Causes and consequences of plasticity in parental and offspring behaviour in the burying beetle *Nicrophorus vespilloides*

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A Ratz T, Nichol T, Smiseth PT (2020) Parental responses to increasing levels of handicapping in a burying beetle. Behavioral Ecology 31:73–80.

B Ratz T, Smiseth PT (2018) Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*. Journal of Evolutionary Biology 31:646–656.

C Ratz T, Stenson S, Smiseth PT (2020) Offspring preferentially beg towards and associate with larger parents in the burying beetle *Nicrophorus vespilloides*. Behavioral Ecology, 31:1250–1256.

D Ratz T, Perodaskalaki A, Moorad J, Smiseth PT (2020) Effects of inbreeding on behavioural plasticity of parent-offspring interactions in a burying beetle. Journal of Evolutionary Biology 33:1006–1016.

E Ratz T, Castel E, Smiseth PT (2018) Male assistance in parental care does not buffer against detrimental effects of maternal inbreeding on offspring. Frontiers in Ecology and Evolution 6:196.

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: These experiments were designed with the help of my supervisor, Dr Per Smiseth. The majority of the data were collected by Thomas Nichol under my supervision. I performed all analyses presented here and wrote the subsequent manuscripts and chapter in collaboration with Dr Per Smiseth.

Chapter 4: The data were collected and analysed in collaboration with Katy Monteith. I wrote the subsequent manuscript and chapter in collaboration with Dr Pedro Vale and Dr Per Smiseth.

Chapter 5: The data were collected with Katerina Kremi and Lyndon Leissle. I performed all the analyses presented here and wrote the subsequent manuscript and chapter in collaboration with Dr Per Smiseth.

Chapter 6: The majority of the data were collected by Sophie Stenson under my supervision. I performed all the analyses presented here and wrote the subsequent manuscript and chapter in collaboration with Dr Per Smiseth.

Chapter 7: The data were collected in collaboration with Anastasia Perodaskalaki. I performed all the analyses presented here and wrote the subsequent manuscript and chapter in collaboration with Dr Jacob Moorad and Dr Per Smiseth.

Chapter 8: The data were collected in collaboration with Elise Castel. I performed all the analyses presented here and wrote the subsequent manuscript and chapter in collaboration with Dr Per Smiseth.

Tom Ratz

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Lay Summary

Animals inhabit an environment that is constantly undergoing changes: weather conditions and temperature depend on the season and the time of the day; access to food and exposure to predators vary across places. Flexible behaviours allow animals to quickly take into account these changes to make an appropriate decision in a given situation. Making the right decision at the right time heavily influences the ability to survive, grow, and reproduce. To ensure success in rearing a brood, animals have evolved the ability to adjust the amount of care they provide according to changing conditions in the surrounding environment. In turn, the young have evolved the ability to adjust how much care they demand from their parents according to their hunger and needs for protection. In this dissertation, I examine how such adjustments in behaviour come about, and what is their impact on growth and survival of parents and their young. My study system was the burying beetle (*Nicrophorus vespilloides*), which uses the carcass of a small bird or a rodent for breeding. In this species, parents remain with the brood after hatching and provide elaborate care that includes feeding the larvae with regurgitated carrion. Larvae can obtain more food by begging from a parent. I first characterise behavioural adjustments to various aspects of the environment, such as the energy expenditure, number of young in the brood, exposure to infection, food abundance and adult body size. I found that behavioural adjustments oftentimes reflect a strategy to make the most of changing conditions, but that these adjustments can also take an unexpected turn depending on prospects for survival and future reproduction. I next focus on the consequences of adjustments in parental and offspring behaviours. I found that the ability to adjust behaviour has generally an impact on growth and survival. Interestingly, I also found that behavioural adjustments commonly impact on the growth and survival of other individuals, such as young interacting with parents and vice versa. Overall, my findings highlight that flexibility is at the core of complex parental and offspring behaviour, and provides a major way to take advantage of new conditions in an environment that is constantly changing.

Abstract

Behavioural plasticity, the environmentally induced change in behaviour, is a reversible response that allows a rapid switch in activity to best match the environment. Behavioural plasticity is a widespread mechanism influencing the ability to find resources, reproduce and survive. Behavioural plasticity is particularly important in parent-offspring interactions because it allows parents and offspring to finely tune costly behaviours, such as parental care or offspring begging, to avoid unnecessary expenditure and obtain the highest returns from the interaction. In this thesis, I examined the role of plasticity in parental and offspring behaviour in response to changes in various aspects of in the intrinsic and environmental conditions in the burying beetle *Nicrophorus vespilloides*: energetic costs, infection status, resources availability, and parent's body size. I first showed how females unexpectedly increase parental care with higher energetic costs and that females do so irrespectively of variation in brood size. Next, I showed that infected females maintain their level of care despite suffering from high mortality. I further showed that resource availability has a positive effect on biparental cooperation over care, as males tend to provide care for longer when resources are more abundant. I also showed how larvae preferentially beg towards larger females as they spend more time associating with larger females over smaller ones. I focused the final part of the thesis on the consequences of behavioural plasticity and tested whether inbreeding can alter plasticity in adult and larval behaviour, and how parent-offspring and male-female interactions mediate the effects of inbreeding depression. I found evidence that inbreeding can increase plasticity in offspring behaviour. Moreover, I found that maternal inbreeding has detrimental effects on offspring survival, and that these effects remain regardless of the presence or the inbreeding status of the male parent. Collectively, these findings confirm that behavioural responses oftentimes allow balancing the costs and benefits of a behaviour, but that the direction of behavioural adjustments can also change unexpectedly depending on prospects for survival and future reproduction. These findings provide further evidence indicating that the intrinsic and environmental

conditions not only shape the behavioural responses and fitness of focal individual, but also influence the behavioural responses and fitness of social partners. Overall, these studies provide additional support to the idea that behavioural plasticity might be a key step in the emergence of complex behavioural phenotypes and a major source of behavioural diversity.

Résumé vulgarisé[1]

Les animaux vivent dans un environnement qui change constamment : les conditions climatiques et la température dépendent de la saison et du moment de la journée ; l'accès à la nourriture et le risque de prédation varient en fonction de la situation géographique, etc. La flexibilité des comportements permet de répondre rapidement à ces changements et de prendre une décision adéquate dans une situation donnée. Cette capacité à modifier son comportement est primordiale puisque les différentes décisions ont une influence profonde sur la survie, la croissance et la reproduction d'un individu. Pour assurer le succès de leurs jeunes, les parents ajustent leur attention parentale en fonction des conditions environnantes qui fluctuent. Les jeunes ajustent également leur demande en soin parental en fonction de leurs besoins en nourriture et protection. On comprend encore mal comment de tels ajustements du comportement face aux changements environnementaux se produisent et quel est leur impact sur la reproduction et la survie. Dans cette thèse, mon objectif est de clarifier la cause et les conséquences des ajustements comportementaux des parents et des juvéniles. Mon modèle d'étude est le scarabée nécrophore (*Nicrophorus vespilloides*), qui utilise le cadavre d'un petit oiseau ou rongeur comme source de nourriture pour ses larves. Chez cette espèce, les parents s'occupent de la portée et alimentent les larves avec de la nourriture prédigérée, prélevée directement sur le cadavre. De leur côté, les larves obtiennent de la nourriture en quémandant aux parents. Dans un premier temps, je caractérise les ajustements comportementaux en réponse à différents aspects liés à l'environnement, tels que la dépense en énergie, la taille de portée, le risque d'infection, l'abondance en nourriture et la taille corporelle à l'âge adulte. Les résultats montrent que les ajustements comportementaux reflètent le plus souvent une stratégie visant à tirer profit de nouvelles conditions environnementales, mais que la direction de ces ajustements peut aussi être inattendue suivant les perspectives de survie et de reproduction. Je me focalise ensuite sur les conséquences des ces ajustements comportementaux. Ici, mes résultats montrent que l'habilité

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[1]This is a translation of the lay summary in French.

à ajuster le comportement a, en général, un impact important sur la croissance et la survie. De façon intéressante, les ajustements comportementaux ont aussi souvent un impact sur la croissance et la survie d'autres individus, tels que les larves interagissant avec les adultes, et vice versa. Dans l'ensemble, mes résultats soulignent le rôle central de la flexibilité dans les comportements complexes, comme les interactions parent-progéniture, et comme moyen d'exploiter au mieux un environnement en changement constant.

To my parents and siblings,

for their flexibility and all their care.

1 General Introduction

When we observe animals, we quickly notice that they display sequences of behaviours that vary in nature and intensity: organisms switch between resting and activities, such as foraging, feeding, interacting with conspecifics, moving away from a predator or towards a shelter. These activities involve one or a set of behavioural traits that constantly change in manner. For instance, an individual that interacts with others might vary in its level of aggressiveness, from simple behavioural displays to physical aggression. This temporal variation in behavioural traits is known as behavioural plasticity (West-Eberhard 1989, Komers 1997). Behavioural plasticity is a form of phenotypic plasticity, which corresponds to the expression of a phenotype that varies according to the environment. Behavioural plasticity is remarkable in the sense that it is generally a quick and a reversible change in the phenotype (Mery and Burns 2010), allowing to rapidly switch activity to best match the environment. As such, behavioural plasticity is an important mechanism influencing population dynamics and the phenotype/genotype relationship, which could have a profound impact on evolution (Snell-Rood 2013). Although behavioural plasticity is well-documented, its role in evolution remains poorly understood (Snell-Rood 2013, Foster 2013). This is unfortunate because behavioural plasticity is widespread among animals and tightly linked to the ability to find resources, reproduce and survive (see below).

Behavioural plasticity is a key feature of social interactions because, in addition to environmental conditions, organisms have to adjust their response to social partner's behaviour, which is often unpredictable. Behavioural plasticity is thus crucial in behavioural interactions such as courting and mating (Jennions and Petrie 1997, Ingleby et al. 2010), intraspecific competition (e.g. Brenowitz et al. 1994, Marshall et al. 2003), and parenting (Royle et al. 2014). Furthermore, the

impact of a social behaviour might not be limited to the individual expressing the behaviour, but can also impact social partners and have more widespread consequences at the population level (Sih et al. 2004, Dingemanse et al. 2010, Snell-Rood 2013). From a mechanistic perspective, behavioural plasticity in social interactions is interesting because social interactions require high cognitive abilities (the social complexity hypothesis, Humphrey 1976). According to the social complexity hypothesis, social interactions are important drivers in the evolution of cognition (Seyfarth and Cheney 2015). Yet most animals show some form of sociality (at least during sexual reproduction) and behavioural plasticity should be a key mechanism underlying complex traits such as those mediating social interactions (Taborsky and Oliveira 2012). In species with overlapping generations, parents and offspring often interact repeatedly. Offspring might then seek to stay in close proximity to parents to benefit from their protection and interact by begging towards the parents to obtain food from them. Likewise, parents might seek to remain with the brood after hatching to protect offspring against predators or environmental hazards and supply them with food. Parent-offspring social interactions provide an ideal system to study behavioural plasticity in the context of social interactions for two main reasons. Traits mediating parent-offspring interactions – typically parental care and offspring begging – often have dramatic effects on the both parental and offspring fitnesses. Second, in species where offspring survival and growth depend on parental care, parent-offspring interactions tend to be repeated, stable and extended in time, making them a readily observable form of social interactions.

Behavioural plasticity is at the core of parent-offspring social interactions. It provides a mechanism for parents and offspring to finely tune costly behaviours, such as parental care or offspring begging, to avoid unnecessary expenditure and obtain the highest returns from the interaction. Offspring are selected for their ability to obtain the maximum amount of parental care whilst keeping the costs associated with begging as low as possible. In turn, parents are selected for their ability to provide care to a maximum number of young whilst keeping the

costs associated with care as low as possible. Behavioural plasticity in parent-offspring interactions also allows balancing investment into competing functions of the organism. Offspring begging might take up a large amount of the energy expenditure of developing offspring (Chappell and Bachman 2002). By adjusting begging and mitigating this expenditure, offspring are able to balance their investment in begging relative to investment in other functions, such as growth, survival and immunity, and this under a wide range of nutritional and environmental conditions. For example, starvation, extreme temperatures or infection might suppress offspring body condition. Under these circumstances begging for food may become more costly if, for example, offspring in poorer condition have to make a greater effort to beg. Alternatively, begging may be more beneficial to offspring in poorer condition if it allows to compensate for their lower state by obtaining more care. Flexibility begging allows accounting for such variation in the costs and benefits of begging. Likewise, parental care is an important component of a parent's reproductive investment. Being able to adjust their provisioning to offspring and balance it with allocation towards other reproductive components, parents can compensate for changes in their ability to provide care. For example, poor nutrition, predation risk or infection might suppress parental condition. Under these circumstances, it might pay off to reduce parental care to shift resource allocation towards somatic maintenance, or in contrast shift resources towards reproduction and parental care, as a form of terminal investment. Hence, flexible parental care allows behavioural adjustment to account for variation in the costs and benefits of care.

Behavioural plasticity is thus a way to make the best of a bad situation and handle a reduction in condition by, for example, mediating the impact of starvation, infection, or ecological hazards. This is particularly the case in parent-offspring interactions because parents and offspring not only have to deal with variation in intrinsic and ecological conditions, but also to unpredictable variation in the social environment that relies on other forms of interactions: siblings cooperation and competition (Mock and Parker 1997), and biparental cooperation and conflict

(McNamara et al. 2000). As well as responding to variation in the ecological environment, individuals should thus also respond to social factors that depend on the level of sibling competition (e.g. brood size, asymmetry in sibling competitive ability) and the level of biparental cooperation (e.g. partner's presence or contribution to care). From a broader perspective, parent-offspring interactions are closely linked to parent-offspring conflict, which can have a profound impact on evolution. This is because parent-offspring conflict generates opposing selection on parental provisioning (i.e. selection operating at the juvenile stage favours individuals that obtain more parental provision, whereas selection operating at the adult stage favours individuals that reduce their provisioning per offspring), it can have dramatic consequences on the evolutionary process. For example, Rollinson and Rowe (2015) propose that parent-offspring conflict could explain why juvenile body size in birds often does not evolve despite heritability and directional selection, thus showing evolutionary stasis. There is, however, little knowledge about the role of behavioural plasticity in such conflict (Kilner and Hinde 2012). The conflict resolution and the direction of the potential co-adaptation between parental and offspring behaviour should depend whether the offspring or parents control the supply of care (Smiseth et al. 2008, Hinde et al. 2010). Yet the predictions are complicated by the high degree of plasticity in the interacting traits (Kilner and Hinde 2012) and a more realistic model should focus on the link between the parental and offspring behavioural reaction norm (Smiseth et al. 2008).

In this thesis, I explore the role of behavioural plasticity in parent-offspring interactions. I ask how parents and offspring adjust their behaviour in response to variation in the ecological and social environment. I further investigate how flexible parental care relates to the broader life history strategy of an individual and how it allows parents to adjust their allocation in current versus future reproduction, or between competing functions, such as between reproduction, survival and immunity. Finally, I touch on the consequences of flexible decisions for fitness-related traits.

1.1 Plasticity in parental behaviour

Parental behaviour encompasses parental care, which can be defined as any parental behaviour that enhances offspring fitness and negatively impacts the parent's ability to invest in future offspring (Smiseth et al. 2012). Parental care is the rule in birds and mammals, but it is also common in many other species of vertebrates, such as of fish, reptiles and amphibians (Balshine 2012) and is present in some species of invertebrates (Trumbo 2012). Parental care is a key component of a parent's investment in reproduction. Furthermore, parental care has a great impact on fitness because of its positive influence on offspring survival and future reproduction. Caring parents incur a direct cost as care reduces the chance to survive and reproduce again, and obtain indirect (fitness) benefits in terms of enhanced offspring survival and growth. In order to obtain the greatest returns from care, parents must then balance their care according to these costs and benefits.

However, environmental conditions are likely to change the benefits and costs of care, which forces parents to respond dynamically and continuously readjust care to environmental variable such as temperature, resource availability, and predation or pathogen threats. For instance, parents in Kentish plovers spend more time incubating the clutch under higher temperatures to ensure that the eggs are kept under a temperature suitable for embryonic development (Vincze et al. 2013), females in the European earwig increase food provisioning when food resources are more abundant (Wong and Kölliker 2012), and females in lizards spend more time guarding the clutch in the presence of predators (Huang et al. 2013). These behavioural adjustments reflect that the costs and benefits of care are not fixed but prone to variation, to which parents respond. Temporal and spacial variation in food abundance, temperature or predation occurs within a short timescale, driving parents to adjust their level of care between breeding seasons and reproductive attempts (e.g. Ratz et al. 2016), offspring developmental stages (e.g. Smiseth et al. 2007a), or to rapid changes in offspring nutritional needs (e.g. Hinde and Kilner 2007). Thus, in species where parents live long

enough to experience multiple environments, parental behaviour should evolve to become highly flexible, reflecting that parental care is sensitive to environmental variation (Westneat et al. 2011, Royle et al. 2014).

1.1.1 Biparental behaviour

Oftentimes parents do not care in isolation but receive help from a partner. In this case, each parent will not only have to adjust its own care to changes in the ecological environment, but also to its partner's contribution to care. Because, as I will explain below, the contribution of a partner affects the costs and benefits of care in a different way than do other environmental variables, it is necessary to take a different approach to understand flexible care in biparental situations.

When both parents provide care, parents have to coordinate their behaviour to best match offspring needs and obtain the highest benefits from care. Given that the benefits to parents are measured in terms of success in raising the common brood, these benefits are shared between the two parents. In contrast, each caring parent reduces it own, but not its partner's (at least not directly), future reproduction and/or survival. Thus, while the benefits of care are shared between parents, the costs are decoupled and independently paid by each individual parent. As a consequence, each parent should try to minimise the costs by reducing its own level of care, while maintaining necessary levels of care to the brood by having the partner increasing care. This is the essence of the evolutionary sexual conflict over care (Trivers 1972, Houston et al. 2005). How much care a focal parent provides will thus likely depend on how much care its partner is providing. For instance, empirical work has shown that parents in some species fully compensate for a reduction in partner's contribution to care (Sanz et al. 2000, Stoehr and Hill 2000), whereas in other species parents show partial compensation (Schwagmeyer et al. 2005, Lendvai et al. 2009) or even a reduction in care (Hinde 2006). Rather than just asking what is the optimal strategy of a parent in a given environment, theoretical studies on biparental care need to take on a game-theoretic approach. This approach assumes strategies that depend on the

strategy displayed by other individuals (who are breeding partners in the case of care) in the population (Maynard Smisth 1977).

