply and demand for resources (Mock & Parker, 1997; Roulin & Dreiss, 2012). Yet, we find that sibling competition does not exacerbate inbreeding depression in *N. vespilloides*, which appears to contradict the argument made by Yun & Agrawal (2014). One potential explanation for this discrepancy is the difference in experimental designs between our study and that of Yun & Agrawal (2014). We studied the effects of sibling competition within homogeneous broods comprised of either outbred or inbred larvae, whereas

Table 1 Effects of offspring inbreeding status (inbred or outbred) and sibling competition (small, medium-sized or large brood) on offspring fitness traits: average larval mass at dispersal (mg), survival rate from hatching to dispersal, survival rate from dispersal to eclosion and survival rate from hatching to eclosion.

	Larval mass (mg)		Survival to dispersal		Survival to eclosion		Total survival	
	F-value	*P*-value	LR χ^2	*P*-value	LR χ^2	*P*-value	LR χ^2	*P*-value
Inbreeding status	2.27	0.13	4.54	**0.03**	37.79	**< 0.0001**	17.17	**< 0.0001**
Brood size	4.93	**< 0.01**	6.72	**0.03**	12.23	**< 0.01**	6.07	**0.048**
Interaction	0.09	0.91	0.31	0.86	4.00	0.14	0.38	0.83

Larval mass data were analysed using a linear model. Survival rate data were analysed using generalized linear models fitted with a binomial distribution corrected for overdispersion. LR refers to likelihood ratio. Statistically significant *P*-values are in bold.

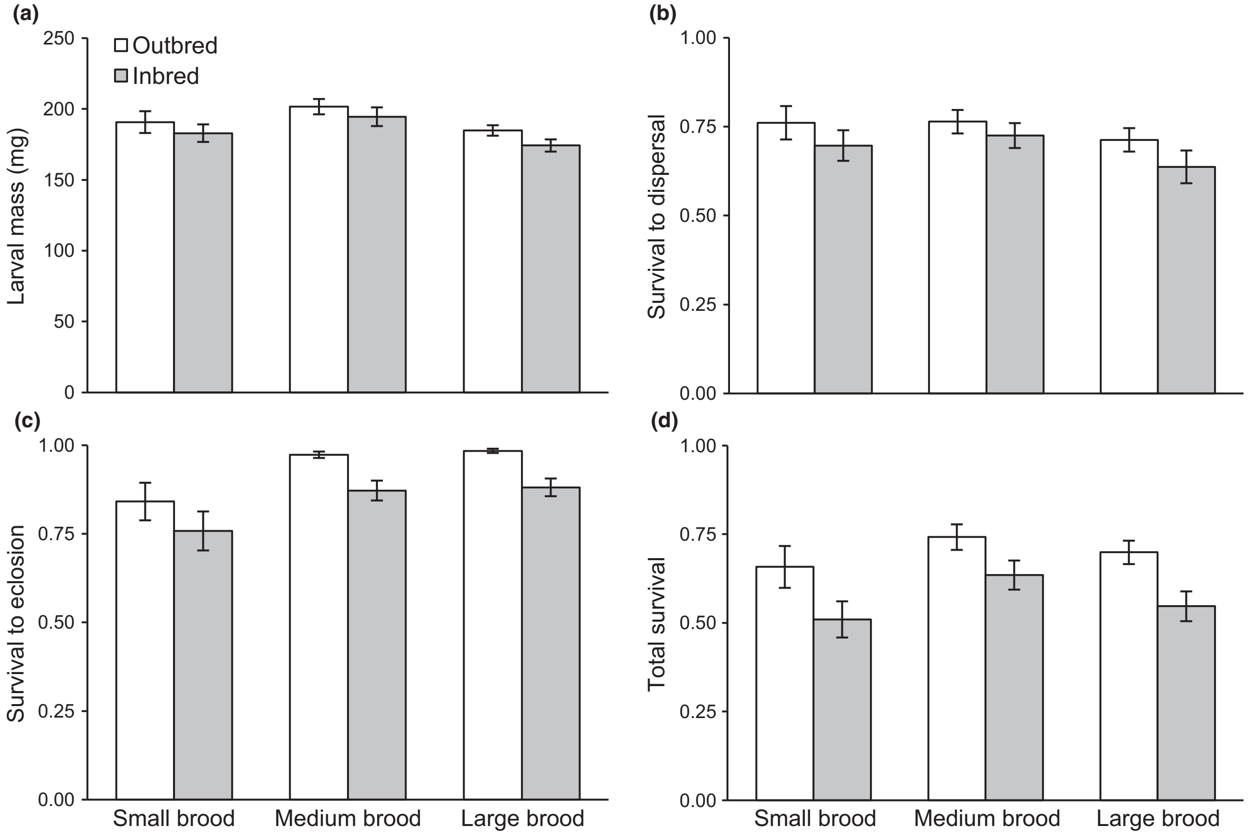

Fig. 1 Mean (± SE) average larval mass at dispersal (a), survival from hatching to dispersal (b), survival from dispersal to eclosion (c) and survival from hatching to eclosion (d) for outbred (white) and inbred (grey) larvae reared in small, medium-sized or large broods (5, 20 or 40 larvae, respectively).

Table 2 *Post hoc* pairwise comparisons (Tukey test) for the effect of sibling competition (small, medium-sized or large brood) on offspring fitness traits: average larval mass at dispersal (mg), survival rate from hatching to dispersal, survival rate from dispersal to eclosion and survival rate from hatching to eclosion.

	Larval mass (mg)				Survival to dispersal				Survival to eclosion				Total survival			
Brood size	Est	SE	*t*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*	Est	SE	*z*	*P*
Small–Medium	−12.37	5.75	−2.15	0.08	−0.10	0.26	−0.38	0.92	−1.15	0.34	−3.36	**< 0.01**	−0.49	0.25	−1.97	0.11
Small–Large	6.65	6.38	1.04	0.55	0.29	0.25	1.13	0.49	−1.13	0.33	−3.40	**< 0.01**	−0.18	0.24	−0.73	0.74
Medium–Large	19.02	6.39	2.98	**< 0.01**	0.39	0.15	2.52	**0.03**	0.02	0.27	−0.06	> 0.99	0.31	0.15	2.08	0.09

We provide information on the parameter estimates (Est), standard errors (SE), test statistics (*t*- and *z*-values) and *P*-values. Parameter estimates represent the difference when subtracting the mean fitness of larvae in the second group listed from the mean fitness of larvae in the first group on each row. Statistically significant *P*-values are in bold.

Yun & Agrawal (2014) studied the effects of competition within heterogeneous groups of unrelated inbred and outbred fruit flies. Thus, in our study, any effect of sibling competition on the severity of inbreeding depression would be mediated through an increase in the level of stress. In contrast, in the latter study, such effects would be mediated through direct competitive interactions between inbred individuals (inferior competitors) and outbred individuals (superior competitors). Homogeneous broods comprised of either outbred or inbred larvae are likely to be the norm in *N. vespilloides* given that caring males sire over 90% of the offspring in their brood (Müller & Eggert, 1989). In such homogeneous broods, all larvae may be equally disadvantaged by stresses caused by a shortage of resources. However, we cannot rule out possible effects of sibling competition on the severity of inbreeding depression in mixed paternity broods comprising of both inbred and outbred larvae. Under those conditions, higher-quality (i.e. outbred) offspring may outcompete their lower-quality (i.e. inbred) half-siblings, thus magnifying any fitness differences. We encourage future studies to investigate this question in the context of family groups that comprise of both outbred and inbred offspring, as in socially monogamous birds where the female is closely related to her social partner and has extra-pair matings with nonrelatives (e.g. Blomqvist *et al.*, 2002; Foerster *et al.*, 2003; Brouwer *et al.*, 2011; Reid *et al.*, 2015).