Parental behaviour in biparental care situations is relevant to the study of behavioural plasticity because parents tend to adjust care according to variation in partner's contribution, which might change between partners and across different breeding attempts (e.g. Nakagawa et al. 2007), or even during the course of a single breeding attempt as (e.g. Schwagmeyer et al. 2003, Westneat et al. 2011). Each parents is thus expected to adjust its care continuously to variation in its partner's contribution (McNamara et al. 2000).

1.1.2 Plastic parental behaviour

From what I described above, it should become clear that phenotypic plasticity is a fundamental feature of parental behaviour. Accordingly, we should give equal importance to the average level of care and to variation around the average. The average parental care or the total amount of care provided per offspring should of course influence offspring growth and survival. Yet if parental care is not provided at the right time, i.e. when offspring most need it, providing a large amount of care might be a poor strategy. Therefore, in parallel to the amount of care, parents should pay attention to changes in offspring needs and in other environmental conditions when adjusting care. As such responsiveness in parental behaviour should often reflect adaptive phenotypic plasticity (Royle et al. 2014).

In order to study the consequences of parental behaviour plasticity on parents and offspring, a powerful experimental approach is to manipulate the ability of parents to respond plastically. Although manipulating the average amount of care is in general straightforward, for example by using handicapping experiments (e.g. Wright and Cuthill 1989, Harrison et al. 2009, Suzuki and Nagano 2009), experimentally altering plasticity in parental behaviour is a difficult task. This is because rather than interfering with the ability to express the behaviour, experimenters have to find a way to interfere with the ability to change the behaviour

while expressing it at similar levels. One way to alter the expression of phenotypic plasticity is to reduce the ability to detect a change in the cues that stimulate a plastic response and/or the ability to mount a plastic response. Suppressing the condition or quality of experimental individuals can provide a way to alter behavioural plasticity, while not necessarily altering trait value of the behaviour. In this respect, the mating between related individuals – inbreeding – could produce a generation of individuals of lower quality. In addition to providing a potential experimental tool to manipulate plasticity, inbreeding is an important issue in evolutionary biology and conservation biology (Charlesworth and Charlesworth 1987, Reed et al. 2012). This is because inbreeding is associated with reduced genetic variation and evolutionary potential (Charlesworth 2003, Charlesworth and Willis 2009), and has crucial implications for the evolution of mating systems and reproductive strategies (Escobar et al. 2011, Liu et al. 2013, Szulkin et al. 2013). There is thus additional interest in studying inbreeding as it can have useful applications in other fields of biology.

1.2   Plasticity in offspring behaviour

When receiving care, offspring are generally not just passive but might try to influence parental care by actively associating with and displaying behaviour directed towards parents. Begging displays are generally striking signals through which the offspring advertise their nutritional need, involving visual (e.g. Weygoldt 1980), auditory (e.g. Redondon and Castro 1992, Kilner 1997, Haskell 1999) or tactile displays (e.g. Tretzel 1961, Milne and Milne 1976). Begging signals are key for the offspring in many organisms to obtain food and enhanced growth and survival. In most cases, begging is costly in terms of reduced growth (e.g. Kilner 2001, Takata et al. 2019) or reduced survival (e.g. Haskell 1994, Redondo and Castro 1992, Andrews and Smiseth, 2013). Offspring are thus expected to mitigate such costs by begging only when they need food and by begging in accordance to their nutritional need. The costs and benefits of begging vary with environmental variables such as food availability and parental food provisioning, sibling competition, or predation risk. For example, Haskell (1994) showed that

experimentally increasing begging in birds nesting on the ground, where preda-
tion risk is high, is associated with increased chick mortality due to predation.
In contrast, experimentally increasing begging in birds nesting in trees, where
predation risk is low, did not change chick mortality due to predation. These
findings show that the costs of begging for a chick vary with the risk of preda-
tion. Thus, offspring should plastically adjust their behaviour to the costs and
benefits of care, which are susceptible to change constantly according to offspring
nutritional needs and environmental conditions.

To interact with parents, offspring must stay physically close to them. There are
two main reasons explaining why offspring associate with parents and why this is
a key aspect of offspring behaviour. First, to be able to beg effectively, offspring
must ensure that their begging signals reach their parents. Although parents
might perceive vocal signals from a short distance, visual displays and tactile
signals require the offspring to be in close contact with parents. Second, in species
where the juvenile stage is mobile, such as in precocial birds, offspring often
benefit from staying closely associated with parents as they benefit from receiving
other forms of care, such as protection, brooding or guiding towards a source
of food. Thus, an important part of offspring social interactions with parents
involves physical association. As for begging, this behaviour comes at some costs
and benefits to the offspring, who should adjust their behaviour accordingly.

1.2.1   Plastic offspring behaviour

As explained above with parental care, the average or total level of begging and
time spent associating reflects only one aspect of offspring behaviour. Another
key aspect is the responsiveness of offspring behaviour. In other words, how
quickly and to which degree offspring adjust begging and time spent associating
with parents in response to environmental variation. The optimal behaviour, i.e.
the behaviour that maximises the difference between the costs and benefits of
begging or time spent associating, varies according to environmental conditions
and plasticity in offspring behaviour might be crucial to understand the associa-

tion between offspring behaviour and fitness. As such, offspring that accurately adjust begging and association in response to nutritional needs should do better than offspring that do not adjust their behaviour and beg or associate at a constant level. This is because the former will always be able to beg to obtain the necessary amount of provisioning from the parents, while keeping the costs as low as possible. In contrast, offspring not adjusting their behaviour might on average obtain more food, but will also pay higher costs, even when begging is not necessary.

Again here, inbreeding could offer a useful experimental tool to alter plasticity in offspring behaviour. This is because, in order to adjust behaviour to variation in hunger and environmental conditions, offspring need to be able to assess these changes and mount an appropriate plastic response. Inbreeding is often associated with inbreeding depression, which arises from the expression of rare, recessive and deleterious alleles in inbred individuals (Davenport 1908, East 1908). Given that inbreeding and inbreeding depression could interfere with both pathways and reduce the ability to detect and/or respond to this variation, inbreeding could provide a way to manipulate the ability of expressing normal level of behaviour plasticity.

1.3   Study System: Burying Beetle *Nicrophorus vespilloides*

Burying beetles (Figure 1.1a) belong to the genus *Nicrophorus*, which includes over 75 species distributed in temperate areas, mainly in North America, and Northern Europe and Asia, albeit some species live in South East Asia and Central and South America (Portevin 1926, Peck 1982, Sikes et al. 2002). Burying beetles are particularly suitable for investigating behavioural plasticity in parent-offspring interactions : they have a relatively short life cycle of 6 weeks at 20°C and are readily maintained under standard laboratory conditions, in which they breed and provide care to the brood in a similar manner as in natural populations (Scott 1998). This allows conducting experimental manipulations and behavioural observations under controlled, laboratory conditions. In addition,

laboratory-based setups facilitate repeated measures on the same individuals and thus experiments quantifying behavioural plasticity within the same individuals.

In order to breed, a male and female burying beetle have to secure the carcass of a small vertebrate, typically a rodent or a small bird, which will serve as the sole food source for the larvae and breeding parents. Although burying beetles can breed multiple times in the laboratory (e.g. Creighton et al. 2009, Cotter et al. 2010), given intense competition for access to carcasses (Otronen 1988, Scott 1990, 1994) it seems likely that most individuals in wild populations rarely breed more than once. In addition to resource provisioning, parents provide a number of pre- and post-hatching forms of care. They first prepare the carcass removing the fur (or feathers) and roll it into a ball, while burying it under the ground. The female lays eggs in the soil around the carcass, which will develop within three days at 20°C (Smiseth et al. 2006). Given that egg laying is asynchronous and take place over a mean period of 27 h, there is often asymmetry in body size of offspring in the early stages of their development (Müller 1987, Smiseth et al. 2006). Parents coat the surface of the carcass with anal and buccal antimicrobial exudates that reduce fungal and bacterial growth (Hoback et al. 2004, Rozen et al. 2008). To enable larvae to access inside the carcass after hatching, parents cut an opening in the skin around the abdomen. Larvae then start feeding from the carcass, albeit they initially receive a large part of their food from the parents (see next section; Figure 1.1b). Larval development inside the carcass lasts for a period of 5 days, after which larvae leave the carcass to disperse in the surrounding soil (hereafter referred to as dispersal) and start pupation. The average brood size on large carcasses (i.e. 20–25g) is 21 larvae (Smiseth and Moore 2002). At dispersal, larvae stop feeding and have reached a size that largely determines their future adult size (Lock et al. 2004). Before initiating pupation, larvae spend several days wandering in the soil looking for a site suitable for pupation, and will then form a pupal chamber by rolling their body and pack the soil around them to shape an ovoid chamber. Approximately three weeks elapse between dispersal and the emergence of newly eclosed adult beetles.
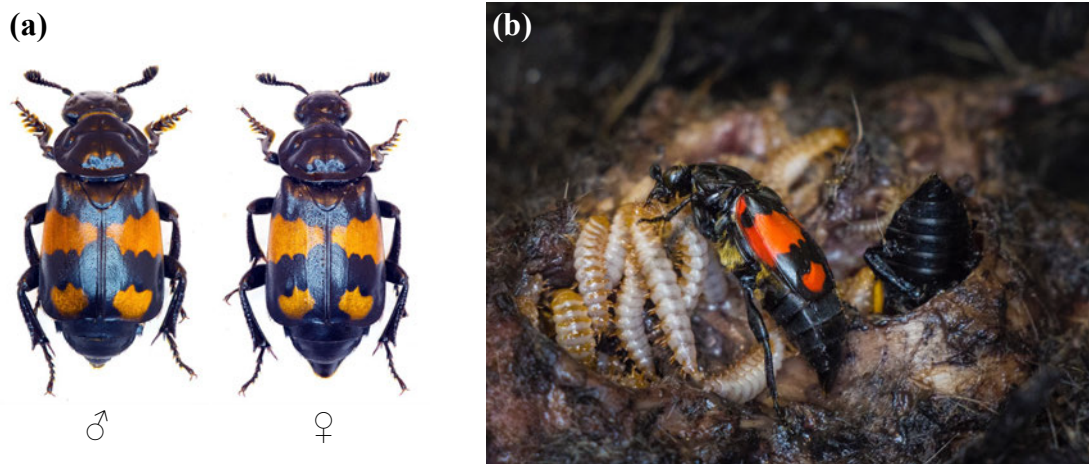
Figure 1.1: The burying beetle *Nicrophorus vespilloides*. The two sexes are of similar size and show little morphological difference (a). Both parents provide care to the brood, which includes regurgitating pre-digested carrion to begging larvae (b). Females sometimes breed communally on large carcasses. In communal breeding situations, females lay the eggs as a single batch and provide care to the joint brood. Here, two co-breeding females are caring for the joint brood.

1.3.1 Parental and offspring behaviour in *N. vespilloides*

Both parents provide care continuously during larval development as the form of carcass maintenance, guarding, and food provisioning to the brood. Carcass maintenance includes the spreading of antimicrobial exudates on the surface of the carcass and the excavation of the soil surrounding the carcass forming a depression (hereafter referred to as the crypt). Parents provision the brood by regurgitating pre-digested carrion via mouth-to-mouth contact with larvae. Females generally remain at the carcass and care for the brood until larval dispersal, whereas males tend to desert the brood several days earlier (Bartlett 1988, Scott 1998, Scott and Traniello 1990, Trumbo 1991). When both parents are present, the female spend on average more time than the male on food provisioning (Smiseth and Moore 2004a, Trumbo 2007). Under laboratory conditions, male removal has no detectable effects on larval growth or survival (Smiseth et al. 2005). However, it seems that male parental care would be highly advantageous in the wild as two parents might be better able to deter conspecific intruders or predators, which can greatly reduce offspring survival (Trumbo 1991). Biparental care in burying beetles might thus be crucial to offspring survival in the presence of competitors

or predators.

Larvae beg for food by associating with a parent, raising their body and waving their legs at the parent's mouth parts (Rauter and Moore 1999). Begging in burying beetles is thus a tactile signal. Begging is costly as begging larvae incur a growth cost (Takata et al. 2019), as well as a potential survival cost as the female targets individuals that beg more when culling larvae (Andrews and Smiseth 2013). Given that larvae mainly beg at early and middle stages of their development, it seems likely that begging enables obtaining food that is otherwise difficult to access and/or process for young larvae. Larvae also obtain parental bacteria that are essential to constitute their microbiota from regurgitated food (Wang and Rozen 2018). Larvae would beg towards a dead adult beetle, which can be used as a stimulus in experiments investigating larval behaviour. Using a dead adult as stimulus allows avoiding any potential confounding effects of parental behaviour on offspring (Smiseth and Parker 2008, Mäenpää et al. 2015, Paquet et al. 2017, Ratz et al. 2020c).

Females occasionally tolerate the presence of other females on larger carcasses and breed communally (Komdeur et al. 2013, Eggert and Müller 2000, Richardson and Smiseth 2020). Communal breeding occurs in burying beetles presumably because a single female is not able to fully defend a large carcass and because a large amount of resources allows raising multiple broods successfully. When multiple females lay their eggs, it is also impossible for parents to identify their own offspring. This is because females simply use temporal kin discrimination, whereby they kill any larvae arriving on the carcass before their own eggs would have hatched (Müller and Eggert 1990). In a communal breeding situations, several females cooperate to provide care to the joint brood and larvae beg indifferently towards all females.

1.3.2 Behavioural plasticity in *N. vespilloides*

Offspring and parental behaviours in burying beetles are highly flexible in response to variation in intrinsic, ecological and social conditions. For instance, larvae beg more at younger ages (Smiseth et al. 2007a), under limited access to food (Smiseth and Moore 2004b, 2007), and in smaller broods than in larger ones (Smiseth et al. 2007a). Likewise, parents adjust their behaviour to brood size, spending more time on food provisioning towards large broods relative to small ones (Rauter and Moore 2004, Smiseth et al. 2007a, Ratz and Smiseth 2018). Parents adjust their behaviour to larval age and partner's presence, spending more time provisioning food when larvae are about one day old compared with younger or older larvae (Smiseth et al., 2003, Smiseth et al. 2007a) and in the absence of a partner (Smiseth et al. 2005, Suzuki and Nagano 2009). There is also evidence that parents adjust care to variation in ecological conditions, such as resource availability (Smiseth and Moore 2002), intraspecific competition (Hopwood et al. 2015) and the presence of intruders at the carcass (Georgiou Shippi et al. 2018).

1.4 Aims

As highlighted above, behavioural plasticity plays a fundamental role in parent-offspring interactions because individuals have to adjust behaviour to others, as well as variation in intrinsic, social and ecological conditions. I also alluded that parent-offspring social interactions among conspecifics can have a profound impact on population dynamics and evolutionary processes. Given these two statements, clarifying how interacting individuals make flexible decisions is a first step towards integrating more realistic responses of organisms in models of ecological and evolutionary dynamics. This step requires knowledge about the aspects of the social and ecological environment that triggers a shift in the social behaviour, how this shift affects the fitness of the focal individual and the fitness of non-focal conspecifics via social interactions. The goal of this thesis is to contribute to this task.

My first aim is to clarify the role of behavioural plasticity in parental and offspring behaviour in response to a number of intrinsic, social and ecological variables. My second aim is to determine the consequences of behavioural plasticity on the behaviour, life histories and fitness of both focal and non-focal individuals. Chapters 2 and 3 mainly focus on the energetic costs of care. Chapters 4 and 5 focus on the effects of ecological variables on parental care: pathogenic infection and resource availability. Chapter 6 is about offspring responses to parental body size. In Chapter 7, I examine the potential effects of inbreeding on plasticity of offspring and parental behaviours. Chapter 8 goes a step further by looking at the consequences of male contribution to care and maternal inbreeding on offspring performance. In Chapter 9, I discuss the main findings stemming from the different data chapters and the broader implications for ecology and evolutionary biology. Below, I provide an overview of each of the data chapters.

Chapter 2: Parental response to increasing energetic costs

The aim of this chapter is to clarify how females adjust their care in response to different levels of impairment caused by a handicap imposing a physical constraint on movement. Given that providing care is often associated with energetic expenditures, parents facing higher energetic or physiological costs should reduce their care. Parents could also perceive such costs as a constraint reducing their chance to successfully reproduce in the future and respond to handicapping by shifting their investment towards current reproduction, thus providing more care. In this chapter, I investigate how caring females respond to different levels of handicapping. I further test whether the effects of the handicapping procedure and the potential shift in investment affect female life span and reproductive output.

Chapter 3: Parental responses to variation in energetic costs and brood size

In this chapter, I test if females respond independently to an increase in both the energetic costs of care and the indirect benefits of care. Whether

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the responses to variation in the costs and benefits of care are independent from one another depends on the shape of the cost and benefit functions of care. Assuming that females respond in a nonlinear manner to variation in both the costs and the benefits of care, a large change in the costs of care should also impact on how females respond to the benefits of care, and vice versa. In contrast, if the change in the costs of care is relatively small, it should have no impact on how females respond to a change in the benefits of care. I investigated this issue here using a handicapping experiment and a brood size manipulation to alter simultaneously the direct costs and the indirect benefits of care.

Chapter 4: Parental responses to infection

In this chapter, I explore whether caring females change or maintain their level of care when facing an infection by a bacterial pathogen. In general, an infection caused by a pathogen induces a reduction in the host's activity and social interactions, which can reflect a response of the host to redirect resources towards immunity and potentially mitigate the risk of disease transmission to close kin. In burying beetles, however, there is evidence that females increase their reproductive investment in response to infection as a form of terminal investment, and could thus potentially increase their care. Here I test these contrasting predictions by estimating female investment in immunity, reproduction and maternal care.

Chapter 5: Biparental responses to resource availability

When two parents cooperate to provide care to the joint brood, the benefits of care are shared between parents, whereas each parent pays its own costs of caring. Each parent should then reduce its own contribution to care, while expecting its partner to increase their contribution. This creates a conflict between the two parents over care. How this conflict is resolved and the degree to which each parents cooperate to provide care highly depends on environmental conditions, such as resource availability, that influence

the costs and benefits of biparental care. In this chapter I investigate how resource availability shifts the balance between cooperation and conflict between caring parents. I also test how the abundance of resources and the balance between cooperation and conflict affect sex differences in parental care.

Chapter 6: Offspring responses to parental body size

The aim of this chapter is to test whether offspring adjust their begging and association with parents based on attributes reflecting how much food the parents are likely to provide. Whenever offspring can beg to obtain food from multiple parents, there should be selection on offspring to maximise the returns on costly begging and beg preferentially towards the parent that provisions the most food. Given that larger parents are often able to provision more food than smaller ones, offspring should show a preference to associate with and beg towards a larger parent over a smaller one. I test this prediction here, giving experimental broods of larvae a simultaneous choice between a smaller female and a larger one. I then test between two potential mechanisms underpinning offspring choice for begging more towards a specific parent.

Chapter 7: Effects of inbreeding on parent and offspring plasticity

Here I aim at investigating whether inbreeding status alters behavioural plasticity in traits mediating parent-offspring interactions. Inbreeding commonly has negative effects on performance and can reduce sensory and cognitive abilities. This could ultimately reduce the ability to assess and mount an appropriate behavioural response to environmental changes. This assumption has never been tested in the context of behavioural plasticity and it was unclear whether inbreeding could alter social interactions by interfering with behavioural plasticity. I tackle this issue in this chapter and test whether inbreeding alters behavioural plasticity in parental care and

offspring begging and association with parents.

Chapter 8: Effects of biparental care and inbreeding on offspring performance

After having shown the effects of inbreeding on flexible parent-offspring interactions, I focus in this chapter on the indirect consequences of maternal inbreeding on offspring. The poor condition of a parent, reflected in this study by a higher degree of inbreeding, could have indirect detrimental effects on the offspring via parent-offspring interactions. Here I confirm this prediction and test whether the presence and inbreeding status of the other parent could offset the adverse effects of maternal inbreeding on offspring.

2 Parental response to increasing energetic costs

Abstract

Parental care is highly variable, reflecting that parents make flexible decisions about how much care to provide in response to variation in the cost and/or benefit of care. Handicapping has traditionally been used as a tool for increasing the energetic cost of care, thereby inducing a reduction in care by handicapped parents. However, recent evidence shows that handicapped parents sometimes provide more care, suggesting that handicapping can trigger terminal investment. Here, I investigate responses to different levels of handicapping in the burying beetle *Nicrophorus vespilloides* by comparing handicapped female parents fitted with a wide range of handicaps, as well as control females without a handicap. I found that handicapped females spent more time provisioning food and less time being absent from the crypt than control females, while there was no detectable effect of the level of handicapping among handicapped females. I found no effect of handicapping on larval begging behaviour, larval performance (mean larval mass and brood size at dispersal), or female investment in future reproduction (i.e., weight gain while breeding and life span after breeding). My findings provide no support for the widely held assumption that handicapping simply increases the cost of care. Instead, these results are consistent with the suggestion that handicapping triggers terminal investment by suppressing the condition of parents below the threshold at which terminal investment is triggered.

2.1   Introduction

Parental care encompasses any parental trait that enhances the survival and/or growth of a parent's offspring, often at a cost to the parent's ability to invest in other current or future offspring (Clutton-Brock 1991, Royle et al. 2012). Parental care is highly variable (Clutton-Brock 1991, Royle et al. 2012), reflecting that parents make flexible decisions about how much care to provide due to variation in the cost of care to themselves and/or the benefit to their offspring (Royle et al. 2014, Ratz and Smiseth 2018). For example, as shown by handicapping experiments on birds and insects, parents are expected to provide less care given an increase in the cost of care (Wright and Cuthill 1989, Harrison et al. 2009, Suzuki and Nagano 2009). Handicapping experiments are used to study negotiation between parents in birds with biparental care (Harrison et al. 2009), and their rationale is to increase the energetic cost of providing care at a given level by attaching a lead weight to the base of the handicapped parent's tail feathers (Wright and Cuthill 1989) or by clipping some of its flight feathers (Slagsvold and Lifjeld 1988, 1990). Most such experiments find that handicapped parents provide less care than control parents (e.g., Wright and Cuthill 1989, Harrison et al. 2009), confirming that parents plastically reduce the amount of care they provide when the cost of care increases. However, a recent study on the burying beetle *Nicrophorus vespilloides* found that handicapped females provided more care than control females (Ratz and Smiseth 2018). This finding contradicts the implicit assumption that handicapping simply increases the cost of care. In light of this, there is now a need to improve our understanding of how parents respond to handicapping given its important role in the study of parental care.

One potential explanation for why handicapped parents sometimes provide more care than control parents is that handicapping can trigger a shift towards greater investment in current reproduction (Ratz and Smiseth 2018), often referred to as terminal investment (Williams 1966, Clutton-Brock 1984). Theory suggests that terminal investment is triggered when an individual's condition deteriorates below a certain threshold value, thereby reducing its future survival prospects (Duffield

et al. 2017). Handicapping could trigger terminal investment if it suppresses the parent's condition below this threshold value by, for example, reducing its foraging ability or increasing its energy expenditure. Thus, current evidence suggests that handicapping might influence the parent's behaviour either by increasing the energetic cost of care or by triggering terminal investment. I note that these two effects are not mutually exclusive, as handicapping could both increase the cost of care and trigger terminal investment. If so, I might expect more complex responses to handicapping that are determined by a combination of whether or not the handicap suppresses the parent's condition below the threshold triggering terminal investment and the extent to which the handicap increases the energetic cost of care. As outlined below, in order to advance our understanding of the effects of handicapping, we now need novel experimental designs that monitor how caring parents respond to different levels of handicapping.

In this study, I investigated how female parents responded to different levels of handicapping in a burying beetle. Burying beetles of the genus *Nicrophorus* are ideal study systems to explore this issue because they show highly elaborate forms of parental care, including provisioning of predigested carrion to the larvae and depositing antimicrobial secretions to preserve the small vertebrate carcass used for breeding as a food source throughout larval development (Scott 1998). Unlike birds, burying beetles walk while provisioning for their current brood, whereas they fly while searching for carcasses for use in future reproductive attempts (Scott 1998). Furthermore, these beetles have been subject to handicapping experiments, showing that handicapped parents either provide less care, as reported in studies on *Nicrophorus quadripunctatus* and *N. orbicollis* (Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016), or more care, as reported in *N. vespilloides* (Ratz and Smiseth 2018). One potential explanation for why studies have reported contrasting effects of handicapping is that these studies used different levels of handicapping. For example, studies showing that handicapped parents provide less care used larger weights that were about 40–50% of a parent's body mass (Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki

2016), whereas the study reporting that handicapped parents provide more care used smaller weights that were about 20–30% of a parent's body mass (Ratz and Smiseth 2018). Although this pattern suggests that parents provide more care in response to a relatively small handicap but less care in response to a relatively large handicap, there is now a need for experimental work monitoring how parents respond to different levels of handicapping within a single species.

My aim was to investigate how single female parents respond to different levels of handicapping in the burying beetle *N. vespilloides*. I handicapped females by attaching a small weight to their pronotum (Suzuki and Nagano 2009). The weights weighed 0.037–0.242 g, corresponding to 11–103% of a female's body mass. I also included a control treatment, where females were not fitted with a weight but otherwise were handled in the same way as handicapped females. Prior work shows that females respond by providing more care when fitted with a 0.05 g weight (Ratz and Smiseth 2018), suggesting that the threshold triggering terminal investment is below this level of handicapping. I then tested for subsequent effects on the amount of care provided by females (i.e., time spent provisioning food and maintaining the carcass) during the period where females provide direct care for larvae, as well as on offspring performance (i.e., mean larval mass, number of larvae at dispersal, and larval begging behaviour) and female investment in future reproduction (i.e., weight change while breeding and life span after breeding).

If handicapping primarily increased the cost of care, I predicted that females should provide progressively less care as the level of handicapping increased (Figure 2.1a). Furthermore, offspring performance should gradually decline as the level of handicapping increases, and females should pay a progressively higher cost in terms of their investment in future reproduction. Conversely, if handicapping primarily triggered terminal investment, I predicted that the effects of the level of handicapping should be discontinuous with handicapped females providing more care than control females provided that the handicap suppressed the parent's condition below the threshold value (Figure 2.1b). Below this thresh-

old, handicapped parents should provide as much care as control parents. Above the threshold, handicapped parents should provide more care than control parents, but the former should provide the same level of care regardless of the level of handicapping (Figure 2.1b). Furthermore, offspring performance should be higher, while female investment in future reproduction should be lower, above the threshold than below. Finally, if handicapping both elevates the cost of care and triggers terminal investment, I predicted that the effects of the level of handicapping should be discontinuous with a marked increase in care by handicapped parents at the threshold value (Figure 2.1c). However, above this threshold, handicapped parents should provide progressively less care as the level of handicapping increases. Furthermore, offspring performance and female investment in future reproduction should gradually decline with the level of handicapping above the threshold.

2.2 Methods

The beetles used in this experiment came from a laboratory stock population originating from beetles collected at Corstorphine Hill Local Nature Reserve and Hermitage of Braid and Blackford Hill Local Nature Reserve, Edinburgh, UK. Nonbreeding adult beetles were housed in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil. All beetles were fed organic beef twice a week and maintained under a constant temperature (20°C) and a 16:8 h light:dark photoperiod.

2.2.1 Experimental design

I manipulated the level of handicapping by attaching a nontoxic fishing weight (Dinsmores, Aldridge, UK and DGT, Shirley, UK) to the pronotum of caring females (see below for further details). The weights used in my experiment weighed 0.037–0.242 g, corresponding to 11–103% of the initial body mass of females. I used this range to ensure that the handicaps overlapped the range used in prior
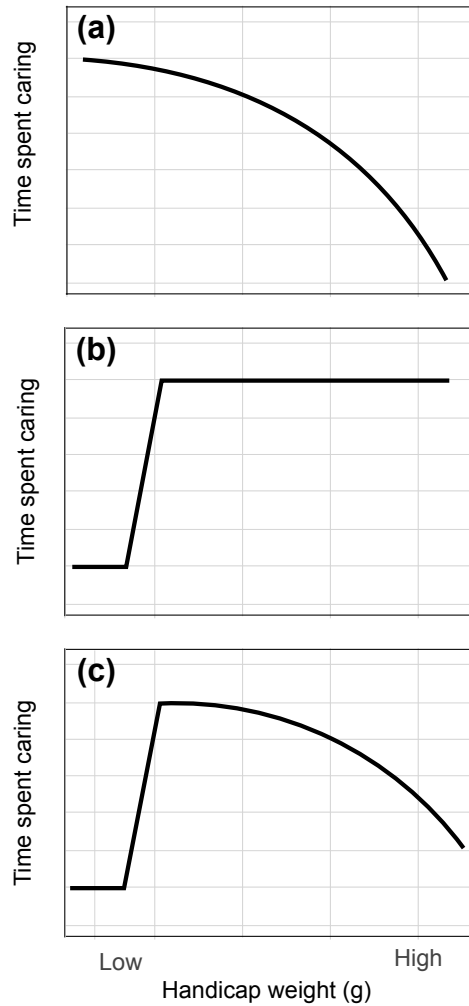
Figure 2.1: Predicted effects of the level of handicapping on the amount of care provided by parents. If handicapping primarily elevates the cost of care, parents should provide progressively less care as the level of handicapping increases (a). If handicapping primarily triggers terminal investment, the effects of the level of handicapping should be discontinuous with a marked increase in care by handicapped parents at the threshold value (b). Handicapped parents should provide as much care as control parents below this threshold, while they should provide more care than control parents above the threshold. Handicapped parents should provide the same level of care regardless of the level of handicapping above the threshold. If handicapping both elevates the cost of care and triggers terminal investment, the effects of the level of handicapping should also be discontinuous with a marked increase in care by handicapped parents at the threshold value (c). However, in this case, handicapped parents should provide progressively less care as the level of handicapping increases above the threshold.

work on this species (20–30%, Ratz and Smiseth 2018) and on *N. quadripunctatus* and *N. orbicollis* (40–50%, Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016). I also included weights that went beyond this range to ensure that

the handicaps were large enough to induce a potential increase in the energetic cost of care. My design included a control treatment, where females were not fitted with a weight but were otherwise handled and treated in the same way as handicapped females. In this experiment, I focused on the response of a single parent to exclude potential compensatory responses by its partner. I did this given that my aim was to establish whether handicapping increases the cost of care, triggers terminal investment, or both. I specifically focused on single female parents because females provide more parental care than males in this species (Eggert et al. 1998, Rauter and Moore 2004) and because the experimental removal of the male has no effect on offspring fitness under laboratory conditions (Smiseth et al. 2005).

I began the experiment by pairing females and males at random, transferring each pair into a larger plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass (Livefoods Direct, Sheffield, UK) of a standardized size (14.68–19.98 g). One day before the expected date of hatching (i.e., 2 days after the beginning of egg laying), I randomly assigned each female to the handicapping or the control treatment (i.e., no weight; hereafter referred to as 0 g). Although the nominal mass of the weights was categorical (0.05 g, 0.10 g, or 0.20 g), there was considerable variation in the mass of weights within each category (range, mean ± SE for 0.05 g, 0.10 g, and 0.20 g weights, respectively: 0.0370±0.0757 g, 0.0544 ± 0.0017 g; 0.0716–0.1241 g, 0.0959 ± 0.0019; 0.1702–0.2423 g, 0.1988 ± 0.0026). I weighed all females before and after subjecting them to the handicapping treatment, using the difference in mass as a measure of the mass of the handicap provided to each female. I attached the weight to the pronotum of each handicapped female using instant-adhesive glue (Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016, Ratz and Smiseth 2018). Before attaching the weight, I gently scraped the surface of the apex of the pronotum using fine sandpaper (P600). I did so to remove impurities, thereby improving adhesion of the weight. I treated females assigned to the control treatment in the same way as handicapped females (i.e., I

weighed them before and after handling, handled them, and scraped the surface of, and applied glue to, their pronotum), except that no weight was attached to their pronotum. For further details on the handicapping procedure, I refer to Ratz and Smiseth (2018).

Once handicapped females had been fitted with a weight and control females had been handled, I moved them together with their mouse carcass to a fresh container filled with moist soil. I did this to separate females from their eggs, thereby allowing me to provide them with standardized experimental broods. Once the larvae started hatching, I collected them in a temporary holding container, using them to generate experimental broods comprised of 10 same-aged larvae of mixed maternal origin (Smiseth et al. 2007a). For practical reasons, I allocated females broods comprising some larvae that were their own and some that were foreign. It is unlikely that this would influence my results as there is no evidence that females differentiate between their own and foreign larvae in this species. Instead, females have a temporal kin discrimination mechanism whereby they kill any larvae arriving on the carcass before their own eggs would have hatched (Müller and Eggert 1990). Thus, to avoid infanticide, I ensured that I only provided females with an experimental brood once their own eggs had hatched. I used experimental rather than natural broods in this experiment to control for potential confounding effects due to variation in the number of larvae in the brood and the age of the brood, both of which are known to influence the amount of care provided by females in *N. vespilloides* (Smiseth et al. 2003, Smiseth et al. 2007a, Smiseth et al. 2007b). Note that with this brood size and given the range of carcass size, I expected that there would be ample resources available for the larvae and thus little filial cannibalism from females. I removed male parents at the same time as I moved females to a fresh container.

I recorded data on the amount of care provided by handicapped and control females 24 h ($\pm$15 min) after I placed the larvae on the carcass. This time point corresponds to the peak in time spent providing care towards larvae in this species

(Smiseth et al. 2003). I collected behavioural data using instantaneous sampling every 1 min for 30 min under red light, in accordance with established protocols (e.g., Smiseth and Moore 2002, 2004b, Ratz and Smiseth 2018). Although the 30 min sampling period is a relatively small part of the period when females provide direct care for the larvae (larvae become nutritionally independent 72 h after hatching), there are positive correlations between different measures of parental care in *N. vespilloides* (Andrews et al. 2017), and the amount of time spent providing care 24 h after hatching is positively correlated with the time at which the parents desert the brood (Pilakouta N, Hanlon B, Smiseth PT personal communication). Thus, this sampling period is representative of the total amount of care provided by females. At each scan, I recorded whether the female was engaged in the following behaviours: provisioning food, defined as when there was mouth-to-mouth contact between the female and at least one larva, maintaining the carcass, defined as when the female was excavating the soil around the carcass or coating the carcass with secretions or absent from the crypt, defined as when the female was away from the crypt (i.e., the depression surrounding the carcass). I conducted the behavioural observations blind with respect to treatments as far as this was practically possible. The observations were blind for the different levels of handicapping, as it was not possible for the observer to identify the size of the handicap in the dim light conditions under which the observations were conducted. However, it was not possible to keep the control treatment (i.e., 0 g) blind, as the observer could tell whether females had been provided with a weight or not.

At the same time as I recorded data on the amount of care provided by females, I also recorded data on larval begging to test for potential effects of handicapping on larval behaviour. In burying beetles, larval begging is tactile and begging larvae raise their bodies towards the female and touch the female with their legs (Smiseth and Moore 2002). Larval begging only occurs when the parent is in close contact with the larvae, defined as a distance less than or equal to the width of the female's pronotum (Rauter and Moore 1999, Smiseth and Moore

2002). At each scan, I counted the number of larvae that were begging. I calculated the average proportion of time spent begging per larva in the brood as $B = (\Sigma b/n)/p$, where Σb is the cumulative number of begging events during the 30-min observation period, n is the brood size at the time of observation, and p is the number of scans during which the female was near the larvae. This metric provides a measure of larval begging that is largely independent of variation in female behaviour towards the larvae (Smiseth and Moore 2004a).

At the time of larval dispersal from the carcass, which normally takes place about 5 days after hatching, I recorded the number of surviving larvae in the brood and weighed the brood. I did this to test for potential effects of handicapping on offspring performance. I calculated mean larval mass by dividing the total brood mass by the number of surviving larvae in the brood. In this species, body size is a key determinant of an individual's reproductive success and adult body size is highly correlated with larval mass at dispersal (Otronen 1988, Safryn and Scott 2000). At the time of larval dispersal, I also removed the weights from the female's pronotum by gently twisting the weight or lifting it off using soft forceps. I removed the weights at this time to obtain information on the potential fitness cost of handicapping during the period when females provided care for their larvae. I then recorded the postbreeding body mass of each female, which I used to calculate the female's weight change while breeding as the difference between post- and prebreeding body mass. Finally, I recorded female life span after breeding. To this end, I moved all females into individual containers and I then checked each container twice a week and recorded the date of death for each female.

I set up 137 pairs in total in the course of this experiment. I excluded 3 females that did not lay any eggs, 11 females whose eggs did not hatch, and 3 females for which the weight of the handicap was recorded incorrectly, yielding the following final sample sizes for female parental behaviour, larval begging, mean larval mass at dispersal, and female weight change: control females (0 g weight: N = 30), and handicapped females (0.037–0.242 g: N = 90). I further excluded two females

from my analyses on brood size at dispersal because the number of larvae was uncertain, yielding the following final sample sizes for brood size: control females (N = 29), and handicapped females (N = 89). For female life span, I excluded 35 females for the reasons stated above and because I could not remove their weights, yielding the following final sample sizes for this trait: control females (N = 28), and handicapped females (N = 67).