Even though understanding the factors that drive the observed variation in the severity of inbreeding depression across species and across environments could have important implications for the conservation of many endangered populations, these dynamics are still not well understood. Our findings suggest that the intensity of sibling competition may not contribute towards variation in the severity of inbreeding depression, at least within homogeneous broods of inbred offspring. Determining whether and when sibling competition might play a role in the expression of inbreeding depression may help us better understand the causes underlying temporal and spatial patterns of variation in inbreeding depression in natural populations.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Effects of additional factors on offspring fitness traits: larval mass at dispersal (mg), survival rate from hatching to dispersal, survival rate from dispersal to eclosion, and survival rate from hatching to eclosion. ‘Foster parent relatedness’ refers to whether the foster mother rearing the brood had been mated to a full sibling or an unrelated male.

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Parental care buffers against inbreeding depression in burying beetles

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When relatives mate, their inbred offspring often suffer a reduction in fitness-related traits known as "inbreeding depression." There is mounting evidence that inbreeding depression can be exacerbated by environmental stresses such as starvation, predation, parasitism, and competition. Parental care may play an important role as a buffer against inbreeding depression in the offspring by alleviating these environmental stresses. Here, we examine the effect of parental care on the fitness costs of inbreeding in the burying beetle *Nicrophorus vespilloides*, an insect with facultative parental care. We used a 2 × 2 factorial design with the following factors: (*i*) the presence or absence of a caring female parent during larval development and (*ii*) inbred or outbred offspring. We examined the joint influence of maternal care and inbreeding status on fitness-related offspring traits to test the hypothesis that maternal care improves the performance of inbred offspring more than that of outbred offspring. Indeed, the female's presence led to a higher increase in larval survival in inbred than in outbred broods. Receiving care at the larval stage also increased the lifespan of inbred but not outbred adults, suggesting that the beneficial buffering effects of maternal care can persist long after the offspring have become independent. Our results show that parental care has the potential to moderate the severity of inbreeding depression, which in turn may favor inbreeding tolerance and influence the evolution of mating systems and other inbreeding-avoidance mechanisms.

parental care | environmental stress | fitness | inbreeding depression | inbreeding tolerance

Inbreeding is an important issue in evolutionary biology and ecology because of its profound implications for genetic variation and the evolution of mating systems and reproductive strategies (1–5). Inbreeding results from matings between related individuals and can cause a reduction in offspring fitness because the higher degree of homozygosity associated with inbreeding increases the risk that deleterious recessive alleles are expressed (6). Evidence for such fitness costs of inbreeding, known as "inbreeding depression," has been documented in a wide range of taxa, including mammals, birds, insects, and plants (7). However, there is substantial variation in the severity of inbreeding depression among species as well as among and within populations of a species (7–10). This variation may be driven in part by differences in the physical or social environment, which can have a major effect on the severity of inbreeding depression (11, 12). For example, environmental stresses such as starvation and competition are expected to exacerbate inbreeding depression (9, 11).

Parental care is an important component of the social environment in many birds, mammals, and insects (13). It is thought to have evolved as a means by which parents enhance their offspring's fitness by neutralizing the detrimental effects of a wide range of environmental stresses, including starvation, predation, parasitism, and competition (13). Thus, parental care may indirectly buffer against inbreeding depression by alleviating these stresses (14), but currently there is little empirical evidence in support of this suggestion. A study on a subsocial spider (*Anelosimus cf. jucundus,* currently *Anelosimus arizona*) proposed that the absence of detectable inbreeding depression in the offspring of this species could be caused by the buffering effects of either parental care or group living (14). The only experimental test of this hypothesis, conducted on the European earwig (*Forficula auricularia*), found no evidence that maternal care reduced the fitness costs of inbreeding depression (9).

Here, we tested whether parental care can buffer against inbreeding depression in the burying beetle *Nicrophorus vespilloides*, an insect with facultative parental care. Although there is no prior information on the effect of the interaction between parental care and the offspring's inbreeding status, the independent effects of parental care and inbreeding status on offspring fitness are well established in this species (15, 16). Parental removal experiments show that larval growth is reduced if the caring parents are removed during the early stages of development when offspring are too young to self-feed efficiently (15). Furthermore, inbred offspring suffer reduced survival at the larval stage (16).

To test for a causal effect of parental care on the severity of inbreeding depression, we used a 2 × 2 factorial design with the following factors: (*i*) presence or absence of posthatching maternal care and (*ii*) inbred or outbred offspring. All parents used in this experiment were outbred. Because inbreeding depression can affect traits across the entire life cycle of an organism, we assessed the joint effects of inbreeding status and maternal care on the following fitness-related offspring traits: (*i*) time to dispersal from the carcass (corresponding to the end of the parental care period); (*ii*) larval survival to dispersal; (*iii*) average larval mass at the time of dispersal; (*iv*) survival from dispersal to eclosion as an adult; and (*v*) posteclosion lifespan. We predicted that if maternal care can buffer against inbreeding depression, the presence of the mother would have a stronger fitness effect on inbred offspring than on outbred offspring.

Significance

When relatives mate, their inbred offspring often suffer a reduction in fitness-related traits known as "inbreeding depression." Environmental stresses such as starvation and competition can exacerbate these fitness costs of inbreeding. However, caring parents could mitigate the fitness costs of inbreeding by neutralizing the effects of these environmental stresses. We tested the hypothesis that maternal care can buffer against inbreeding depression in the offspring in burying beetles. Indeed, the female's presence led to a higher increase in larval survival in inbred than in outbred broods, and it increased the lifespan of inbred but not outbred adults. Our findings suggest that parental care can moderate the severity of inbreeding depression, possibly affecting how parental care strategies and inbreeding avoidance mechanisms evolve.

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Results

Maternal care shortened the time to dispersal from the carcass, but there was no difference in time to dispersal between inbred and outbred broods, and there was no effect of the interaction between maternal care and the offspring's inbreeding status (Tables 1 and 2). Time to dispersal was significantly shorter when the female parent was younger (estimate = 0.038 d, SE = 0.008, $t_{84} = 4.87$, $P < 0.0001$). Finally, there was no effect of carcass size ($t_{84} = 1.21$, $P = 0.23$) or the number of larvae dispersing ($t_{84} = -0.127$, $P = 0.90$) on time to dispersal.

Maternal care significantly increased the probability that at least one larva in a brood survived to dispersal (zero hurdle model: estimate = 0.964, SE = 0.241, $z_{84} = 4.00$, $P < 0.0001$). The offspring's inbreeding status did not have a significant effect on this component of larval survival (zero hurdle model: $z_{84} = 1.91$, $P = 0.056$), and there was no effect of the interaction between maternal care and inbreeding status (zero hurdle model: $z_{84} = 1.09$, $P = 0.28$). For broods in which at least one larva was present at dispersal, both maternal care and offspring inbreeding status had an effect on larval survival (Tables 1 and 2). The interaction between these two factors also had a significant effect (Table 1), because maternal presence improved the survival of inbred larvae more than that of outbred larvae (Table 2). There were no detectable effects of carcass size ($z_{84} = -0.62$, $P = 0.54$) or female age ($z_{84} = 1.77$, $P = 0.076$) on larval survival to dispersal.

Maternal care significantly increased larval mass at dispersal, but there was no significant effect of inbreeding status on larval mass and no significant interaction between maternal care and inbreeding status (Tables 1 and 2). Furthermore, larval mass did not depend on carcass size ($t_{84} = -0.15$, $P = 0.88$) or female age ($t_{84} = -1.08$, $P = 0.28$).

Both maternal care and inbreeding status had significant effects on the offspring's survival to eclosion. Maternal care increased survival to eclosion, and outbred larvae had higher survival than inbred ones (Tables 1 and 2). Survival to eclosion was not influenced by the interaction between maternal care and inbreeding status (Table 1).

Last, maternal care increased the lifespan of offspring after eclosion, and outbred offspring had longer lifespans than inbred ones (Tables 1 and 2). The statistically significant interaction between inbreeding status and maternal care indicated that receiving care improved the lifespan of inbred adults but not of outbred adults (Tables 1 and 2). There was no evidence for a difference in lifespan after eclosion between males and females ($z_{448} = -0.33$, $P = 0.74$).

Overall, we found evidence for inbreeding depression in survival to dispersal, survival from dispersal to eclosion, and lifespan after eclosion (Table 1). Moreover, we found evidence for a significant interaction between maternal care and offspring inbreeding status for survival to dispersal and posteclosion lifespan, indicating a buffering effect of maternal care (Table 1). This conclusion is supported by our estimates of inbreeding depression (δ), which show that maternal care moderates the severity of inbreeding depression in these two traits (Fig. 1 and Table 2).

Discussion

We provide experimental evidence supporting the suggestion that parental care can buffer against the deleterious effects of inbreeding on offspring fitness (14). First, we found that maternal care had a greater positive effect on larval survival to dispersal in inbred broods than in outbred broods. Second, we found that maternal care increased the lifespan of inbred offspring but not of outbred offspring. Thus, our results show that the buffering effects of parental care are detectable not only during the period when offspring depend on parental care (i.e., from egg laying to dispersal from the carcass) but also later in life when offspring have become independent. To our knowledge, this is the first study to show a causal effect of parental care on the severity of inbreeding depression. We provide a more detailed discussion of our results below.

Our first key finding was that maternal care had a stronger effect on survival to dispersal in inbred than in outbred larvae. This finding provides clear evidence that maternal care buffers against inbreeding depression during the period when larvae depend on maternal care. Before independence, larvae benefit directly from various components of maternal care, such as food provisioning (16, 17), defense against conspecific intruders (18), and defense against bacterial and fungal competitors through antimicrobial secretions (19). Thus, during this period, caring parents are in a position to neutralize directly the negative effects of the environmental stresses that are otherwise expected to exacerbate the fitness costs of inbreeding depression (11, 12).

Our second key finding was that maternal care increased the adult lifespan of inbred offspring but not of outbred offspring. This finding shows that parental care can buffer against inbreeding depression in offspring long after they have become independent of their parents. The extended adult lifespan of inbred offspring resulting from maternal care may indicate that maternal care increases the general condition of inbred offspring, thereby enhancing their survival prospects after the end of the parental care period. Our results show that the buffering effects of parental care against inbreeding depression can occur across different life stages, reinforcing the importance of measuring fitness consequences across an individual's whole life span (6).

Our finding that maternal care in *N. vespilloides* buffers against inbreeding depression in larval survival and adult lifespan contrasts with a recent study that found no evidence for a buffering effect on larval survival in the European earwig (9). One potential explanation for these opposing results is that no inbreeding depression in larval survival was observed in European

Table 1. Effects of maternal care and offspring inbreeding status on fitness-related offspring traits

	Maternal care				Inbreeding status				Interaction			
Offspring trait	Est	SE	*z*/*t*	*P*	Est	SE	*z*/*t*	*P*	Est	SE	*z*/*t*	*P*
Time to dispersal	−1.00	0.25	−4.0	**<0.001**	0.26	0.41	0.64	0.52	−0.64	0.51	−1.3	0.21
Survival to dispersal	0.67	0.10	6.8	**<0.0001**	0.77	0.10	7.6	**<0.0001**	−0.49	0.12	−4.2	**<0.0001**
Larval mass	0.027	0.008	3.5	**<0.001**	0.006	0.007	0.85	0.40	0.011	0.011	1.0	0.31
Survival to eclosion	1.22	0.41	3.0	**0.004**	1.88	0.50	3.7	**<0.001**	−0.12	0.80	−0.15	0.88
Posteclosion lifespan	0.53	0.09	5.8	**<0.0001**	0.36	0.09	4.2	**<0.0001**	−0.53	0.11	−4.8	**<0.0001**

We provide information on the parameter estimates (Est), SEs, test statistics (*z* and *t* values), and *P* values for time to dispersal (days), larval survival to dispersal (%), larval mass (grams), survival from dispersal to eclosion (%), and posteclosion lifespan (days). The data were analyzed using general linear models for time to dispersal and larval mass and generalized linear models for survival to eclosion (fitted with a quasibinomial error structure) and lifespan (fitted with a negative binomial error structure). We used a ZAP regression to analyze the zero-inflated data on survival to dispersal, and here we present the results for the count model (see text for zero-hurdle model results). Statistically significant *P* values are indicated in bold.

Table 2. Means ± SE and estimates of inbreeding depression (δ) for fitness-related traits for offspring that did or did not receive maternal care during the larval stage

	Means ± SE		δ	
Offspring trait	Care	No care	Care	No care
Time to dispersal				
Outbred	9.57 ± 0.23	10.90 ± 0.26	−0.028	0.054
Inbred	9.84 ± 0.24	10.31 ± 0.43		
Survival to dispersal				
Outbred	23.28 ± 2.56	12.67 ± 2.74	0.36	0.69
Inbred	14.97 ± 2.20	3.94 ± 1.27		
Larval mass at dispersal				
Outbred	0.180 ± 0.006	0.147 ± 0.005	0.072	0.048
Inbred	0.167 ± 0.004	0.140 ± 0.006		
Survival to eclosion				
Outbred	96.54 ± 1.44	90.33 ± 3.09	0.14	0.35
Inbred	82.72 ± 3.98	58.7 ± 10.7		
Posteclosion lifespan				
Outbred	32.93 ± 1.34	39.15 ± 1.92	−0.006	0.41
Inbred	33.12 ± 1.19	23.11 ± 2.29		

We provide information on time to dispersal (days), larval survival to dispersal (%), larval mass (grams), survival from dispersal to eclosion (%), and lifespan (days). For each of these traits, we used the equation $\delta = (w_o - w_i)/w_o$ to calculate inbreeding depression as a proportional change in mean fitness of outbred and inbred offspring.

earwigs. In contrast, we found evidence for substantial inbreeding depression in this trait, as is consistent with previous work on *N. vespilloides* (16). We suggest that parental care can buffer against inbreeding depression only when the following two conditions are met: (*i*) offspring suffer from inbreeding depression in a particular trait, and (*ii*) parental care can improve offspring performance with regard to that trait. Our results confirm that both conditions were met in *N. vespilloides*, whereas only the second condition was met in European earwigs (9).