2.2.2 Data analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team 2019). Behavioural traits were recorded as the total number of scans out of a maximum of 30 scans and were therefore analysed assuming a binomial error structure. Given that the data on time spent provisioning food, maintaining the carcass and absent from the crypt by females showed over-dispersion and minor zero-inflation, I analysed these data using a Bayesian approach with the $MCMC_{GLMM}$ R package (Hadfield 2010), fitting the models with a binomial error structure using "multinomial2" and a flat improper prior. I analysed data on offspring performance and female investment in current and future reproduction using general linear models with a Gaussian error structure for normally distributed traits (mean larval mass at dispersal and female weight change), and using generalized linear models with a binomial error structure for larval begging and a Poisson error structure for other traits representing count data (female life span and brood size at dispersal).

Given that my main aim was to test for an overall effect of the level of handicapping on the traits of interest and given the considerable variation in mass of fishing weights (see above for further details), I treated handicapping as a continuous linear predictor, including a quadratic term to test for possible nonlinear effects of handicapping. Whenever handicapping had significant linear and quadratic effects, I presented the data with a polynomial regression ± 95% CIs (see Results section below). I included the initial weight of the female at the

time of treatment as a predictor in the models to account for potential variation among different-sized females in their response to the level of handicapping. I also included brood size at the time of observation as a covariate in the model on female parental behaviour, and I included brood size at dispersal in the model on female weight change because brood size influences food provisioning in this species (e.g., Smiseth et al. 2007a, Ratz and Smiseth 2018). Finally, I included female weight change as a covariate in the model on female lifespan given that prior work shows that life span is positively correlated with weight change (Gray et al. 2018). Parameter estimates for the Bayesian model are given as posterior means $\pm$ 95% CIs of 1499 samples ran for 1.5×10^6 iterations with a thinning interval of 1.0×10^3 and a burn-in of 1.0×10^3.

2.3 Results

2.3.1 Female parental behaviour

Handicapping had a positive linear effect on the amount of time females spent provisioning food to the brood, while there was a negative effect of the quadratic term of handicapping (Figure 2.2a; Table 2.1). Visual inspection of confidence intervals suggests that handicapped females spent more time provisioning food than control females, but that there was no effect of the level of handicapping among handicapped females (Figure 2.2a). This interpretation is supported by posthoc tests, showing that handicapped females spent more time provisioning food than control females (estimate = 1.129, lower 95% = 0.416, upper 95% = 1.940, $P_{MCMC} = 0.001$) and that there was no effect of the level of handicapping when restricting the analysis to handicapped females (estimate = 18.4, lower 95% = 15.07, upper 95% = 50.9, $P_{MCMC} = 0.278$). Handicapping had a negative linear effect on the amount time females were absent from the crypt (thus near the brood), and there was a positive effect of the quadratic term of handicapping (Figure 2.2b, Table 2.1). Visual inspection suggests that control females were more likely to abandon the brood temporarily than handicapped females,

while there was no effect of the level of handicapping among handicapped females (Figure 2.2b). This interpretation is supported by posthoc tests, showing that handicapped females spent less time being absent than control females (estimate $= -6.510$, lower 95% $= -10.6$, upper 95% $= 2.000$, $P_{MCMC} = 0.001$) and that there was no effect of the level of handicapping when restricting the analysis to handicapped females (estimate $= -184.7$, lower 95% $= -451.1$, upper 95% $=$ 65.1929, $P_{MCMC} = 0.108$). There was no linear effect of handicapping and no effect of the quadratic term on time spent maintaining the carcass (Table 2.1).

There was no effect of brood size at the time of observation on time spent provisioning food (estimate $= 0.136$, lower 95% $= -0.026$, upper 95% $= 0.288$, $P_{MCMC} = 0.092$), time spent absent from the crypt (estimate $= 0.036$, lower 95% $= -0.882$, upper 95% $= 0.973$, $P_{MCMC} = 0.925$), or time spent maintaining the carcass (estimate $= 0.108$, lower 95% $= -0.070$, upper 95% $= 0.282$, P_{MCMC} $= 0.235$). Likewise, there was no effect of the initial weight of females on time spent provisioning food (estimate $= -4.63$, lower 95% $= -10.4$, upper 95% $=$ 1.84, $P_{MCMC} = 0.111$), time spent absent from the crypt (estimate $= 22.6$, lower 95% $= -18.8$, upper 95% $= 65.3$, $P_{MCMC} = 0.273$), or time spent maintaining the carcass (estimate $= 4.25$, lower 95% $= -2.69$, upper 95% $= 11.0$, $P_{MCMC} =$ 0.272).

2.3.2   Offspring performance

There were no effects of either the linear or the quadratic terms of handicapping on larval begging (Table 2.2). Likewise, there were no effects of the linear or the quadratic terms of handicapping on mean larval mass at dispersal (Table 2.2) or brood size at dispersal (Table 2.2). Thus, there was no evidence that larvae spent less time begging in response to handicapping of their female parent even though handicapped females spent more time provisioning food, and there was no evidence that handicapping of the female affected offspring performance. There was no effect of the initial weight of females on larval begging (estimate $= -4.40$,

SE = 7.49, z = −0.588, P = 0.557), mean larval mass (estimate = −0.070, SE = 0.051, t = −1.38, P = 0.171), or brood size (estimate = −0.340, SE = 2.28, t = −0.149, P = 0.882).

2.3.3 Female investment in current and future reproduction

There were no effects of the linear or quadratic terms of handicapping on female weight change while breeding (Table 2.2) or female life span after breeding (Table 2.2). Likewise, brood size at dispersal had no effect on female relative weight change (estimate = −0.412, SE = 0.519, t = −0.795, P = 0.429). The initial weight of females had no effect on female relative weight change (estimate = 25.4, SE = 28.7, t = 0.886, P = 0.378), but it had a significant positive effect on female life span with heavier females living for longer (estimate = 0.823, SE = 0.240, z = 3.43, P = 0.001). Finally, female weight change had no effect on female life span (estimate = −0.0003, SE = 0.0009, z = −0.300, P = 0.764).
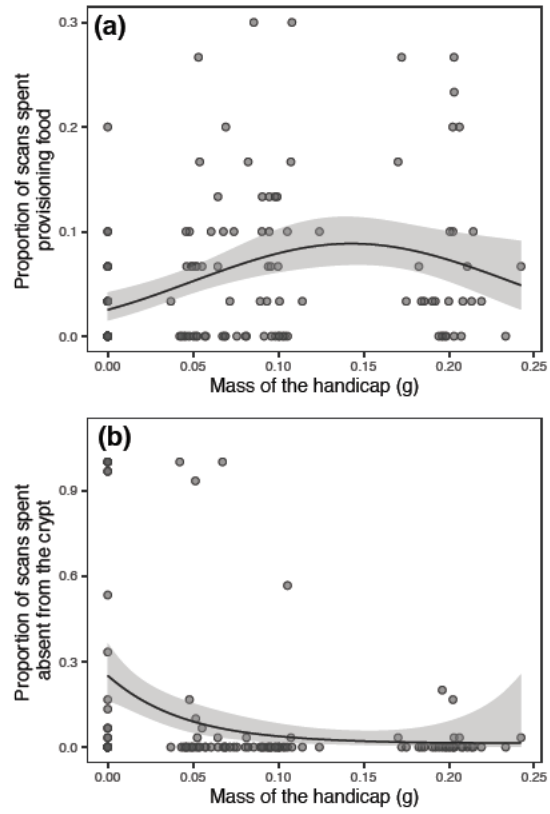
Figure 2.2: Effects of the level of handicapping on the proportion of time spent provisioning by the female (a) and time absent from the crypt (b). Proportions represent the total time spent provisioning or absent from the crypt during the 30-min observation period, divided by 30. The black lines represent polynomial regression lines (± 95% confidence intervals) from GLMs assuming a binomial error structure.

Table 2.1: Effects of handicapping (linear and quadratic terms) on time spent provisioning food, maintaining the carcass and being absent from crypt by females.

	Handicapping				Handicapping^2			
	Estimate	Lower 95%	Upper 95%	P_{MCMC}	Estimate	Lower 95%	Upper 95%	P_{MCMC}
Provisioning food	19.4	4.83	33.2	**0.004**	−66.1	−125.3	1.42	**0.033**
Absent from the crypt	−134.1	−238.6	−49.6	**<0.0001**	491.0	83.5	919.7	**0.008**
Maintenance of carcass	15.2	−1.18	30.7	0.056	−47.5	−124.4	18.8	0.192

Table 2.2: Effects of handicapping (linear and quadratic terms) on larval begging behaviour, larval performance (mean larval mass and brood size), and female investment in current and future reproduction (female weight change and female life span).

	Handicapping				Handicapping^2			
	Estimate	SE	t/z-value	P	Estimate	SE	t/z-value	P
Larval begging	2.17	18.1	0.120	0.904	−3.70	79.5	−0.047	0.963
Mean larval mass	0.051	0.116	0.444	0.658	−0.235	0.519	−0.454	0.651
Brood size	8.94	4.89	1.827	0.070	−35.4	22.3	−1.59	0.115
Female weight change	11.6	63.5	0.182	0.856	132.9	292.3	0.454	0.651
Female life span	0.334	0.526	0.635	0.529	−2.01	2.42	−0.830	0.406

2.4 Discussion

Here, I tested the effects of different levels of handicapping on the amount of care provided by female parents, the performance of their offspring and female investment towards current reproduction in the burying beetle *N. vespilloides*. At the time point in larval development corresponding to the peak in parental care, handicapped females spent more time provisioning food to the brood and less time being away from the crypt than control females. This finding confirms evidence from a recent study on *N. vespilloides* reporting that handicapped females provide more care than control females (Ratz and Smiseth 2018). I found no evidence of females providing less care as the level of handicapping increased. Furthermore, there was no evidence that handicapping influenced time spent maintaining the carcass by females, larval begging behaviour, larval performance (i.e., mean larvae size at dispersal and larval survival until dispersal), or female investment in current and future reproduction (i.e., weight change over the reproductive attempt or female life span after breeding). Below, I provide a more detailed discussion of these results and their implications for our understanding of how handicapping affects parental care decisions.

My main finding was that handicapped females spent more time provisioning food than control females, but that there was no effect of the level of handicapping among handicapped females. The first finding is consistent with prior work on this species showing that handicapped females spend more time provisioning food (Ratz and Smiseth 2018). Handicapped females are predicted to provide more care than control females if handicapping suppresses the female's condition below the threshold value triggering terminal investment (Duffield et al. 2017). Thus, these results provide further evidence that handicapping can trigger terminal investment and suggest that even the smaller handicaps used in my experiment were sufficient to suppress the female's condition below the threshold value. The second finding (i.e., that there was no effect of the level of handicapping among handicapped females) is consistent with what I predicted if handicapping primarily induced a shift towards greater investment in current reproduction (Figure

2.1b). In contrast, if handicapping both induced such a shift and increased the energetic cost of care, I predicted that handicapped females should provide progressively less care as the level of handicapping increased (Figure 2.1c). One potential explanation for why I found no evidence that handicapped females provided less care as the level of handicapping increased is that the handicaps were too small to increase the energetic cost of care. This explanation seems unlikely given that my experiment included handicaps that were substantially larger than those used in prior studies on burying beetles reporting that handicapped females provided less care than control females (Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016), Thus, these results have important implications for our understanding of handicapping by confirming that its effects on parental behaviour cannot be explained simply as a consequence of an increase in the energetic cost of providing a given level care, as implicitly assumed in prior handicapping experiments (Ratz and Smiseth 2018).

An alternative explanation for why handicapped females provide more care than control females is that handicapping might have a differential effect on activities associated with different modes of locomotion. For example, in burying beetles, females walk while caring for their current brood, while they fly while searching for carcasses for use in future reproductive attempts (Scott 1998). Increasing the level of handicapping might trigger a shift towards greater investment in current reproduction if handicapping has a greater impact on the energetic cost of flight than on the energetic cost of walking. There is some support for this suggestion from prior work on the burying beetle *N. quadripunctatus* indicating that handicapped females cease flying but continue walking (Suzuki and Nagano 2009). Handicapping may have limited impact on walking in these beetles given that females have been reported to move vertebrate carcasses weighing up to 30 g (i.e., objects weighing over 100 times more than the largest handicaps used in my experiment) for several meters (Scott 1998). Thus, my results may reflect that handicapping in burying beetles may have a greater impact on the cost of locating a new carcass required for initiating a future reproductive attempt than

on the cost of providing care in the current reproductive attempt.

My finding that handicapped females spent more time providing care than control females contrasts with prior handicapping experiments on birds (e.g., Wright and Cuthill 1989, Harrison et al. 2009) and other species of burying beetles (*N. quadripunctatus*: Suzuki and Nagano 2009, Suzuki 2016, *N. orbicollis*: Creighton et al. 2015) reporting that handicapped parents provide less care than controls. One potential explanation for why my results differ from those of prior studies is that handicapping primarily increases the cost of care in birds and other species of burying beetles, while it primarily triggers a shift towards greater investment in current reproduction in my study species. For example, in altricial birds, parents fly continuously between the nest and the foraging sites in the surrounding environment to provision their nestlings with arthropods or other sources of food. Thus, we might expect handicapping to have greater impact on the energetic cost of care in birds than in my study species. Although this suggestion might explain why my results differ from prior studies on birds, it seems unlikely that it accounts for the difference between my study species and other species of burying beetles. The reason for this is that all burying beetles breed on carcasses of small vertebrates and that, in all species, parents walk rather than fly while caring for their larvae. Instead, the different results from studies on different species of burying beetles might reflect differences in their life histories. For example, a recent study shows that larval survival is more dependent on parental care in *N. orbicollis* than in *N. vespilloides* (Capodeanu-Nägler et al. 2016). Thus, there may be differences between species of burying beetles with respect to the returns on investment in current reproduction. Alternatively, there might be differences in the availability of resources for investment in future reproduction between different species. If so, this might lead to interspecific variation in the trade-off between current and future reproduction. Currently, relatively little is known about differences between species of burying beetles with respect to availability of resources and the trade-off between current and future reproduction. Thus, obtaining such information should now be a priority to help explaining why studies

on different species of burying beetles sometimes find somewhat different results.

One potential explanation for why my results differ from those of prior studies on burying beetles is that females may respond differentially to handicapping depending on whether they are assisted by a male partner or not. In my study, as well as in the prior study reporting that handicapped females provided more care than controls (Ratz and Smiseth 2018), handicapped and control females reared their brood on their own without assistance from a male partner. In contrast, handicapped and control females reared their brood with the assistance from a male partner in studies reporting that handicapped females provided less care than controls and that their male partners partially compensated for the reduction in female care (Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016). Thus, handicapped females might provide less care when assisted by a male partner, while they provide more care when rearing the brood on their own. Such a differential response to handicapping might be expected if the presence of a male partner buffers against any negative effects on offspring should females provide less care. If so, handicapped females could reduce their contribution towards care without harming their offspring's fitness when assisted by a male partner, while this would not be the case when rearing the brood on their own. Thus, there is now a need for studies that investigate whether female burying beetles respond differentially to handicapping depending on whether they are assisted by a male partner or not.

I found that handicapped females spent less time being absent from the crypt than control females. Currently, little is known about why breeding females temporarily leave the crypt in this species, but potential explanations are that females do so to explore the surrounding area for signs of conspecific intruders and/or predators. Thus, my results suggest that handicapped females are less inclined to explore the surrounding area than control females. An alternative explanation is that handicapped females remained within the crypt simply as a consequence of reduced mobility. However, if this was the case, we should also

expect handicapped females to spend less time provisioning food than control females given that this behaviour also requires mobility. Thus, given that I found that handicapped females spent more time provisioning food, this explanation seems unlikely (Figure 2.2). My study highlights that there is a need to investigate why breeding females temporarily leave the carcass.

I found no evidence that handicapping affected larval begging behaviour, larval performance (i.e., mean larval mass or larval survival until dispersal), or female investment in current and future reproduction (i.e., weight change over reproduction and life span after reproduction). These findings are surprising given that handicapped females spent more time provisioning food towards larvae than control females. Prior work shows that larval begging in N. vespilloides reflects larval hunger state (Smiseth and Moore 2004b) and that larvae grow to a larger size when receiving more care from female parents (Andrews et al. 2017). Thus, we might expect larvae reared by handicapped females to be less hungry, therefore spending less time begging, and to grow to be a larger size than larvae reared by control females. One potential explanation for why I found no such effects is that the quality of care (e.g., nutritional quality of predigested carrion transferred to larvae via mouth-to-mouth contact) was lower in handicapped females than in control females. If so, larvae might receive a similar amount of care regardless of whether they are reared by handicapped or control females. An alternative explanation is that handicapping had a differential effect at different times of the larvae's development. My results show that handicapped females spent more time providing care at the time point in larval development corresponding to the peak in parental care (i.e., 24 h after hatching) than control females. Given that I recorded effects on female parental behaviour at a single time point, I cannot rule out the possibility that handicapped females provided less care either earlier or later in development. Finally, I found that handicapping had no effect on female weight change during breeding or female life span. These results contrast with those of most studies on birds, showing that handicapped females lose more weight than control females (e.g., Slagsvold and Lifjeld 1990, Markman et al.

1995, Sanz et al. 2000). As discussed above, the energetic cost of care might be relatively high in birds, in which case we might expect handicapped females to lose more weight than controls. In contrast, the energetic cost of care might be relatively low in burying beetles. There is also evidence that parents forage from the carcass while breeding (Pilakouta et al. 2016), which may allow handicapped females to compensate for the energetic cost of handicapping by consuming more food from the carcass (Ratz and Smiseth 2018).

This study adds to our understanding of the terminal investment hypothesis, that is, the suggestion that parents should increase their investment in reproduction during their final reproductive attempt (Williams 1966, Hirshfield and Tinkle 1975, Clutton-Brock 1984). Traditionally, the terminal investment hypothesis has focused on increases in investment in reproduction with age (Clutton-Brock 1984), but its rationale applies to any factor that suppresses the condition of parents below a certain threshold that reduces their prospects for future reproduction. Indeed, there is mounting evidence that terminal investment is triggered by a range of factors other than age, including immune challenges (e.g., Podmokła et al. 2014), intraspecific competition (e.g., Rebar and Greenfield 2017) and predation risk (e.g., Knight and Temple 1986). Thus, my results suggest that handicapping can be added to the list of factors that can induce terminal investment by suppressing the parent's condition. I suggest that handicapping would provide a useful tool for studying terminal investment as it provides a simple experimental tool for suppressing an individual's condition. Given that handicaps can be removed, such experiments could be used to establish whether individuals reverse their decisions to invest more in current reproduction should their condition improve at a later stage.