Given that matings between close relatives are relatively uncommon in most natural populations of animals (20, 21), it seems unlikely that parental care evolved specifically to buffer against inbreeding depression. Instead, it is generally accepted that parental care evolves as a mechanism for neutralizing the effects of environmental stresses, such as starvation, predation, parasitism, and competition, on the offspring's fitness (13, 22). However, once parental care has evolved, it may inadvertently moderate the severity of inbreeding depression because it alleviates many of the same stresses that are predicted to exacerbate inbreeding depression (11). We therefore expect similar buffering effects against inbreeding to be widespread across species with parental care, regardless of whether they have a history of inbreeding. Furthermore, we suggest that whenever a previously outbred population becomes subject to inbreeding (e.g., because of habitat fragmentation or a population bottleneck), the severity of inbreeding depression may depend on the preexisting form or level of parental care. Thus, the buffering effect of parental care is likely to be nonadaptive in the context of coping with inbreeding but adaptive in the context of neutralizing environmental stresses.

Although it seems unlikely that parental care originated to provide a buffer against inbreeding, the form or level of parental care may be modified subsequently because of its capacity to buffer against the fitness costs of inbreeding depression should the population remain inbred over many subsequent generations. Such evolutionary changes in parental care might occur in animal taxa with inbred mating systems (14, 23). Avilés and Bukowski (14) proposed that parental care or other forms of sociality that buffer against inbreeding depression could facilitate the transition from an ancestral outbred mating system toward an inbred mating system (23). Our finding that parental care buffers against inbreeding depression provides experimental evidence that parental

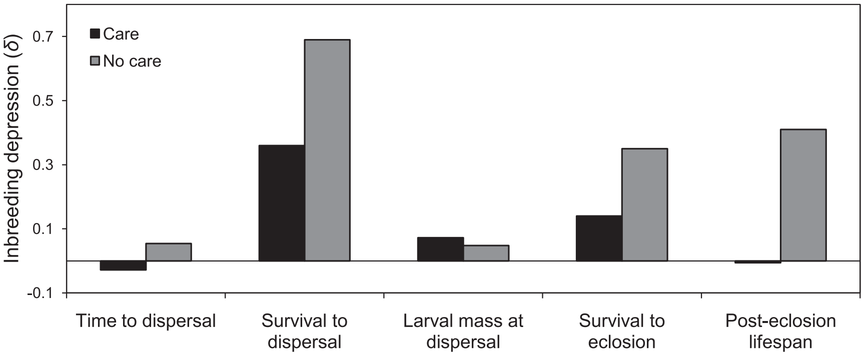

Fig. 1. Inbreeding depression (δ) in offspring when the female parent was present (black bars) or absent (gray bars) during the larval stage. Three of these fitness traits (time to dispersal, survival to dispersal, and mass at dispersal) were measured before independence; the other two traits (survival from dispersal to eclosion and posteclosion lifespan) were measured after offspring became independent. Inbreeding depression was calculated as a proportional change in mean fitness of outbred (w_o) and inbred (w_i) offspring, using the equation $\delta = (w_o - w_i)/w_o$.

care may facilitate the evolution of inbred social systems by reducing the fitness costs of inbreeding depression associated with such a transition. Nevertheless, the argument by Avilés and Bukowski (14) implicitly assumes that parental care itself is not subject to inbreeding depression. Theoretical considerations suggest that this assumption might be violated (24, 25), in which case persistent inbreeding might affect the parents' ability to buffer against inbreeding depression in their offspring. Given these theoretical predictions and some mixed evidence from empirical studies on the effect of inbreeding on parental care (26–28), we encourage further work in this area.

Based on our findings, we expect selection for inbreeding avoidance to be relaxed when parental care can moderate the deleterious effects of inbreeding in the offspring (provided that parental care itself is not subject to inbreeding depression). Under these conditions, the buffering effects of parental care may favor inbreeding tolerance or even inbreeding preference, a possibility that has been overlooked in the literature. Theoretical models emphasize the importance of the costs of dispersal, mating system, mate encounter rate, and kin recognition as important factors shaping the balance between inbreeding tolerance and avoidance (29–31). To our knowledge, the only theoretical study to specifically consider the role of parental care concludes that biparental care should lead to lower inbreeding tolerance, because both parents must put in a substantial amount of parental effort for a relatively small return in the form of inbred offspring (30). However, existing theory has not considered that parental care might moderate the severity of inbreeding depression in the offspring, in which case it could have the opposite effect of leading to higher inbreeding tolerance. For example, in an African cichlid with biparental care (*Pelvicachromis taeniatus*), both sexes preferentially mate with a close relative (32). There is no evidence for inbreeding depression in this species, and it has been suggested that the absence of inbreeding depression results from the occurrence of parental care (9, 32). The buffering effects of parental care on offspring fitness may interact with life-history traits and mating dynamics to determine an organism's inbreeding strategy, which in turn can have profound implications for the maintenance of genetic variation within a population (5, 6). Considering the wider implications of the potential effects of parental care on the severity of inbreeding depression may thus help us better understand and predict when animals should avoid, tolerate, or prefer inbreeding (5).

In summary, our findings have important implications for the understanding of inbreeding, a central topic in ecology and evolutionary biology. First, we show that the buffering effects of parental care were detectable not only during the period when larvae depend on parental care (i.e., from egg laying to dispersal from the carcass) but also long after independence. We expect such buffering to be widespread in species with parental care, even in populations with no history of inbreeding, as long as parental care can alleviate environmental stress and kin matings lead to considerable inbreeding depression in the offspring. Second, the buffering effects of parental care may favor the evolution of inbred mating systems or inbreeding tolerance by reducing the fitness costs to inbred offspring (14). Therefore, a better understanding of how parental care and other forms of sociality can influence the expression of inbreeding depression may help explain the observed variation in animal inbreeding strategies.

Materials and Methods

Study Species. Burying beetles (*N. vespilloides*) breed on carcasses of small vertebrates and have facultative biparental care. Parents bury the carcass in the soil and lay the eggs around it (33). They prepare the carcass by removing any fur or feathers and apply antimicrobial secretions to suppress bacterial and fungal growth (33, 34). After hatching, larvae crawl to the carcass and start feeding in a crater created by the parents. The larvae can self-feed, but parents also provision larvae with predigested carrion (16). In addition, parents defend the brood from predators and conspecific competitors (35). The larvae disperse from the carcass about 5 d after hatching, pupate about 10 d after dispersal, and eclose as adults about 10 d after pupation.

Experimental Design. We used beetles from an outbred laboratory population maintained at The University of Edinburgh. To avoid inbreeding in the stock population, we maintained a large population (500–1,000 individuals per generation) and mated only unrelated or distantly related individuals (i.e., no common ancestors for at least two generations). The beetles used in this study comprised of third- and fourth-generation beetles from lines originally collected in Edinburgh and Warmond, The Netherlands. Similar numbers of beetles were used from each line, and there was no evidence of outbreeding depression. They were housed individually in transparent plastic containers ($12 \times 8 \times 2$ cm) filled with moist soil and kept at 20 °C and constant light. Nonbreeding adults were fed raw organic beef twice a week.

To examine whether parental care buffers against inbreeding depression, we used a 2×2 factorial design with the following treatment groups: (*i*) outbred offspring that received maternal care ($n = 32$); (*ii*) outbred offspring that received no maternal care ($n = 33$); (*iii*) inbred offspring that received maternal care ($n = 33$); and (*iv*) inbred offspring that received no maternal care ($n = 33$). To produce outbred offspring for treatment groups 1 and 2, we paired outbred virgin beetles that did not share ancestors for at least two generations. To produce inbred offspring for treatment groups 3 and 4, we paired outbred virgin beetles that were full siblings. These experimental pairs ($n = 131$) were randomly assigned to treatments (care or no care). They then were transferred to transparent plastic containers ($17 \times 12 \times 6$ cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (Livefoods Direct Ltd.) of a standardized size (24–27 g). In this species, the amount of care provided by the male is highly variable, and male removal has no average effect on offspring fitness under laboratory conditions (36). For this reason, we removed males from all treatments after eggs were laid but before the larvae had hatched. In treatment groups 2 and 4, we also removed females at the same time, but females were left to care for their brood until dispersal in treatment groups 1 and 3.

When all larvae had dispersed from the carcass, we recorded the date, the number of surviving larvae, and the total mass of the brood. These data were used to calculate time to dispersal and average larval mass for each brood. All larvae, up to a maximum of 15 per brood, were placed into large transparent boxes filled with moist soil. At eclosion, we recorded the proportion of individuals that eclosed successfully from each brood and placed up to six beetles into individual containers. We tracked the mortality of these beetles ($n = 449$) by checking them twice a week until death.

Statistical Analyses. All data were analyzed using R version 3.1.1. We used general linear models for traits that had a normal error structure (average larval mass and time to dispersal). For survival to eclosion, we used a generalized linear model fitted with a quasibinomial error distribution, and for posteclosion lifespan we used a generalized linear model fitted with a negative binomial error distribution. Because of a high proportion of zeros in the larval survival data, we ran a zero-adjusted Poisson (ZAP) regression using the hurdle function in the pscl package (37). A Poisson structure was assumed for the count model, and a binomial structure was assumed for the zero-hurdle model. Significant values on the count model indicate that a given variable had an effect on the number of larvae surviving to dispersal, whereas significant values on the zero-hurdle model indicate that a given variable had an effect on the probability of having zero versus nonzero larvae at dispersal.

All models included parental care (maternal presence or absence) and inbreeding status (inbred or outbred offspring) as main effects, as well as an interaction between these two factors. Carcass size was included as an additional covariate in the models for time to dispersal, survival to dispersal, and average larval mass, because the amount of resources available may influence offspring growth and survival. We also added female age to the models for time to dispersal, survival to dispersal, and average larval mass, because the amount of care a female provides may depend on her age. Note that there was no significant difference in female age between treatments [one-way ANOVA; $F(3,127) = 0.26$, $P = 0.86$]. Last, we added sex as a factor in the lifespan model because of the possibility of sex-specific mortality (38). Decisions as to which variables to include in the final model were based on Akaike Information Criterion model-selection criteria.

To compare inbreeding depression in offspring fitness traits between the care and no care treatments, we calculated inbreeding depression as a proportional change in mean fitness of outbred (w_o) and inbred offspring (w_i) based on the equation $\delta = (w_o - w_i)/w_o$ (Table 2) (39).

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Maternal effects alter the severity of inbreeding depression in the offspring

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A maternal effect is a causal influence of the maternal phenotype on the offspring phenotype over and above any direct effects of genes. There is abundant evidence that maternal effects can have a major impact on offspring fitness. Yet, no previous study has investigated the potential role of maternal effects in influencing the severity of inbreeding depression in the offspring. Inbreeding depression is a reduction in the fitness of inbred offspring relative to outbred offspring. Here, we tested whether maternal effects due to body size alter the magnitude of inbreeding depression in the burying beetle *Nicrophorus vespilloides*. We found that inbreeding depression in larval survival was more severe for offspring of large females than offspring of small females. This might be due to differences in how small and large females invest in an inbred brood because of their different prospects for future breeding opportunities. To our knowledge, this is the first evidence for a causal effect of the maternal phenotype on the severity of inbreeding depression in the offspring. In natural populations that are subject to inbreeding, maternal effects may drive variation in inbreeding depression and therefore contribute to variation in the strength and direction of selection for inbreeding avoidance.

1. Introduction

Inbreeding results from matings between relatives and can lead to a general loss of heterozygosity, which increases the likelihood that recessive, deleterious alleles are expressed [1]. As a result, inbreeding is commonly associated with a reduction in the fitness of any resulting offspring, known as inbreeding depression. Inbreeding depression is an important issue in evolutionary biology and ecology because it can exert strong selection pressures on dispersal strategies, mating systems, reproductive strategies, and social behaviours [2]. Furthermore, inbreeding depression is a growing conservation concern, given that increasing rates of habitat loss and habitat fragmentation can increase the likelihood of inbreeding [3,4], thereby contributing to higher local extinction rates [5]. Even though there is abundant evidence for inbreeding depression across a wide range of animal and plant taxa, the severity of inbreeding depression can vary dramatically both among and within species [5,6]. Understanding the factors that underlie this variation could have implications for the conservation of many endangered populations, yet these dynamics are still not well understood. Earlier work has proposed that this variation may be partly attributed to differences in the physical or social environment [7–9]. Environmental stresses, such as starvation and competition, tend to exacerbate inbreeding depression [8,10], whereas benign conditions may moderate inbreeding depression [11,12].

We have recently shown that inbreeding depression is more severe in the absence of maternal care, suggesting that the presence of the mother during offspring development can buffer against inbreeding depression [12]. However, it is still unknown whether such a buffering effect depends on the mother's phenotype. Maternal condition may affect the quantity or quality of care provided to the offspring [13–17], so we might expect the severity of inbreeding depression to be influenced by maternal traits such as body size, age, nutritional condition, and health status. This type of a causal influence of the maternal phenotype on the offspring phenotype would represent a maternal effect [18]. Even though the mechanisms and consequences of maternal effects have been studied extensively [18,19], previous work has overlooked the potential role of maternal effects in the context of inbreeding depression.