In conclusion, I found that handicapped females spent more time providing care than control females, possibly reflecting that handicapping suppresses the condition of females below the threshold triggering terminal investment (Duffield et al. 2017). My results have important implications for our understanding of the

effects of handicapping, which is a key experimental tool used by behavioural ecologists to study negotiation between parents in species with biparental care (Harrison et al. 2009). Such studies are based on the assumption that handicapping primarily increases the energetic cost of care, and my results show that this is not necessarily the case. This conclusion emphasizes that handicapping experiments can lead to different outcomes in different species, presumably reflecting differences in the modes of locomotion of caring parents, differences in life histories, and/or differential responses depending on the presence or absence of a partner. Thus, I encourage further handicapping experiments across a variety of different taxa and social contexts.

3 Parental response to variation in energetic costs and brood size

Abstract

Parental care is highly variable, reflecting that parents make flexible decisions in response to variation in the cost of care to themselves and the benefit to their offspring. Much of the evidence that parents respond to such variation derives from handicapping and brood size manipulations, the separate effects of which are well understood. However, little is known about their joint effects. Here, I fill this gap by conducting a joint handicapping and brood size manipulation in the burying beetle *Nicrophorus vespilloides*. I handicapped half of the females by attaching a lead weight to their pronotum, leaving the remaining females as controls. I also manipulated brood size by providing each female with 5, 20 or 40 larvae. In contrast to what I predicted, handicapped females spent more time provisioning food than controls. I also found that handicapped females spent more time consuming carrion. Furthermore, handicapped females spent a similar amount of time consuming carrion regardless of brood size, whereas controls spent more time consuming carrion as brood increased. Females spent more time provisioning food towards larger broods, and females were more likely to engage in carrion consumption when caring for larger broods. I conclude that females respond to both handicapping and brood size manipulations, but these responses are largely independent of each other. Overall, my results suggest that handicapping might lead to a higher investment into current reproduction and that it might be associated with compensatory responses that negate the detrimental

impact of higher cost of care in handicapped parents.

3.1   Introduction

Parental care is defined as any parental trait that has evolved to enhance the survival and/or growth of the parent's offspring, often at cost to the parent's own fitness (Royle et al. 2012). Typical forms of care include protection against predators and other environmental hazards, and provisioning of food or other resources after hatching or birth (Smiseth et al. 2012). In many species, parental care is highly variable, reflecting that parents make flexible decisions about how much care to provide in response to variation in the cost of care to themselves and the benefit of care to their offspring (Alonso-alvarez and Velando 2012, Royle et al. 2014). In general, parents are expected to provide less care when the cost of care is higher and provide more care when the benefit of care is higher (Grodzinski and Johnstone 2012). Much of the experimental evidence for these two predictions derives from handicapping and brood size manipulations, respectively. For example, handicapping experiments in birds and insects (based on attachments of weights or feather clipping) show that handicapped parents decrease their care, presumably because handicapping elevates the cost of care to parents (Wright and Cuthill 1989, Harrison et al. 2009, Suzuki and Nagano 2009). Likewise, brood size manipulations in birds, fishes and insects show that parents usually provide more care towards enlarged broods, presumably because the benefit of care is higher, while parents provide less care towards reduced broods, presumably because the benefit of care decreases (in a non-linear manner; Trivers 1974) with decreasing brood size (e.g. Ridgway 1989, Sanz 1997, Rauter and Moore 2004, Smiseth et al. 2007a). Thus, handicapping and brood size manipulations have been instrumental in providing experimental evidence showing that variation in the cost and benefit of care are key determinants of how parents make flexible decisions regarding how much care to provide for their offspring.

Although we have a good understanding of the separate effects of handicapping

and brood size manipulations on the amount of care provided by parents, little is known about their joint effects. Despite the lack of formal theory, we can derive predictions from simple graphical models based on assumptions about how handicapping and brood size manipulations influence the cost and benefit functions of care (Figure 3.1). These functions describe the effect that specific levels of parental care have on parental and offspring fitness, respectively (Smiseth 2017). The cost function is assumed either to increase at an accelerating rate or to be linear. In either case, if handicapping increases the cost of care, handicapped parents are predicted to reduce their level of care (Figure 3.1), as reported for birds (Wright and Cuthill 1989, Harrison et al. 2009) and insects (Suzuki and Nagano 2009). Meanwhile, the benefit function is assumed to increase at a decelerating rate to reach an asymptote above which any further increase in care has no effect on offspring fitness (Trivers 1974, Royle et al. 2012). The benefit function describes the fitness effect on an individual offspring. Thus, in order to derive the indirect benefit function to the parent, we need to account for both the coefficient of relatedness between the parent and its offspring and the number of offspring in the brood (Figure 3.1). If brood size enlargement increases the benefit of care, parents should increase their care towards enlarged broods in a non-linear way (Figure 3.1; Trivers 1974), as reported for fishes (e.g. Ridgway 1989), birds (Sanz 1997) and insects (e.g. Rauter and Moore 2004, Smiseth et al. 2007a). Furthermore, this model predicts no interaction effect (or one that is too small to be detected) if handicapping leads to only minor divergence in the cost function at higher levels of care (Figure 3.1a,b). On the other hand, it predicts an interaction effect if handicapping leads to a greater divergence in the cost function at higher levels of care (Figure 3.1c,d). These predictions have never before been tested empirically, and here, I address this gap by conducting a joint handicapping and brood size manipulation experiment in the burying beetle *Nicrophorus vespilloides*.

Burying beetles of the genus *Nicrophorus* are ideal for studying the joint effects of handicapping and brood size as prior studies show that parents respond to
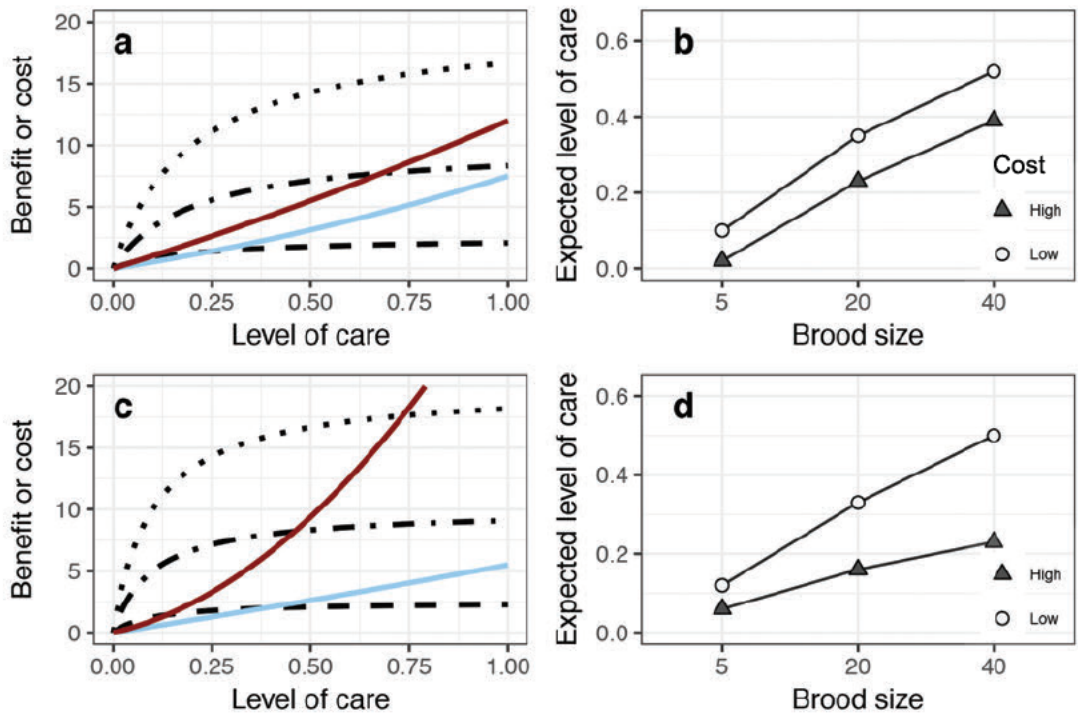
Figure 3.1: Direct cost and indirect benefit functions of parental care in relation to the level of care (a, c). The cost functions (blue and red lines) increase at an accelerating rate when a parent increases its level of parental care c, as Cost $= c + c^2 \cdot k_c$. The benefit functions (black lines) increase at a decelerating rate with c, as Benefit $= r \cdot N \cdot \frac{c}{c + k_b}$. The specific cost and benefit returns to a parent depend on the coefficient of relatedness between the parent and its offspring (here, $r = 0.5$), the brood size N, the intercept b of the cost function, and the shapes kc and kb of the cost and benefit functions, respectively (adapted from Kölliker et al. 2010). The indirect benefit of care to the parent increases with the number of offspring in the brood, which in this case varies between 5 (dashed line), 20 (dashed-dotted line) or 40 offspring (dotted line). The direct cost of care to the parent may be relatively low (blue line, $kc_{Low} = 6$) or high (red line), depending on whether females are handicapped or not. Handicapping may affect the slope of the cost function, shown here as the divergence in functions (red lines) at higher levels of care, here with $kc_{High} = 10$ (a) and $kc_{High} = 24$ (c). This model predicts that parents should provide less care when the cost of care is higher and the benefit of care is lower. The model also predicts that there should be an effect of the interaction between the cost and benefit of care if handicapping leads to a greater divergence in the cost function at higher levels of care (d). On the other hand, there may be no such an effect (or it may not be detectable) if handicapping leads to a minor divergence in the cost function at higher levels of care (b).

both treatments (handicapping: Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016, brood size manipulations: Rauter and Moore 2004, Smiseth et al. 2007a). These beetles breed on carcasses of small vertebrates that serve as the

sole food source for the brood during larval development (Eggert et al. 1998, Scott 1998). Larvae can obtain resources by either feeding directly from the carcass or begging for predigested carrion from the parents (Smiseth and Moore 2002, Smiseth et al. 2003). In *N. vespilloides*, begging reflects the offspring's nutritional need (Smiseth and Moore 2004b) and is costly to the offspring in terms of increased risk of filial cannibalism (Andrews and Smiseth 2013). Prior work on *N. vespilloides* and *Nicrophorus orbicollis* shows that parents respond to brood size manipulations by increasing their food provisioning rate towards larger broods (Rauter and Moore 2004, Smiseth et al. 2007a). Moreover, prior work on *Nicrophorus quadripunctatus* and *N. orbicollis* shows that handicapped parents provide less care than control parents (Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016). Although the reduction in parental care by handicapped parents is generally attributed to an increase in the cost of care, this response may also be caused by deteriorating condition of handicapped parents (Pilakouta et al. 2015b) or by stress induced by handicapping. Regardless of how handicapping leads to a reduction in parental care, there is no information on the joint effects of handicapping and brood size manipulations on the amount of care provided by parents.

My main aim was to examine joint effects of handicapping and brood size on the overall level of care provided by females and on female weight change during breeding. The latter is used as a proxy for how much females consume from the carcass to invest into their future reproduction (Creighton et al. 2009, Billman et al. 2014). I expect an effect of the interaction between handicapping and brood size only if handicapping leads to a greater divergence in the cost function at higher levels of care (Figure 3.1d). I predict main effects of handicapping and brood size, reflecting that weighted females provide less care to the brood than control females and that females provide more care to larger broods than to smaller ones. I predict an effect of the interaction between handicapping and brood size and main effects of handicapping and brood size on the amount of time spent provisioning food by parents. The reason for this is that this form

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of parental care is directed towards individual offspring within the brood (unlike other forms of care, such as carcass maintenance). I also predict that handicapping and an increase in brood size would be associated with a greater loss in weight of females, reflecting that weighted females pay a greater cost from their investment into the current brood and that larger broods require more care. My second main aim was to test for subsequent consequences of handicapping and brood size on offspring begging and offspring performance. I predict that handicapping of females would lead to an increase in larval begging and have a detrimental impact on larval fitness given that weighted females would spend less time provisioning food to the brood. Similarly, I predict that an increase in brood size would lead to an increase in larval begging and have a detrimental impact on larval performance given that larger broods should be associated with more intense sibling competition (Smiseth et al. 2007b).

3.2 Methods

I used the second to the fifth generation of beetles from a laboratory population of outbred beetles descending from a population collected in Corstorphine Hill, Edinburgh, UK. Adult beetles were kept individually in transparent plastic containers (12 × 8 × 2 cm) filled with moist soil. The laboratory conditions were kept constant throughout the experiment; that is, the beetles were kept at 20 C and under a 16:8 h light:dark photoperiod. Nonbreeding beetles were fed small pieces of beef twice a week.

3.2.1 Experimental design

I used a 2 × 3 factorial design to examine effects of handicapping of the female parent (weighted or control females) as one factor and brood size (5, 20 or 40 larvae) as the other factor. Previous work has found that weighted (i.e. handicapped) parents reduce their amount of parental care in the closely related *N. quadripunctatus* (Suzuki and Nagano 2009) and *N. orbicollis* (Creighton et al.

2015). Meanwhile, brood size manipulations on *N. vespilloides* and *N. orbicollis* show that parents provide more care towards larger broods (Rauter and Moore 2004, Smiseth et al. 2007a). In this experiment, I chose brood sizes of 5, 20 and 40 larvae as treatment levels reflecting that broods range in size from 2 to 47 larvae under laboratory conditions with a mean brood size of 21 larvae (Smiseth and Moore 2002).

I selected an initial number of 231 virgin females for use in the experiment. At the start of the experiment, each female was paired with an unrelated virgin male. The pair was placed in a larger plastic container (17×12×6cm) filled with 1cm of moist soil and containing a previously frozen mouse carcass of a standardized size (22.31 ± 0.002 g; range: 20.45–23.51 g; Livefoods Direct, Sheffield, UK). Containers were checked for the presence of eggs the following days, and egg-laying date was recorded as the day where the first eggs were laid. Females were randomly assigned to a handicapping treatment (weighted or control) 1 day before the expected hatching date. At this stage, I moved females and their mouse carcasses into new boxes filled with fresh soil. I did this to separate females from their eggs, such that the larvae hatching from the eggs could be used to generate experimental broods of different sizes (Smiseth et al. 2007a). At this time, I also removed males because males often desert the brood before hatching and the presence or absence of males in *N. vespilloides* has no detectable impact on offspring fitness under laboratory conditions (Smiseth et al. 2005). As soon as the eggs hatched, I randomly allocated each female a brood of newly hatched unrelated offspring made up of either 5, 20 or 40 larvae. I only allocated a female with an experimental brood once her own eggs had hatched given that parents will kill any larvae that emerge on the carcass before their own eggs have hatched (Bartlett 1987, Müller and Eggert 1990). This is because burying beetles use temporal kin discrimination, which is plausibly controlled by physiological mechanisms involving hormonal change during reproduction (e.g. Trumbo and Robinson 2008, Steiger and Stökl 2018)

In parallel with the experimental females used in the experiments, I set up a total of 485 pairs of nonexperimental parents. These parents produced foster larvae that were used to generate the experimental foster broods. The foster broods were always of mixed maternity, which allowed me to eliminate any potential prenatal maternal effects associated with the handicapping treatments that can have had confounding effects on offspring and parental behaviours (Paquet et al. 2015, Paquet et al. 2020).

3.2.2   Handicapping procedure

To test the effects of handicapping on parental care, I weighted breeding females in the gap between the end of egg laying and the beginning of hatching. In this species, this gap occurs during the 2 days following the beginning of egg laying (Müller and Eggert 1990). For weighted (handicapped) females, I attached a small lead weight to the pronotum of the female using instant-adhesive glue, as described in previous studies on the closely related *N. quadripunctatus* (Suzuki and Nagano 2009, Suzuki 2016) and *N. orbicollis* (Creighton et al. 2015). In both species, handicapping reduced mobility of adult beetles and affected parental care behaviours by reducing the frequency of direct and indirect care (Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016). In my study, the mass of the weight together with the glue (0.06 ± 0.0008 g) represented approximately 20% of the initial female body mass ($n = 116$, 0.30 ± 0.004 g) measured shortly before handicapping. During the course of the experiment, I noticed that sizeable amounts of dirt were accumulating around the weight due to the digging behaviour of the burying beetles. This formed a lump on the pronotum and induced handicapped females to carry a total mass (i.e. lead weight + dirt) of approximately 30% their initial body mass (mean $\pm$ SE mass of dirt: 0.014 ± 0.0013 g). I had a control treatment of females that were of a similar body mass to the experimental females ($n = 101$, 0.30 ± 0.005 g). The control females were treated in the same way as the experimental females (i.e. these beetles were handled and disturbed), except that they had no weight attached to them.

Among the initial 231 experimental broods, 41 were excluded from the analysis for the following reasons: females lost their weights ($n = 12$) or died ($n = 3$) before the behavioural observations, females could not be allocated a foster brood ($n = 4$), females failed to produce eggs ($n = 6$), no eggs hatched from the clutch ($n = 9$), or eggs hatched before females were handicapped ($n = 7$). In addition to this, 11 broods were included in the behavioural analysis but excluded from analyses on fitness-related traits because the females had lost their weights or died between the time of observation and the time of larval dispersal. The final sample sizes for the different treatment groups were as follows for the behavioural traits measured 1 day after hatching (n_{d1}) and the fitness traits measured at larval dispersal (n_{disp}): control females with brood size of five larvae: $n_{d1} = n_{disp}$ = 29; control females with a brood size of 20 larvae: $n_{d1} = n_{disp} = 29$; control females with a brood size of 40 larvae: $n_{d1} = n_{disp} = 34$; weighted females with a brood size of five larvae: $n_{d1} = 33$ and $n_{disp} = 29$; weighted females with a brood size of 20 larvae: $n_{d1} = 35$ and $n_{disp} = 31$; and weighted females with a brood size of 40 larvae: $n_{d1} = 30$ and $n_{disp} = 27$.

3.2.3 Female and offspring behaviours

I recorded parental and larval behaviours 24 h (±15 min) after the larvae were placed on the carcass, as this stage corresponds to the period when there is a peak in female food provisioning (Smiseth et al. 2003, 2007a). Behavioural observations were performed under red light using instantaneous sampling every 1 min for 30 min. Both parental and larval behaviours were simultaneously observed and scored following methods described in previous studies (e.g. Smiseth and Moore 2002, 2004a,b). To summarize briefly, I recorded the occurrence of parental food provisioning as the number of scans where there was mouth-to-mouth contacts with larvae, carcass maintenance as the number of scans where the female was spreading secretions on the surface of the carcass or excavating the crypt (i.e. the depression in the soil surrounding the carcass), and carrion consumption as the

number of scans where female was feeding within the crater (i.e. the opening on the top of the carcass).

At each scan, I also recorded the number of larvae that were begging within the brood. I considered a larva to be begging when it raised its head towards the female while waving its legs or when it touched the female with its legs (Smiseth and Moore 2002). I then calculated the average proportion time spent begging per larva in the brood as $B = (\Sigma b/n)/l$, where Σb is the total number of begging events during an observation session, n is the number of larvae in the brood at the time of observation, and l is the number of scans for which the female was near the larvae (Smiseth et al. 2003). I included the latter because larvae only beg when the parent is in close vicinity (i.e. less than or equal to the female's pronotum width; Rauter and Moore 1999, Smiseth and Moore 2002, Smiseth et al. 2007a). Thus, this measure of begging is largely independent of the female's behaviour towards the larvae (Smiseth and Moore 2004a).