Here, we use the burying beetle *Nicrophorus vespilloides* to examine whether maternal body size, an important component of the maternal phenotype, affects the severity of inbreeding depression in the offspring. Burying beetles are a highly suitable study system for addressing this question. They have facultative biparental care, and male removal has no effect on offspring fitness under laboratory conditions [20], allowing us to focus on maternal effects. Moreover, we have previously shown that there is severe inbreeding depression in this species, with respect to survival at the larval and pupal stages, as well as adult lifespan [12,21,22]. We have also shown that inbreeding depression in the offspring is less severe when the mother is present than when she is removed [12]. Given that small females provide less post-hatching care than large females [16,17], we hypothesized that inbred offspring would suffer a greater reduction in fitness (compared with outbred offspring) if they had a small mother than if they had a large mother. To test this hypothesis, we used a 2 × 2 factorial design with the following treatment groups: (i) a large female with outbred offspring, (ii) a small female with outbred offspring, (iii) a large female with inbred offspring, and (iv) a small female with inbred offspring. Because inbreeding depression affects traits across the entire life cycle in this species [12,21,22], we measured several offspring fitness traits at different life stages: (i) hatching success, (ii) larval mass at dispersal, (iii) survival rate from hatching to dispersal, (iv) survival rate from dispersal to eclosion, and (v) lifespan after eclosion.

2. Material and methods

(a) Study species

Burying beetles breed on carcasses of small vertebrates. Parents bury the carcass and lay the eggs in the soil around it [23]. They prepare the carcass by removing any fur or feathers and apply antimicrobial secretions to suppress bacterial and fungal growth [23–25]. After hatching, larvae crawl to the carcass and start feeding in a crater created by the parents. The larvae can self-feed, but parents also provision larvae with predigested carrion. In addition, parents defend the brood from predators and conspecific competitors [26]. Although both parents typically provide care, females stay on the carcass for longer and spend more time provisioning food to the larvae [17,20,27–29]. The larvae disperse from the carcass about 5 days after hatching, which corresponds to the end of the parental care period. They pupate about 10 days after dispersal and eclose as adults about 10 days after pupation.

(b) Beetle husbandry

We used virgin beetles from an outbred laboratory population maintained at The University of Edinburgh. The beetles used in this study comprised of second-generation beetles from lines originally collected in Edinburgh, UK. They were housed individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and kept at 20°C and constant light. All non-breeding adults were fed small pieces of raw organic beef twice a week.

(c) Experimental design

In the first part of our experiment, we generated small and large beetles. Because adult body size is determined by larval mass at the dispersal stage [30,31], it is possible to generate different-sized beetles by removing larvae from the carcass at different times after hatching [16,17,32,33]. Thus, for each of 89 broods, we removed third-instar larvae weighing 100–150 mg and 200–250 mg to generate small and large adults, respectively. The main advantage of this method was that it allowed us to generate small and large females that were full siblings. We were thus able to remove any potential confounding genetic effects that might have arisen if we had selected small and large individuals from our stock population. After each small or large larva was removed from the carcass, it was placed in an individual container (12 × 8 × 2 cm) filled with moist soil. At eclosion, we measured the pronotum width of all female beetles. As intended, there was a substantial difference in the mean (±s.d.) pronotum width of females from the two groups: 4.04 (±0.24) mm for small females and 5.33 (±0.24) mm for large females. There was also no overlap in the range of pronotum widths for small (3.50–4.59 mm) and large females (4.99–6.00 mm). Steiger [16] used similar size classes: 3.97 (±0.21) mm for small females and 5.54 (±0.23) mm for large females. These categories were based on the size range observed in both the laboratory population and beetles collected in the field [16].

In the second part of our experiment, we used a 2 × 2 factorial design with the following treatment groups: (i) a large female with outbred offspring, (ii) a small female with outbred offspring, (iii) a large female with inbred offspring, and (iv) a small female with inbred offspring. To produce outbred offspring for treatments (i) and (ii), we paired outbred virgin beetles that had no common ancestors for at least two generations. To produce inbred offspring for treatments (iii) and (iv), we paired outbred virgin beetles that were full siblings. All male and female parents were mated within 15 days after reaching sexual maturity (i.e. 10–25 days after eclosion). On the day of mating, we measured each female's prebreeding mass, which was later used to estimate the female's mass change over the breeding attempt (see below). Each experimental pair ($n = 276$) was placed in a transparent plastic container (17 × 12 × 6 cm) filled with 1 cm of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd, Sheffield, UK) of a standardized size (24–26 g). After mating, we checked the containers twice a day for the presence of eggs. As soon as the first eggs were laid, we removed the male from the container. In this species, the amount of care provided by the male is highly variable and male removal has no effect on offspring fitness under laboratory conditions [20]. Right before larvae started hatching, we recorded the number of eggs laid (clutch size) by counting the number of eggs visible at the bottom of the transparent breeding box [33,34]. Because each box contained a very thin layer of soil, the number of eggs at the bottom of the box was very close to the actual clutch size [34].

When all larvae had dispersed from the carcass, we weighed the female again. By subtracting each female's prebreeding mass from her postbreeding mass, we calculated her mass change over the breeding attempt, as a measure of somatic investment and thus allocation to future reproduction [32,35]. Females were then transferred to individual containers (12 × 8 × 2 cm) filled with moist soil. They were checked twice a week until death to determine their postbreeding lifespan, as a measure of their residual reproductive value [33].

At the dispersal stage, we also recorded the number of unhatched eggs visible at the bottom of the box, the number of

Table 1. Effects of inbreeding (outbred or inbred offspring), maternal body size (large or small), and their interaction on female reproductive decisions (clutch size and mass change) and residual reproductive value (postbreeding lifespan). We provide information on the test statistic (*F* or LR χ^2_1 for linear models or generalized linear models, respectively) and *p*-value for each variable. Statistically significant *p*-values are indicated in italics.

	offspring inbreeding status		female size		interaction	
	F/LR χ^2_1	*p*-values	*F*/LR χ^2_1	*p*-values	*F*/LR χ^2_1	*p*-values
clutch size	1.90	0.17	263	*<0.0001*	0.03	0.86
female mass change (%)	0.11	0.74	11.1	*<0.01*	0.20	0.65
female postbreeding lifespan (days)	0.09	0.77	9.7	*<0.01*	2.21	0.14

surviving larvae, and the total mass of the brood. By subtracting the number of unhatched eggs from the clutch size recorded earlier, we estimated the number of eggs that hatched. We then divided the number of eggs that hatched successfully by clutch size to calculate hatching success. We also calculated the average larval mass in each brood by dividing total brood mass by the number of larvae. We placed all larvae from each brood into large transparent containers (17 × 12 × 6 cm) filled with moist soil. Approximately 20 days later, we recorded the number of individuals that eclosed successfully from each brood to calculate the survival rate from dispersal to eclosion. At this stage, up to six beetles from each brood were placed into individual containers (12 × 8 × 2 cm). We tracked the adult lifespan of these offspring ($n = 872$) by checking them twice a week until death. The sample sizes for matings with at least one offspring surviving until eclosion were as follows: $n = 46$ for large females with outbred broods, $n = 54$ for small females with outbred broods, $n = 40$ for large females with inbred broods, and $n = 43$ for small females with inbred broods.

(d) Data analysis

All analyses were performed using R v. 3.2.3. We used linear models for continuous traits with normally distributed random errors (average larval mass, adult offspring lifespan, female mass change, and female postbreeding lifespan). For discrete traits, we used generalized linear models fitted with a Poisson error distribution (clutch size). For proportion data, we used generalized linear models fitted with a binomial distribution (survival to dispersal and survival to eclosion) or a binomial distribution corrected for overdispersion (hatching success). Proportion data were entered into the models using the 'cbind' function. In all of these models, we analysed absolute differences rather than log-transformed measures [36], as relative measures of inbreeding depression are potentially biased [37].