3.2.4 Female weight change and offspring performance

To assess the consequences of handicapping and brood size on how much females consume from the carcass to invest in future reproduction, I measured the relative change in mass of females over the reproductive period. I estimated female weight change as the difference between the female's initial weight on the day preceding the hatching of her eggs and her final weight at the time of larval dispersal. I also tested for effects of handicapping and brood size on two measures of offspring performance: larval survival until dispersal and mean larval mass at dispersal. I measured effects on larval mass at dispersal because it determines adult body size, which in turn is known to be a major determinant of competitive ability and breeding success as adult in *Nicrophorus* species (Otronen 1988, Safryn and Scott 2000).

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3.2.5 Data analysis

All statistical analyses were conducted using R v 3.3.3 (R Development Core Team 2019) loaded with the packages *car* (Fox and Weisberg 2017), $MASS$ (Ripley et al. 2017), *aod* (Lesnoff and Lancelot, 2012) and $MCMC_{GLMM}$ (Hadfield 2010). Given that the behavioural traits in my experiment were count data bounded between 0 and 30 scans, I analysed the data using a binomial error distribution. I used general linear models for traits with a Gaussian distribution (female relative mass change and larval body mass at dispersal) and generalized linear models with a quasi-binomial distribution for traits that represent binary or count data with an upper limit (larval survival rate and larval begging). I used Bayesian generalized linear models fitted with a binomial error distribution to analyse food provisioning to the brood and carcass maintenance, whereas I used a Bayesian zero-inflated binomial model for carrion consumption to control for overdispersion and zero inflation. All Bayesian models were run using flat improper priors. I present parameter estimates for the Bayesian models as posterior means with 95% credible intervals of 2600 samples ran for 5.2×10^5 iterations with a thinning interval of 200 and a burn-in of 6×10^4. Outputs from the Bayesian zero-inflated binomial model allow me to test both the probability that females engaged in carrion consumption and, when consuming carrion at least once, how much time (i.e. number of scans) females spent consuming carrion during the observation period. All models included female handicapping treatment (control or weighted) and brood size (5, 20 or 40 larvae) and the interaction between them as fixed effects. Brood size was treated as a categorical predictor in the general linear and generalized linear models, whereas it had to be treated as a continuous predictor in the Bayesian models. In the general linear and generalized linear models, I used post hoc contrasts whenever handicapping and/ or brood size had a significant effect on the variable of interest to test for differences between each treatment group or brood size category. In these tests, I used the Bonferroni correction for multiple testing.

3.3 Results

3.3.1 Female parental behaviour and weight change

There was no evidence of an effect of the interaction between handicapping and brood size on any of the two female parental behaviours (i.e. food provisioning and carcass maintenance) (Table 3.1, Figure 3.2a,b) or on female weight change during the breeding attempt (Table 3.2, Figure 3.2d). However, there was an effect of this interaction on the amount of time spent consuming carrion by females (Count model, Table 3.1). This interaction effect reflected that control females spent more time consuming carrion as brood increased, whereas weighted females spent a similar amount of time at this behaviour regardless of brood size (Figure 3.2c).

Figure 3.2: Effects of handicapping (weighted or control females) and brood size manipulation (5, 10 or 20 larvae) on the time spent (number of scans) by the female provisioning food to the brood (a), maintaining the carcass (b), consuming carrion (c) and on female weight change over the reproductive attempt (d). Mean ± SE.

Handicapping had a significant effect on the amount of time spent provisioning food to the brood and consuming carrion (Table 3.1). Contrary to what I predicted, weighted females spent more time provisioning food to the brood than control females (Table 3.1; Figure 3.2a). Weighted females were also more likely to engage in carrion consumption and spent more time consuming carrion overall

(Table 3.1, Figure 3.2c). There was no evidence that handicapping had an effect on carcass maintenance or female weight change (Tables 3.1 and 3.2, Figure 3.2b,d).

Brood size had a significant effect on the amount of time spent provisioning food to the brood, the probability that females engaged in carrion consumption, as well as on female weight change (Tables 3.1 and 3.2; Figure 3.2). Females spent more time provisioning food towards larger broods (Table 3.1, Figure 3.2a). Likewise, females were more likely to engage in carrion consumption when caring for larger brood (Binary model, Table 3.1). Finally, females lost more weight when caring for broods of 20 than for broods of five larvae (Contrast 20 vs. 5 larvae: Estimate $= -0.024$, SE $= 0.009$, z $= -2.67$, P $= 0.02$), but lost a similar amount of weight when caring for broods of 20 and 40 larvae (Contrast 40 vs. 20 larvae: Estimate $= -0.018$, SE $= 0.009$, z $= -1.98$, P $= 0.14$). There was no effect of brood size on the amount of time spent maintaining the carcass (Table 3.1, Figure 3.2b).

3.3.2   Offspring begging and performance

There was no effect of the interaction between handicapping and brood size on the average amount of time spent begging by the larvae, larval survival or mean larval mass at the time of dispersal (Table 3.2, Figure 3.3). Likewise, there were no effects of handicapping on larval begging, larval survival or mean larval mass (Table 3.2). However, there was an effect of brood size on larval begging, larval survival and mean larval mass (Table 3.2, Figure 3a-c). Larvae spent more time begging in broods of 20 or 40 larvae than in broods of five larvae (Contrast 20 vs. 5 larvae: Estimate $= 0.778$, SE $= 0.183$, z $= 4.25$, P < 0.0001; Contrast 40 vs. 5 larvae: Estimate $= 0.471$, SE $= 0.191$, z $= 2.47$, P $= 0.041$). Likewise, larval survival and mean larval mass were higher in broods of 20 compared to broods of five larvae (Contrast 20 vs. 5 larvae: Estimate $= 0.579$, SE $= 0.232$, z $= 2.50$, P $= 0.038$ and Estimate $= 0.012$, SE $= 0.004$, z $= 2.70$, P $= 0.02$, respectively) or 40 larvae (Contrast 40 vs. 20 larvae: Estimate $= -0.757$, SE $= 0.224$, z $= -3.38$, P $=$
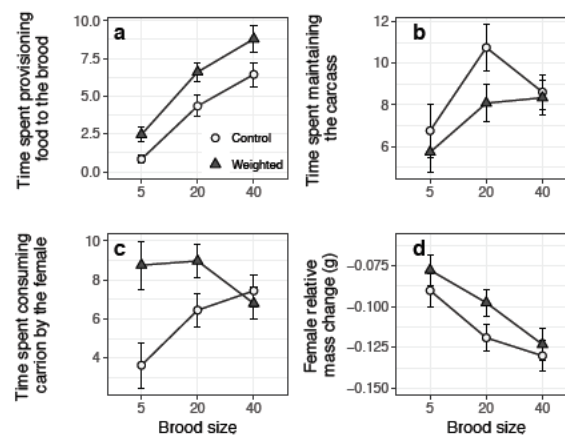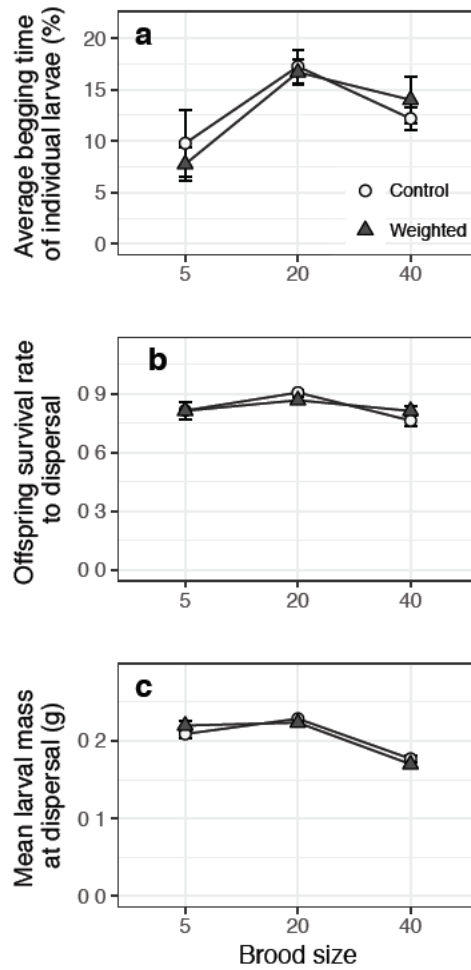
Figure 3.3: Effects of handicapping (weighted or control females) and brood size manipulation (5, 10 or 20 larvae) on the average time spent begging by individual larvae in the brood (a), larval survival to dispersal (b) and mean larval mass at dispersal (c). Mean ± SE.

0.002 and Estimate $= -0.052$, SE $= 0.004$, z $= -12.22$, P < 0.0001, respectively).

Table 3.1: Effects of the interaction between handicapping (weighted or control females) and brood size (5, 20 or 40 larvae) and the main effects of handicapping (weighted vs. control) and brood size (continuous) on female parental behaviours, that is food provisioning, carcass maintenance and female carrion consumption. Values are obtained from Bayesian GLMs using $MCMC_{GLMM}$

	Interaction				Handicapping				Brood size			
	Mean	l-95%	u-95%	P_{MCMC}	Mean	l-95%	u-95%	P_{MCMC}	Mean	l-95%	u-95%	P_{MCMC}
Food provisioning	−0.011	−0.035	0.014	0.368	1.00	0.340	1.74	**0.002**	0.062	0.043	0.080	**<0.001**
Carcass maintenance	0.005	−0.021	0.028	0.699	−0.296	−0.949	0.330	0.380	0.015	−0.003	0.031	0.098
Carrion consumption												
Binary model	0.056	−0.050	0.174	0.239	−1.94	−3.66	−0.309	**0.023**	−0.094	−181	−0.028	**<0.001**
Count model	−0.023	−0.046	−0.001	**0.039**	0.856	0.249	1.45	**0.007**	0.008	−0.009	0.024	0.377

Table 3.2: Effects of the interaction between handicapping (weighted or control females) and brood size (5, 20 or 40 larvae) and the main effects of handicapping and brood size on female mass change, larval begging and offspring performance (larval survival and mean larval mass). Values obtained from general linear models (female mass change and mean larval mass) and generalized linear models (larval begging and larval survival).

	Interaction			Handicapping			Brood size		
	LR_{χ^2}	d.f.	P	LR_{χ^2}	d.f.	P	LR_{χ^2}	d.f.	P
Female mass change	0.645	2	0.724	3.34	1	0.067	21.2	2	**<0.001**
Larval begging	1.15	2	0.535	0.006	1	0.938	21.3	2	**<0.001**
Larval survival	2.34	2	0.310	0.016	1	0.899	12.6	2	**0.002**
Mean larval mass	5.17	2	0.075	0.029	1	0.864	167	2	**<0.001**

3.4 Discussion

The main aim of my study was to investigate effects of the interaction between handicapping and brood size on parental care and offspring performance in the burying beetle *N. vespilloides*. Assuming that handicapping increases the cost of care whereas brood size enlargement increases the benefit of care, I expected such interaction effects if handicapping leads to a greater divergence in the cost function at higher levels of care (Figure 3.1d). I found no evidence for the presence of such an interaction effect on female parental behaviours (food provisioning and carcass maintenance), suggesting that these assumptions were not met in my study. Currently, we have little empirical information on the shape of the cost and benefit functions, and obtaining empirical estimates of these functions should now be a priority to guide future theoretical and empirical work in this field (Smiseth 2017). However, there was an effect of this interaction on female carrion consumption, reflecting that control females consumed more carrion as brood size increased, whereas weighted females consumed a similar amount of carrion regardless of brood size. This finding suggests that weighted females may compensate for the negative effects of handicapping by consuming more food. Moreover, brood size had an effect on most traits; that is, increasing female food provisioning and female probability to engage in carrion consumption, reducing female weight change during breeding, increasing larval begging and decreasing larval performance (larval survival and mean larval mass). In contrast, I found that handicapping had an effect on two female parental behaviours only; that is, increased carrion consumption and, contrary to what I predicted, increased time provisioning food to the brood. These results imply that handicapping can lead to an increase in parental care, suggesting that the effects of handicapping on parental care may be more complex than has been assumed in prior work using such experimental designs. Below, I provide a more in-depth discussion of my results and their implications for our understanding of flexible parental care.

A surprising finding of my study was that weighted females spent more time provisioning food than control females. This finding contradicts the widely held

assumption that handicapping causes a reduction in parental care by increasing the cost of care. Handicapping experiments are traditionally used to study negotiation between parents in birds with biparental care, and their rationale is to increase the flight cost of care to the handicapped parent, thereby forcing it to reduce its contribution towards care (Harrison et al. 2009). Such experiments are based on several types of handicapping treatments, including attachment of lead weights (e.g. Wright and Cuthill 1989), clipping of flight feathers (Slagsvold and Lifjeld 1988) and hormone manipulation (Hegner and Wingfield 1987b). There is good evidence that handicapped parents provide less care than control parents regardless of which handicapping treatment is used (Harrison et al. 2009). More recently, handicapping based on attachment of lead weights has been used to study negotiation between parents in two species of burying beetle, *N. quadripunctatus* and *N. orbicollis*, and these studies show that, as in birds, weighted females provide less care than control females (Suzuki and Nagano 2009, Creighton et al. 2015, Suzuki 2016). The opposite effects of handicapping on parental care reported in studies on *Nicrophorus* species might reflect differences in the level of handicapping as the weights were of 20–30% relative to body mass of the beetles, whereas studies in *N. quadripunctatus* (Suzuki and Nagano 2009, Suzuki 2016) and *N. orbicollis* (Creighton et al. 2015) used weights of 40% and about 50%, respectively. As I discuss in greater detail below, handicapping may not only increase the cost of care, but also impact upon parental decisions through its effect on the parent's state (Pilakouta et al. 2015b). For example, the relatively minor handicaps used in my study might have a greater impact on the parent's state than its costs of care, whereas the relatively major handicaps used in previous work might have greater impact on the cost of care. This is unlikely given that evidence that greater handicaps have similar effects on maternal care and no effect on life span in our study system (Ratz et al. 2020a). An alternative explanation is that these differences reflect species-specific response to handicapping due to divergent life-histories.

As hinted at above, handicapping may alter parental decisions about how much

care to provide if it causes a decline in the parent's state (i.e. its condition, energy reserves or stress level; Pilakouta et al. 2015b). This in turn may lead to a reduction in parental care by weighted parents given that a decline in the parent's state should be associated with lower resources for investment in parental care and other priorities. Why then did I find that weighted females provided more care? One potential explanation for this finding is that weighted females responded to a decline in their state by shifting their investment towards their current brood at the expense of future reproduction. The terminal investment hypothesis predicts that parents should increase their investment into current reproduction when their prospects of future reproduction are lower (Clutton-Brock 1984).

I would expect an increase in care by weighted females if this shift towards current investment more than outweighs the impact of the higher cost of care. There is some evidence for terminal investment from prior studies on species within the genus *Nicrophorus*. For example, in *N. vespilloides*, immune-challenged parents, which face higher risks of death from pathogens, increase their investment into current reproduction (Cotter et al. 2010, Reavey et al. 2015). Likewise, inbred males, which have a shortened lifespan, invest more into current reproduction and are more likely to risk injury in fights with conspecific competitors (Richardson and Smiseth 2017). Finally, there is evidence that investment into current reproduction increases with the age of the female parent in *N. orbicollis* as predicted by the terminal investment hypothesis (Creighton et al. 2009). Thus, if handicapping leads to terminal investment, we might have expected weighted females to gain less weight during breeding, as this trait is used as a proxy for investment in future reproduction (Creighton et al. 2009, Billman et al. 2014). I found no evidence that weighted females lost more weight during the breeding period than control females, suggesting that my results provide no overall support for terminal investment triggered by handicapping. However, as argued below, the lack of evidence for terminal investment based on data on female weight gain might reflect that handicapping also causes an increase in female food consumption.

I found that weighted females consumed a similar amount of carrion regardless of brood size, whereas control females consumed more carrion as brood size increased. In *N. vespilloides*, parents consume carrion partly to provision food in the form of predigested carrion to their larvae and partly to replenish their own energy reserves (Mattey and Smiseth 2015). Thus, my results suggest that control females increased their carrion consumption with brood size (Figure 3.2c) to match the increase in food provisioning towards larger broods (Figure 3.2a). In contrast, weighted females consumed a similar amount of carrion regardless of the brood size (Figure 3.2c), presumably reflecting that these females adjusted their carrion consumption based on their own state rather than the brood size. Thus, control females consumed more carrion when they spent more time provisioning food to the brood, while there was no association between carrion consumption and food provisioning for weighted females. This finding also indicates that handicapping might trigger a compensatory response, whereby weighted females attempt to counteract the detrimental effects of handicapping due to an increase in the cost of care by increasing their energy reserves. For example, if handicapping increases the energetic cost of care, females might reduce this cost by building greater energy reserves. In *N. vespilloides*, it is relatively straightforward for females to increase their energy reserves as they can simply consume more from the carcass that is used for breeding (Boncoraglio and Kilner 2012, Pilakouta et al. 2016). If females increase their energy reserves to reduce the energetic cost of care, this may mask the expected effect of terminal investment on female mass gain.

As predicted, females provided more care and lost more weight when caring for larger broods. Meanwhile, I found that larvae in medium-sized broods spent more time begging, gained more weight and had higher survival than larvae in either small or large broods. These results are consistent with findings from previous work showing that parents tend to provide more care as brood size increases in insects, including *N. vespilloides* (e.g. Rauter and Moore 2004, Smiseth et al.

2007a), fishes (e.g. Ridgway 1989) and birds (e.g. Hegner and Wingfield 1987a, Sanz 1997). Thus, my results are in line with the prediction that females provide more care when the indirect benefit of care is higher due to an increase in the number of offspring in the brood (Figure 3.1). The finding that females lost more weight when caring for larger broods is likely to reflect that larger broods require more care from females and that it is more costly for parents to care for such broods. Finally, the fact that larvae performed best in broods of intermediate size suggests that larval growth and survival are higher in broods closer to the average size in this species (i.e. 21 larvae, Smiseth and Moore 2002). This finding may reflect a balance between sibling competition and sibling cooperation (Forbes 2007, Falk et al. 2014, Schrader et al. 2015), whereby individual offspring in small broods benefit from the presence of other siblings through cooperative begging whereas individual offspring in large broods pay a cost in terms of increased competition (Johnstone 2004). To sum up, my results confirm that variation in the benefit of care influences female decisions about how much care to provide to the current brood and how much resources to invest into current vs. future reproduction.