All models included the following factors: offspring inbreeding status (outbred or inbred), maternal body size (large or small), and the interaction between the two. A statistically significant interaction would suggest that maternal body size affects the severity of inbreeding depression in the offspring (i.e. the extent to which inbred offspring perform less well compared with outbred offspring). Carcass size was added as a covariate in all models, because the amount of resources available may influence female reproductive decisions and offspring performance. Indeed, we found that females laid more eggs on larger carcasses (LR $\chi^2_1 = 8.87$, $p < 0.01$), and larvae had a higher survival rate on larger carcasses (LR $\chi^2_1 = 6.47$, $p = 0.01$). There was also a non-significant trend for females to gain more mass on larger carcasses ($F = 3.20$, $p = 0.08$). In addition, we added maternal age as a covariate in all models, because it can influence female reproductive decisions and offspring performance. Accordingly, we found that younger females laid fewer eggs (LR $\chi^2_1 = 8.56$, $p < 0.01$) and their offspring had a higher survival rate from hatching to dispersal (LR $\chi^2_1 = 28.8$, $p < 0.0001$). Lastly, we added sex as a covariate in the model for adult lifespan of the offspring and found that male offspring had a shorter lifespan after eclosion than female offspring ($F = 9.16$, $p < 0.001$). Decisions on which covariates to include in the final models were based on Akaike information criterion (AIC) scores.

3. Results

(a) Effects of inbreeding

There was no difference in clutch size, mass change, or postbreeding lifespan between females that were mated to their brothers and females that were mated to unrelated males (table 1; electronic supplementary material, table S1). However, inbreeding had significant effects on the offspring's fitness (table 2; electronic supplementary material, table S1). Inbred larvae suffered substantial inbreeding depression in three of the five traits we measured: survival from hatching to dispersal, survival from dispersal to eclosion, and adult lifespan (figure 1). There was no evidence for inbreeding depression in hatching success or larval mass at the dispersal stage (table 2; electronic supplementary material, table S1).

(b) Effects of female body size

Small females laid fewer eggs, gained relatively less mass over the breeding attempt, and had a shorter postbreeding lifespan than large females (table 1; electronic supplementary material, table S1). Small females also produced larvae that had a lower mass at the dispersal stage than larvae of large females (table 2; electronic supplementary material, table S1). However, female body size had no effect on hatching success, survival to dispersal, survival to eclosion, or adult lifespan of the offspring (table 2; electronic supplementary material, table S1).

(c) Interaction between inbreeding and female size

There was a significant interaction between offspring inbreeding status and female size on survival to dispersal (table 2). This interaction reflected that offspring of large females suffered a greater reduction in fitness due to inbreeding than offspring of small females (figure 2). In other words, inbreeding depression in survival to dispersal was more severe for offspring of large mothers than those of small mothers (figure 1). There was no such interaction on hatching success, larval mass, survival to eclosion, or offspring lifespan after eclosion (table 2). Similarly, there was no interaction on female reproductive decisions or residual reproductive value (table 1).

Table 2. Effects of inbreeding (outbred or inbred offspring), maternal body size (large or small), and their interaction on offspring fitness traits. Survival to dispersal refers to the offspring survival rate from hatching to dispersal, and survival to eclosion refers to the offspring survival rate from dispersal to eclosion. We provide information on the test statistic (*F* or LR χ^2_1 for linear models or generalized linear models, respectively) and *p*-values for each variable. Statistically significant *p*-values are indicated in italics.

	offspring inbreeding status		female size		interaction	
	F/LR χ^2_1	*p*-value	*F*/LR χ^2_1	*p*-value	*F*/LR χ^2_1	*p*-value
hatching success (%)	1.83	0.18	1.09	0.30	3.01	0.08
average larval mass (mg)	0.11	0.74	30.3	*<0.001*	1.05	0.31
survival to dispersal (%)	17.8	*<0.0001*	0.05	0.82	9.49	*<0.01*
survival to eclosion (%)	21.5	*<0.0001*	2.24	0.13	1.01	0.32
offspring adult lifespan (days)	24.9	*<0.001*	1.40	0.24	0.32	0.57

4. Discussion

In this study, we tested whether the mother's phenotype can influence the severity of inbreeding depression in her offspring. We found evidence for inbreeding depression in three of the five traits we measured: survival from hatching to dispersal, survival from dispersal to eclosion, and post-eclosion lifespan (table 1). In addition, we found a significant interaction between inbreeding and maternal body size on survival to dispersal. This interaction reflected that inbreeding depression in this trait was more severe for offspring of large females than offspring of small females (figure 2). There was no such interaction on survival to eclosion or post-eclosion lifespan. Although we found an interaction in only one of these fitness traits, we note that this trait was also the one with the highest level of inbreeding depression (figure 1). In summary, our key finding was that offspring of large females suffered a lower survival rate from hatching to dispersal if they were inbred than if they were outbred, whereas inbred and outbred offspring of small females had a similar survival rate (figure 2). To our knowledge, this is the first evidence for a causal effect of the maternal phenotype on the severity of inbreeding depression in the offspring.

Until now, very few studies have considered maternal effects in the context of inbreeding depression, and all of these were conducted on plants [38–41]. Moreover, none of these studies established a causal link between maternal effects and the magnitude of inbreeding depression. For example, Wolfe [38] found that maternal effects influenced early-life fitness traits in *Hydrophyllum appendiculatum*, while inbreeding depression affected late-life fitness traits. He proposed two plausible explanations for this pattern: (i) maternal effects substantially reduce the severity of inbreeding depression in early-life fitness traits, so no inbreeding depression is detected during this life stage or (ii) inbreeding depression is more severe in later life stages because of the cumulative effect of smaller fitness reductions in earlier life stages [38]. Nevertheless, Wolfe [38] could not distinguish between these two explanations, so it is unclear whether the mother's phenotype influences the severity of inbreeding depression in this species.

Here, we demonstrate that maternal body size can alter the severity of inbreeding depression in larval survival in the burying beetle *N. vespilloides*. Inbred offspring of large females suffered lower survival from hatching to dispersal than outbred ones, whereas offspring of small females had the same survival rate regardless of their inbreeding status. The fact that there was no detectable inbreeding depression in this trait for offspring of small females suggests that maternal effects completely masked the deleterious effects of inbreeding on early-life offspring performance. In a population where the majority of females are small, such a masking effect could effectively hide the inbred genotype from natural selection, with potential consequences for the level of genetic load in the population [38].

We expected that inbreeding depression would be less severe for offspring of large females than those of small females, because females that are in better condition might have the capacity to provide more care. Yet, we found evidence for the opposite pattern. One plausible explanation is that large females have larger clutches (table 1), resulting in more intense sibling competition, which might in turn exacerbate the severity of inbreeding depression. We think this is unlikely given our earlier work showing that sibling competition does not exacerbate inbreeding depression in this species [22]. Additionally, our mean brood size was relatively small (potentially due to seasonal variation in reproductive success [42]), suggesting a low level of sibling competition in our study. An alternative explanation is that small and large females differ in how they invest in an inbred brood because of their different prospects for future breeding opportunities. Large females are more successful at acquiring and defending a carcass against conspecific competitors [30], so they have a higher residual reproductive value than small females, which may only breed once. Thus, a small female might maximize her reproductive effort during a breeding attempt regardless of her offspring's inbreeding status. On the other hand, when a large female mates with a relative and produces a brood of inbred, low-quality offspring, she might reduce her investment in current reproduction in order to take advantage of additional breeding opportunities in the future. Such adjustments in maternal investment could be mediated through changes in prehatching effort (e.g. egg size, nutrients deposited into the eggs) and/or post-hatching effort (e.g. provisioning rate), leading to a reduction in the survival of inbred offspring reared by large mothers. Nevertheless, this interpretation assumes that *N. vespilloides* females have the ability to recognize their relatives and/or the inbreeding status or overall quality

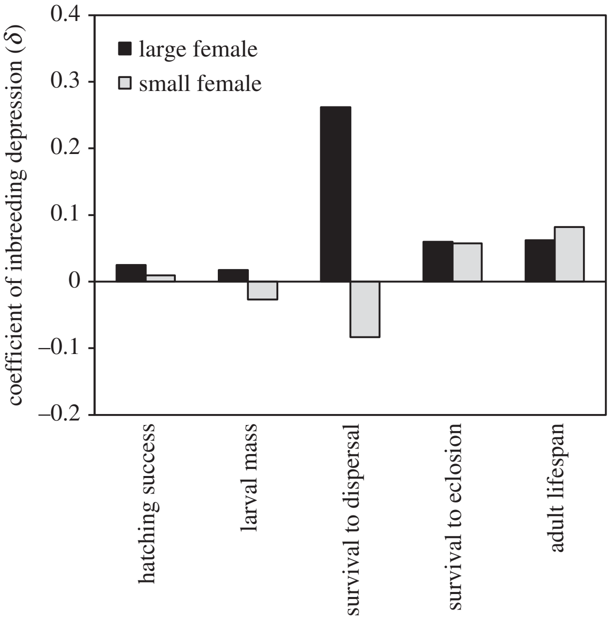

Figure 1. Inbreeding depression (δ) in offspring with large mothers (black bars) or small mothers (grey bars). Inbreeding depression was calculated as a proportional change in mean fitness of outbred (w_o) and inbred (w_i) offspring, using the equation $\delta = (w_o - w_i)/w_o$. Survival to dispersal is the offspring survival rate from hatching to dispersal, which corresponds to the end of the parental care period. Survival to eclosion is the offspring survival rate from dispersal to eclosion. Adult lifespan refers to the number of days an individual was alive after eclosion.

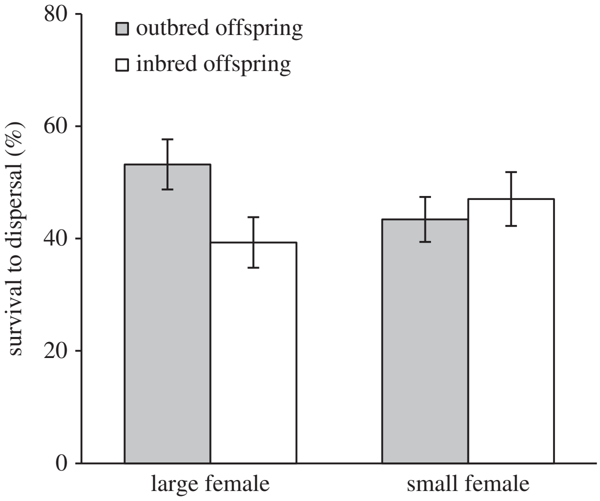

Figure 2. Mean (± standard error (s.e.)) survival rate from hatching to dispersal for outbred (grey) or inbred (white) offspring of small or large females. Inbred offspring of large females suffered lower survival from hatching to dispersal than outbred ones, whereas offspring of small females had the same survival rate regardless of their inbreeding status.

of their offspring. Further work is needed to determine whether females behave differently towards inbred and outbred offspring.

We expect inbreeding to be relatively rare in *N. vespilloides*, which makes this species a good model for understanding how the fitness costs of inbreeding are influenced by maternal effects in species that do not regularly inbreed. There are two important reasons it is useful to focus on species where inbreeding is relatively rare: (i) inbreeding depression is a greater concern for species with no prior history of inbreeding because deleterious, recessive alleles have not yet been purged from the population and (ii) once a species has a significant history of inbreeding, parental behaviours and other family interactions might be modified by selection due to inbreeding. Thus, species with a history of inbreeding might not be appropriate as models for endangered species that have only recently become subject to inbreeding. In principle, all populations are potentially at risk of inbreeding in the future, given increasing habitat loss and other human-induced disturbances that increase the chances of inbreeding [3]. It is therefore important to improve our understanding of how populations that become subject to inbreeding may cope with inbreeding depression.

Our study shows that maternal effects have the potential to influence the magnitude of inbreeding depression in the offspring. We encourage future research to investigate this previously unexplored issue in other taxa, because this pattern may be widespread in natural populations that suffer from inbreeding depression. If that is the case, it could have important implications for conservation efforts. Maternal effects are inextricably linked to maternal condition, which may vary over time within a population due to seasonal changes in weather and food availability [43–45]. Maternal effects may therefore contribute to temporal variation in inbreeding depression in natural populations that are subject to environmental variability. In species where estimates of inbreeding depression (δ) are sensitive to maternal condition, a better understanding of the role of maternal effects may be important for the conservation and management of endangered populations. The presence of maternal effects might cause researchers to overestimate or underestimate inbreeding depression as a threat to population viability depending on the state of females at the time of data collection and on the particular subsample of females used in the study.

Our findings also have general implications for evolutionary biology, because if maternal condition influences inbreeding depression in the offspring, we might expect selection for inbreeding avoidance to depend on the average maternal condition in the population. Depending on the parents' capacity to moderate the deleterious effects of inbreeding in the offspring, there might be selection for inbreeding avoidance, tolerance or even preference. Existing theory [46–48] has overlooked the possibility that maternal effects might influence animal inbreeding strategies. Until now, theoretical models have focused on how the costs of dispersal, mating system, mate encounter rate, and kin recognition might shape the balance between inbreeding tolerance and avoidance [46–48]. We propose that incorporating maternal effects into such models may help us better understand and predict when animals should avoid, tolerate, or prefer inbreeding, which has so far been challenging [2].

In summary, our study provides novel insights into the role of maternal effects in altering the expression of inbreeding depression. We show that inbreeding depression in larval survival was less severe for offspring of small females than for offspring of large females. This pattern might be driven by differences in how small and large females invest in an inbred (low-quality) brood because of their different prospects for future reproduction. We recommend that future research investigates how other maternal traits, such as age, nutritional condition, and health status, might affect the severity of inbreeding depression within or among populations. In natural populations that are subject to inbreeding, maternal effects may contribute to both variation in the magnitude of inbreeding depression and variation in the strength and direction of selection for inbreeding avoidance.

Data accessibility. The raw data are available on the Dryad Digital Repository [49].

Authors' contributions. N.P. conceived the study, designed the study, collected data, carried out the statistical analyses, and wrote the manuscript. P.T.S. helped design the study and provided feedback on the manuscript.

Competing interests. We have no competing interests.

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