Parental care is a highly variable trait (Royle et al. 2012), and this variation reflects that parents make flexible decisions about how much care to provide in response to variation in the cost and benefit of care. Here, I show that parents respond to both handicapping and brood size and that these responses are largely independent of each other. In my experiment, females appear to respond more strongly to variation in brood size than to handicapping, which might reflect that brood size manipulations have a greater impact on the benefit of care compared to the impact of handicapping on the cost of care. Furthermore, weighted females spent more time provisioning food to the brood and consuming carrion than control females. This finding supports the view that parents may respond to handicapping by increasing their investment into the current brood at the expense of investment in future reproduction and/or by increasing their energy reserves to compensate for the increased energetic cost of care. I suggest that

future work on parental care based on handicapping should consider that this treatment may not only affect the cost of care, but that it may also lead to an increase in investment into current reproduction and compensatory responses that counteract the increased cost of care.

4   Parental response to infection

Abstract

Parental care is a key component of an organism's reproductive strategy that is thought to trade-off with allocation towards immunity. Yet it is unclear how caring parents respond to pathogens: do infected parents reduce care as a sickness behaviour or simply from being ill, or do they prioritise their offspring by maintaining high levels of care? Here I explored the consequences of infection by the pathogen *Serratia marcescens* on mortality, time spent providing care, reproductive output, and expression of immune genes of female parents in the burying beetle *Nicrophorus vespilloides*. I compared untreated control females with infected females that were inoculated with live bacteria, immune-challenged females that were inoculated with heat-killed bacteria, and injured females that were injected with buffer. I found that infected and immune-challenged females mounted an immune response and that infected females suffered increased mortality. Nevertheless, infected and immune-challenged females maintained their normal level of care and reproductive output. There was thus no evidence that infection led to either a decrease or an increase in parental care or reproductive output. My results show that parental care, which is generally highly flexible, can remain remarkably robust and consistent despite the elevated mortality caused by infection by pathogens. Overall, these findings suggest that infected females maintain a high level of parental care; a strategy that may ensure that offspring receive the necessary amount of care but that might be detrimental to the parents' own survival or that may even facilitate disease transmission to offspring.

4.1   Introduction

When infected by a pathogen, animals often alter their behaviours and social interactions (Hart 1988, Kelley et al. 2003, Adelman and Martin 2009, Vale et al.

2018). This change in behaviour may occur as a side effect of lethargy (Adelman and Martin 2009) or it may represent what is known as sickness behaviour; a strategic decision to adaptively shift resources towards immune defence by reducing activity levels (Lopes et al. 2016, van Kerckhove et al. 2013) and costly social interactions (Bos et al. 2012). Lethargy may be a consequence of the pathogen negatively impacting on the host's ability to remain active, thus leading to reduced mobility (e.g. Bradley et al. 2005, Cameron et al. 1993), foraging (e.g. Levri and Lively 1996, Venesky et al. 2009) and social activity (Lopes et al. 2016). Lethargy may also be associated with sickness behaviour, an adaptive adjustment to fight the infection that allows the host to diverge resources from non-essential activities, such as social interactions, to the immune system (Hart 1988, Exton 1997, Johnson 2002). When individuals interact with family members, sickness behaviour may also help reduce the risk of disease transmission to close kin (Heinze a,d, Walter 2010, Stroeymeyt et al. 2018) as a possible kin-selected behaviour (Shakhar and Shakhar 2015, Shakhar 2019). However, recent empirical evidence shows that sick individuals often maintain their social interactions with close kin (Lopes et al. 2018, Stockmaier et al. 2020). Yet empirical studies testing the effects of infection on social behaviour towards close kin, such as offspring, are still scarce. In addition, most studies investigating the effects of infection on parent-offspring interactions are based on immune challenges (injecting with heat-killed pathogens or products from pathogens; e.g. Aubert et al. 1997, Bonneaud et al. 2003, Stockmaier et al. 2020) that exclude potential effects of the pathogen on host's behaviour.

Parental care is a key component of an organism's reproductive strategy in many birds, mammals, and insects (Royle et al. 2012) that is thought to trade-off with allocation of resources towards immunity (Richner et al. 1995). Caring parents incur costs of care in terms of increased energy expenditure, reduced opportunities for additional reproductive attempts, reduced survival, and/or reduced future reproductive success (Williams 1966). Parental care enhances offspring growth and/or survival by neutralising environmental hazards to offspring, including risks

associated with starvation, predation, parasitism, and competition (Royle et al. 2012). Thus, when infected by a pathogen, parents face the dilemma of whether to shift allocation towards immunity at the expense of maintaining their level of parental care, or maintain the level of parental care at the expense of increasing their allocation towards immunity. Parents that reduce their level of care to increase their immune response would risk impairing their offspring's growth and survival, whereas parents that maintain their level of care would risk falling ill by not mounting an adequate immune response. Experimental studies using immune-challenges found that female laboratory mice tend to maintain their level of care and maintain normal offspring growth and survival (Aubert et al. 1997), while house sparrows drastically reduce their food provisioning at the cost of reduced offspring survival (Bonneaud et al. 2003). Such contrasting findings might reflect differences in how caring parents balance allocation towards parental care and immunity in response to infection. Thus, it is unclear how caring parents should respond to infection: do infected parents reduce or maintain their level of care, and is there a trade-off between the magnitude of the immune responses and the level of parental care?

Here, I investigated how parents balance their allocation towards parental care and immunity in response to infection in the burying beetle *Nicrophorus vespilloides*. This is an ideal system to investigate this issue because it is one of the few insects with extensive parental care. Parental care includes provisioning of food to larvae, defence against predators and infanticidal conspecific intruders and production of antimicrobials and enhances the offspring's growth and survival (Scott 1998, Eggert et al. 1998, Smiseth et al. 2003, Rozen et al. 2008). Burying beetles show changes in immunity during parental care (Steiger et al. 2011), which include differential expression of antimicrobial peptides (Jacobs et al. 2016, Ziadie et al. 2019). Parents may mount a personal immune response that helps them deal with pathogens. However, there is also evidence that parents invest in social immunity that benefits the offspring but is costly to the parents (Cotter and Kilner 2010b, Ziadie et al. 2019). In contrast to personal immunity that benefits the

challenged individual, social immunity is associated with fitness benefits to others individuals (Cotter and Kilner 2010b). Social immunity in burying beetles occurs as parents coat the carcass with exudates with potent antibacterial activity (Cotter and Kilner 2010b), which reduces microbial competition and improves the offspring's survival (Rozen et al. 2008).

To test for a causal effect of infection on parental care and immunity, I monitored the amount of care provided by infected females that were inoculated with live bacteria, immune-challenged females that were inoculated with heat-killed bacteria, injured females that were injected with buffer, and untreated control females. I also monitored their life span and overall reproductive output. In parallel, I quantified the personal and social immune responses of females in each treatment by measuring the expression of genes encoding antimicrobial peptides, namely *attacin-4*, *cecropin-1*, *coleoptericin-1* and *PGRP-SC2*. I included a three genes involved in personal immunity (i.e. *attacin-4*, *cecropin-1*, and *coleoptericin-1*) because their is some knowledge about their function in the study system (Jacobs et al. 2016). I included *PGRP-SC2* because there is good evidence that it has a role in social immunity in the study system (Parker et al. 2015, Ziadie et al. 2019). If females respond to infection by shifting their allocation towards immunity, I would expect infected and/or immune-challenged females to show a reduction in their reproductive output and parental care, and an increase in the overall expression of immune genes. This is because, given a trade-off between reproduction and immunity, females would have to reduce reproduction investment (including parental care) in order to increase their investment in immunity. Alternatively, if females respond to infection and/or immune-challenges by shifting allocation towards current reproduction, I would expect infected and/or immune-challenged females to maintain their level of parental care and show a reduction the overall expression of immune genes. Assuming there is a trade-off between personal and social immunity and that females are limited in their investment in personal and social immunity (Cotter and Kilner 2010a), I expect an increase in the expression of genes involved in personal immunity relative to the expression of genes involved

in social immunity if infected and/or immune-challenged females shift allocation towards their own immunity. Alternatively, I would expect a reduction in the expression of genes involved in personal immunity relative to the expression of genes involved in social immunity if infected and/or immune-challenged females shift allocation towards current reproduction.

4.2 Methods

Experimental beetles originated from wild individuals collected in the Hermitage of Braid and Blackford Hill Local Nature Reserve, Edinburgh, U.K. The beetles had been maintained in a large outbred population (200–300 individuals were bred per generation) under laboratory conditions for at least 5 generations before the start of the experiment. Non-breeding adult beetles were housed in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil, under constant temperature at 20°C, 16:8h light:dark photoperiod and ad libitum access to organic beef as food supply.

4.2.1 Experimental design

To investigate the effects of infection on parental care, reproductive output and immunity, I used a group of untreated control females ($N_{Control} = 61$) and three groups of experimental females: infected females that were inoculated with the pathogenic bacteria *Serratia marcescens* ($N_{Infected} = 58$) which is known to be pathogenic in burying beetles (Ratz et al. unpublished data), immune-challenged females that were inoculated with heat-killed bacteria ($N_{Challenged} = 70$), and injured females that were injected with buffer ($N_{Injured} = 56$). These four treatments allows to separate potential effects caused by the infection, activation of the immune system or the injury. At the beginning of the experiment, each individual virgin female was randomly assigned an unrelated male partner and transferred to a larger plastic container (17 cm × 12 cm × 6 cm) lined with moist soil and containing a freshly thawed mouse carcass of a standardized size (19.97–

23.68g) (Livefoods Direct, Sheffield). I weighed each female on the day before the anticipated hatching date (i.e. two days after the onset of egg-laying; Smiseth et al. 2006). I then placed females in an individual plastic vial plugged with cotton. Females remained in this vial until I applied the treatment (see details below), after which they were transferred into a new large container containing fresh soil and supplied with their original carcass. I left the eggs to develop in the old container, while males were discarded. I separated the females from the eggs so that I could allocate each female with an experimental brood of 15 same-aged larvae of mixed maternal origin. I removed the male to avoid any potential effects of male parental care buffering against effects of the experimental treatment on the female. Male removal has no effect on the developing brood under laboratory conditions (Smiseth et al. 2005). I next set up experimental broods of 15 larvae by collecting newly hatched larvae emerging in the soil, starting the day following the separation of females and eggs. I generated experimental broods by pooling larvae that had hatched from eggs laid by multiple females (Smiseth et al. 2007a). I used a standardized brood size that was comprised of 15 larvae of a known age to avoid any potential confounding effects of variation in the number and age of the larvae on maternal behaviour (Smiseth et al. 2003, Ratz and Smiseth 2018). Given that parents will kill any larvae that emerge on the carcass before their own eggs have hatched (Müller and Eggert 1990), I only allocated an experimental brood to a female once her own eggs had hatched.

4.2.2 Bacterial preparation

I chose *Serratia marcescens* (strain DB11) as an appropriate natural bacterial pathogen for *N.vespillodies*. *Serratia marcescens* is a gram-negative bacterium commonly found in the soil and on decomposing carrion (Hejazi and Falkiner 1997, El Sanousi et al. 1987). It has been shown to infect several insect species and is known to cause mortality in both eggs and larva of *N. vespilloides* (Wang and Rozen 2018, Jacobs et al. 2014). Pilot tests confirmed that *S. marcescens* increased female mortality (Ratz et al. unpublished data), but only when in-

jected above a certain concentration and volume (see below). I also note that my pilot tests showed that stabbing with *Pectobacterium carotovorum*, *Pseudomonas aeruginosa*, and injections with *Pseudomonas entomophila* had no detectable effect on female mortality as stabbed females showed similar life span as control females.

To grow the *S. marcescens* culture, I inoculated 10 mL of Luria-Bertani (LB) broth (Fisher Scientific) with 200 μL of a frozen 25% glycerol suspension from a single isolated *S. marcescens* colony. The culture was aerobically incubated overnight in an orbital shaker at 140 rpm and 30°C. On the day of infection, the overnight culture was diluted 1:10 into fresh LB broth and incubated under the same conditions until the culture had reached the mid-log growth phase (OD_{600} 0.6–0.8). Optical density was checked using a microplate absorbance reader at an absorbance of 600 nm. The mid-log phase culture was pelleted by centrifugation (15 min, 4°C, 2500 rpm) and the supernatant removed. The pellet was then re-suspended in sterile Phosphate Buffer Saline (PBS, pH 7.4) and adjusted to OD_{600} 1. The final inoculum OD_{600} was calculated as described in Siva-Jothy et al. (2018). The final inoculum was split into two tubes; one tube was heated to 70°C for 45 min killing the bacteria and allowing for an immune-challenged treatment group while the other tube was kept as a live culture for the infected treatment group.

4.2.3 Infection procedure

On the day preceding the expected date of hatching, I randomly allocated each female to an experimental treatment group. Females from all treatment groups were first anesthetised by releasing CO_2 into their individual tube for 40 s. Control females were then returned to their vials to recover for 30 min, while experimental females were placed on a CO_2 pad under a dissecting microscope. I used a glass needle attached to a microinjector (Nanoject II, Drummond Scientific Co) to inject injured females with 0.552 μL of sterile PBS buffer, immune-challenged

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females with 0.552 μL of heat-killed *S. marcescens* solution, and infected females with 0.552 μL of OD_{600} 1 live *S. marcescens* solution ($\sim$1.3 million colony forming units). I performed the injection by introducing the needle through the soft cuticle that joins the thorax and the abdomen on the ventral side (Reavey et al. 2014). Once injected, experimental females were returned to their vials to recover for 30 min. Following recovery, I next moved control and injected females back to the large containers containing their carcasses.

4.2.4 Maternal care, female weight change, female mortality, and off-spring performance

I recorded the amount of care provided by each female 24 h ($\pm$15 min) after I placed the larvae on the carcass, which corresponded to 48 h ($\pm$4 h) after females were handled and/or injected. I performed direct observations under red light for 30 min, recording maternal behaviour every 1 min in accordance with established protocols (e.g., Smiseth and Moore 2002, 2004b, Ratz and Smiseth 2018). I recorded maternal care as food provisioning, defined as when there was mouth-to-mouth contact between the female and at least one larva, and carcass maintenance, defined as when the female was excavating the soil around the carcass or coating the carcass with antimicrobial secretions. I conducted the behavioural observations blindly with respect to treatment, as it was not possible for the observer to identify the experimental treatments.

Females and their broods were then left undisturbed until larvae completed their development, at which stage they left the mouse carcass to disperse into the soil. At dispersal, I weighed the female, counted the number of larvae and weighed the brood. I estimated weight gain over the reproductive attempts by the female as the difference in body mass between egg-laying and larval dispersal. I estimated larval survival as the difference between the final brood size at dispersal and the initial brood size at hatching (i.e. 15 larvae), and mean larval mass as the total brood mass divided by brood size.

4.2.5   Hemolymph sampling, RNA extraction, and RT-qPCR

To examine the effects of the treatment on the female's immune response, I quantified the expression of genes coding for antimicrobial peptides (AMPs) by quantitative real-time polymerase chain reaction (qRT-PCR). I focused on the expression of the four following genes: *attacin-4*, *cecropin-1*, *coleoptericin-1* and *PGRP-SC2*. I focused on these genes because they are known to have a role in the immune response against gram-negative bacteria, such as *S. marcescens* (Imler and Bulet 2005, Vilcinskas et al. 2013a,b) and there is some knowledge about their function in personal or social immunity in this species (Jacobs et al. 2016, Parker et al. 2015, Ziadie et al. 2019): *attacin-4*, *cecropin-1*, and *coleoptericin-1* seem to play a role mainly in personal immunity (Jacobs et al. 2016), while *PGRP-SC2* plays a role in social immunity (Parker et al. 2015, Ziadie et al. 2019). The expression of the *PGRP-SC2* is higher in female during parental care (Parker et al. 2015) and lower in larvae in the presence of parents (Ziadie et al. 2019).

In parallel with the behavioural observation, I randomly selected a subset of females for RNA extraction, which included 13 control, 14 injured, 17 immune-challenged, and 14 infected females. I removed each of these females from their containers 48 h ($\pm$4 h) after infection, and placed them in an individual plastic vial plugged with cotton. I then anesthetised each female with CO_2 as described above. Once anesthetised, I extracted hemolymph from each female placed on a CO_2 pad by puncturing the soft cuticle behind the thorax with a micro-pine and then drawing hemolymph with a 10 μL-glass capillary. I sampled 2 muL to 10 μL of hemolymph per female and transferred it into 1.5μl-micro-tubes containing 100 L of TRIzol reagent (Invitrogen, Life Technologies). All hemolymph samples were then stored at -70°C until RNA extraction.

RNA extractions were performed using the standard phenol-chloroform method and included a DNase treatment (Ambion, Life Technologies). The RNA purity

of eluted samples was confirmed using a Nanodrop 1000 Spectrophotometer (version 3.8.1). cDNA was synthesized from 2 μL of the eluted RNA using M-MLV reverse transcriptase (Promega) and random hexamer primers, and then diluted 1:1 in nuclease free water. I performed quantitative RT-PCR on an Applied Biosystems StepOnePlus machine using Fast SYBR Green Master Mix (Applied Biosystems). I used a 10 μL reaction containing 1.5 μL of 1:1 diluted cDNA, 5 μL of Fast SYBR Green Master Mix and and 3.5 μL of a primer stock containing both forward and reverse primers at 1 μM suspended in nuclease free water (final reaction concentration of each primer 0.35 μM). For each cDNA sample, two technical replicates were performed for each set of primers and the average threshold cycle (Ct) was used for analysis.

Primers were designed based on amino acid sequences provided on Kyoto Encyclopedia of Genes and Genomics (KEGG) or supplementary information provided by Jacobs et al. (2016) (KEGG: *PGRP-SC2*, *Rlp7*; Jacobs et al. 2016: *Attacin-4*, *Coleoptericin-1*, *Cecropin-1*). Briefly, the amino acid sequence was entered into the Basic Local Alignment Search Tool (BLAST) on NCBI.gov, the accession number producing the most similar alignments within *N. vespilloidies* was selected and the corresponding nucleotide sequence used for primer design in Primer3 (version 4.1.0) and Beacon Designer (Premier Biosoft International). All primers were obtained from Sigma-Aldrich Ltd; Attacin-4_Forward: 5' GCATTTACACGCACAGACCT 3', Attacin-4_Reverse 5' CGGCAACTTTACTTCCTCCG 3'; Cecropin-1_Forward 5' CGAGCACACAACAGTTCCTT 3', Cecropin-1_Reverse 5' ATCAAAGCTGCGATGACCAC 3'; Coleoptericin-1_Forward 5' GAAACGGTGGTGAACAGGTG 3', Coleoptericin-1_Reverse 5' GAGTCTTGGGGAACGGGAA 3'; PGRP-SC2_Forward 5' CGAAGGTCAAGGTTGGGGTA 3', PGRP-SC2_Reverse 5' GTTCCGATGACACAGATGCC 3'. I used *Rpl7* as an endogenous reference gene, following Jacobs et al. (2014, 2016) and Cunningham et al. (2014); Rpl7_Forward 5' GTCGGCAAGAACTTCAAGCA 3', Rpl7_Reverse 5' TCCCTGTTACCGAAGTCACC 3'. For each pair of primers the annealing temperature (T_a) was optimised and the efficiency (Eff) of each primer pair calculated

by 10-fold serial dilution of a target template (each dilution was assayed in duplicate); *Attacin-4*: T_a= 59°C Eff= 102.21%, *Cecropin-1*: T_a= 59.5°C Eff= 102.26%, *Coleoptericin-1*: T_a= 61.6°C Eff= 101.86%, *PGRP-SC2*: T_a= 60.2°C Eff= 99.84%, *Rpl7*: T_a= 60°C Eff= 98.25%.

4.2.6 Data analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team, 2019) loaded with the packages *car* (Fox et al. 2016), *MASS* (Ripley et al. 2017), and *glmmTMB* (Brooks et al. 2017). I analysed data on parental care using a zero-inflated binomial model. I used ANOVA models to analyse normally distributed data; that is, female weight change over breeding and mean larval mass at dispersal. I used a quasi-Poisson model to analyse data on female life span and a binomial model to analyse data on larval survival. Note that I did not use a Cox Proportional-Hazards model to analyse female survival as this was not necessary given that I had data on life span of all females, allowing me to compare the life spans of females in the different treatment groups, and because the data did not satisfy the assumption of proportional hazards (Therneau 2015; $\chi^2 = 12.0$, P = 0.007). All models included the treatment as a fixed effect with four levels (i.e. infected, immune-challenged, injured and control females). To account for potential effects of brood size on maternal care (Smiseth et al. 2003, Ratz and Smiseth 2018), I also included brood size at the time of observation as covariate in the model analysing maternal care. I ran pairwise comparisons using a Tukey's test with the Bonferroni correction whenever the treatment had a significant effect.

To analyse data on gene expression, I first calculated the expression of a gene of interest relative to the reference gene *Rpl7* to obtain ΔCT values (Livak and Schmittgen 2001). I then used ANOVA models to for effects of the experimental treatment on the ΔCT values of each gene. Whenever the treatment had a significant effect on gene expression, I ran pairwise comparisons using a Tukey's

test with the Bonferroni correction.

Among the 245 females, I sacrificed a subset of 59 females to sample hemolymph, of which one was excluded because not enough hemolymph was obtained. Among the remaining females, I excluded 55 additional females from my analysis on maternal care, life span and larval survival because their eggs fail to hatch (N = 10), there were not enough larvae to allocate them a brood (N = 25), the female or the whole brood died before the observation (N = 12), no behavioural data were collected (N = 1), or the heat-kill treatment failed (N = 7). The final sample of the behavioural and life history data included 33 control females, 32 injured females, 33 immune-challenged females, and 33 infected females. Likewise, I excluded 9 broods (control females: N = 4; injured females: N = 3; immune-challenged females: N = 2) from my analysis on mean larval mass at dispersal because no larvae survived to dispersal.

4.3 Results

There was a significant effect of treatment on female life span (Figure 4.1a; $\chi^2 = 52.1$, df = 3, P < 0.001), which reflected that infected females had an average life span that was 75% shorter than females from any other treatment group (Table 4.1). There was no significant effect of treatment on the amount of care provided by females (Figure 4.1b; $\chi^2 = 6.63$, df = 3, P = 0.085), showing that females maintained a similar level of care to control females regardless of whether they were infected, immune-challenged or injured. There was no effect of brood size at the time of observation on maternal care ($\chi^2 = 2.62$, df = 1, P = 0.105). There was no effect of treatment on mean larval mass at dispersal (Sum Sq = 0.003, df = 3, F = 0.613, P = 0.608) or survival of the larvae until dispersal ($\chi^2 = 5.66$, df = 3, P = 0.129), suggesting that infected, immune-challenged or injured females maintained a similar level reproductive output to control females. There was no difference in weight change between females in the different treatments (Sum Sq = 174.7, df = 3, F = 1.42, P = 0.239).
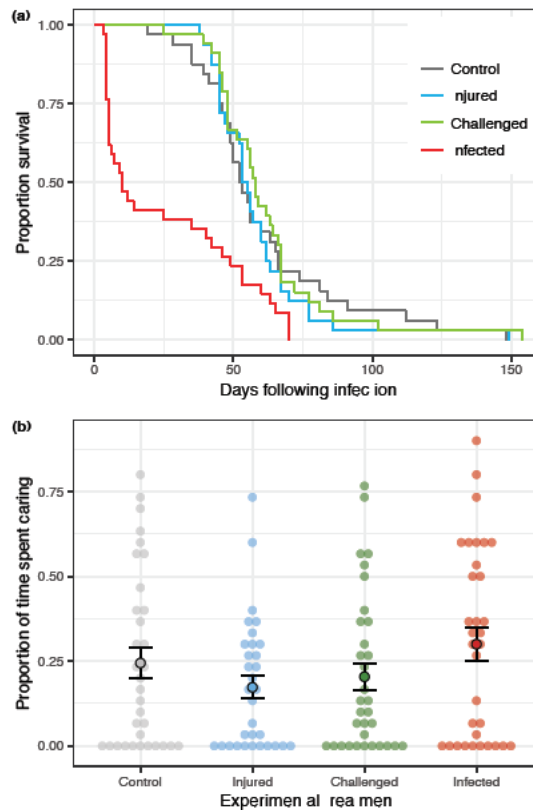
Figure 4.1: Proportion of females alive over time after the day the treatment was applied (a). Effects of the experimental treatment on maternal care (b). Open circles represent individual data, closed circles and bars represent Means ± SEs.

I next investigated the effects of the experimental treatments on the expression of four immune genes. Treatment had a significant effect on the expression of *coleoptericin-1* (Figure 4.2a; Sum Sq = 780.3, df = 3, F = 42.9, P < 0.0001). The expression of this gene was lower in injured females than in control females (Table 4.2), lower in immune-challenged females than in injured females (Table 4.2), and similar in immune-challenged and infected females (Table 4.2). Treatment also had a significant effect on the expression of *PGRP-SC2* (Figure 2b; Sum Sq = 266.7, df = 3, F = 3.47, P = 0.022). The expression of this gene was reduced in injured females compared with infected ones (Table 4.2), while there was no difference in expression between females in any of the other treatment groups (Table 4.2). I found no significant effect of treatment on the expression of *attacin-4* (Figure 2c; Sum Sq = 45.7, df = 3, F = 1.55, P = 0.211) or *cecropin-1* (Figure

Table 4.1: Pairwise comparisons between treatments for the post-infection life span. P-values were obtained using Tukey's HSD test and adjusted using the Bonferroni correction.

	Post-infection life span			
	Estimate	SE	z	P
Injured − Control	−0.035	0.117	−0.299	0.991
Challenged − Control	0.014	0.115	0.123	0.999
Infected − Control	−0.866	0.148	−5.83	<**0.001**
Injured − Challenged	−0.049	0.116	−0.424	0.974
Infected − Injured	−0.831	0.149	− 5.57	<**0.001**
Infected − Challenged	−0.880	0.147	−5.97	<**0.001**

4.2d; Sum Sq = 21.1, df = 3, F = 1.57, P = 0.206).
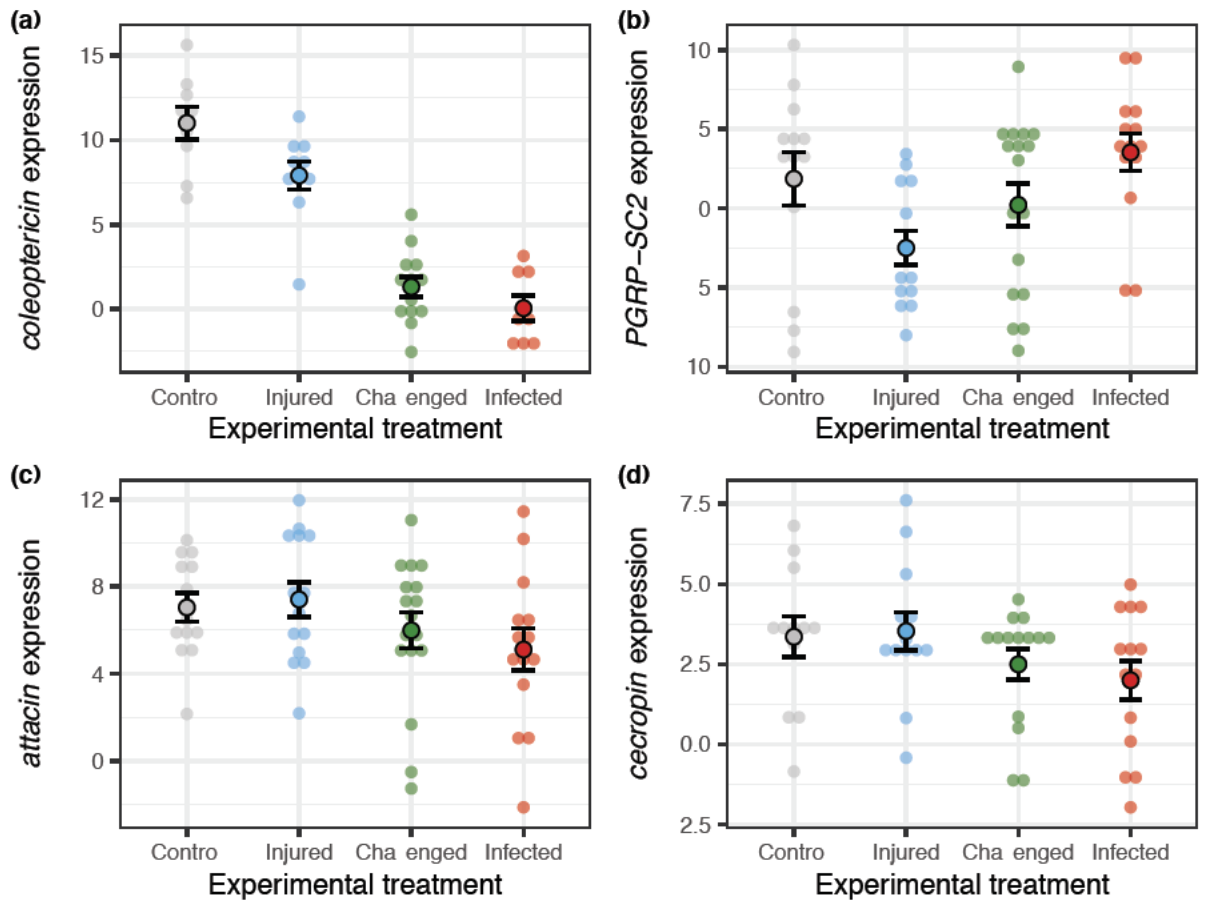
Figure 4.2: Effects of the experimental treatment on the expression of *coleoptericin-1* (a), *PGRP-SC2* (b), *attacin-4* (c), and *cecropin-1* (d). Open circles represent individual data, closed circles and bars represent Means ± SEs.

Table 4.2: Pairwise comparisons between treatments for the level of gene expression. P-values were obtained using Tukey's HSD test and adjusted using the Bonferroni correction.

	attacin-4				cecropin-1				coleoptericin-1				PGRP-SC2			
	Estimate	SE	t	P	Estimate	SE	t	P	Estimate	SE	t	P	Estimate	SE	t	P
Injured − Control	0.365	1.20	0.304	0.990	0.176	0.844	0.209	0.997	−3.10	1.13	−2.74	**0.045**	−4.36	1.98	−2.19	0.136
Challenged − Control	−1.05	1.15	−0.914	0.798	−0.859	0.816	−1.05	0.720	−9.68	1.06	−9.07	**<0.001**	−1.62	1.86	−0.886	0.811
Infected − Control	−1.93	1.20	−1.60	0.385	−1.35	0.829	−1.63	0.367	−10.9	1.19	−9.15	**<0.001**	1.66	1.94	0.856	0.826
Injured − Challenged	1.41	1.12	1.25	0.594	1.03	0.799	1.29	0.569	6.58	1.03	6.36	**<0.001**	−2.71	1.86	−1.45	0.471
Infected − Injured	−2.29	1.18	−1.94	0.222	−1.53	0.812	−1.88	0.245	−7.84	1.16	−6.72	**<0.001**	6.03	1.94	3.09	**0.016**
Infected − Challenged	−0.879	1.12	−0.778	0.864	−0.498	0.788	−0.636	0.920	−1.26	1.10	−1.14	0.666	3.32	1.82	1.81	0.275

4.4 Discussion

Here I show that infected and immune-challenged females altered their expression of immune genes, and that infected females had a shortened life span compared to other females. Despite the heightened mortality of infected females, I found no evidence for a difference between infected, immune-challenged, injured and control females in their level of care or their reproductive output. Altogether, my findings indicate that infected females maintained their level of care despite indication that they mounted an immune response against the pathogen and clear evidence that the pathogen shortened their life span. This strategy may allow infected females to provide the necessary amount of care to ensure the growth and survival of their offspring but might be detrimental to the parents by increasing their mortality and may potentially even facilitate disease transmission to offspring. Below I discuss the broader implications of these findings to our understanding of the effects of infection on parental behaviour and social interactions between caring parents and their dependent offspring.

As expected, I found that infected females altered their expression of immune genes and had a considerably shortened life span, confirming that infection with *Serratia marcescens* had the intended effect of triggering a change in immunity and making infected females sick. Immune-challenged females showed a similar change in the expression of immune genes as infected females, but suffered no corresponding reduction in their life span. Thus, my results confirm that the shortened life span of infected females was caused by the pathogen rather than being a by-product of females mounting an immune response. Taken together, my results confirm that *Serratia marcescens* is a potent pathogen in *N. vespilloides*. I am not aware of any prior studies on *N. vespilloides* reporting elevated mortality as a result of an infection, which may reflect the difficulty in establishing experimental infections in this species. This may reflect that this species breeds on decomposing carcasses, which means they regularly are in close contact with potential pathogens (Jacobs et al. 2014, Wang and Rozen 2018). My study species might thus be resistant to a wide variety of bacterial strains, such as *Bacillus*

subtilis (Reavey et al. 2015), *Pectobacterium carotovorum*, *Pseudomonas aeruginosa*, *P. entomophila*, or *S. marcescens* at low doses and concentrations (Ratz et al. unpublished data) that are pathogenic in many others insect species. My results show that, as long as *S. marcescens* is injected in relatively high dose and concentration based on prior knowledge of the pathogen (Ratz et al. unpublished data), it can successfully establish an infection in *N. vespilloides*, activate the immune system, and greatly increase mortality.

My main finding was that infected females maintained their level of care and their reproductive output, despite showing changes in immune gene expression and suffering negative fitness consequences of infection as indicated by their shortened life span. My results suggest that infected females maintained their level of care at the expense of allocating more resources towards immunity. My results are similar to those of a recent study on the amphipods *Crangonyx pseudogracilis* and *Gammarus duebeni* (Arundell et al. 2014). In this study, infection by a microsporidian did not affect brood care behaviour or the duration of brooding of females. By maintaining their level of care, infected females may ensure that offspring receive the necessary amount of care and produce offspring with a similar survival and body size as offspring of uninfected females. This strategy might allow infected females to maintain their reproductive output (Arundell et al. 2014), but might come at a cost in terms of reduced survival and future reproductive success. Burying beetles can produce multiple broods (Creighton et al. 2009) and tend to gain mass during first reproduction, which is positively correlated with life span (Gray et al. 2018). My results suggest that infected females would have lower fitness because it seems unlikely that the infected females in my study could reproduce again. The reason for this is that approximately 60% of infected females had died by 17 days after the infection (compared with 0% of control females; Figure 4.1a). Thus, many infected females had died before they would have been able to produce an additional brood. In order to breed again, females must first remain with the current brood until larvae complete their development, which would take about 7 days (Smiseth et al. 2003, 2005). They then need to

search for and secure a new carcass, which are thought to be rare (Scott 1998), and produce eggs and care for the new brood, which would take another 10 days (Ford and Smiseth 2017). An alternative explanation for my results is that infected females perceived their chance to survive and reproduce again to be very low, and that they therefore maintained a high level of care as a terminal investment response (Williams 1966). This is suggested by other studies in the species reporting high reproductive output in response to immune-challenges (e.g. Cotter et al 2010, Reavey et al. 2014, Reavey et al. 2015, Farchmin et al. 2020). I found no evidence for an increase in reproductive investment as would be expected under terminal investment. However, this may reflect that infected females were simply not able to increase their level of care. I would have expected immune-challenged females, exposed to pathogen cues but not infected, to be able to increase care given that they did not show any evidence of shortened life span. I did not find such a response in immune-challenge females. Thus, I suggest that, rather than mounting a terminal investment response, infected females maintained their level of care to provide the necessary amount of care to ensure offspring growth and survival, which might come at a cost to females in terms of reduced survival.

My finding that infected females maintained their level of care also shows that infections do not necessarily induce sickness behaviour. Infections are often associated with a reduction in the host's social interactions (Hart 1988, Kelley et al. 2003), which was not the case in my study as there was no evidence for a reduction in maternal care. Infected hosts often show reduced social interactions (Vale et al., 2018), which may be the result of lethargy (i.e., reduced activity levels) of the host associated with sickness (Adelman and Martin 2009), the host actively avoiding costly social interactions (Sah et al. 2018, Lopes et al. 2016), uninfected individuals avoiding an infected host (Curtis 2014), or the pathogen manipulating the host's behaviour (Moore 2002, Hughes et al. 2012). Yet this reduction in social behaviour is not always observed, depending on the social context (Lopes et al. 2012, Adamo et al. 2015), and parents that are sick might maintain their level of care and interactions with offspring (Stockmaier et al. 2020). Because

parental care and parent-offspring interactions can have a large impact on the reproductive output of organisms, I propose that infected parents might prioritise their allocation in reproduction by maintaining necessary care and social interactions with their offspring. In species with biparental care, infected females might be able to reduce their level of care (and thereby increase their immune response) without harming their offspring if the male parent compensate for the reduction in female care. If so, male compensation could temper the negative effect of infection on female life span. Thus, I encourage future studies to compare the responses of infected females in the contexts of biparental care and uniparental care.

My last finding was that females from the different treatment groups showed different levels of expression in two immune genes (i.e. *coleoptericin-1* and *PGRP-SC2*), while there was no difference in the expression of other immune genes (i.e. *attacin-4* and *cecropin-1*). The expression of *coleoptericin-1*, a gene seemingly having a systemic role inhibiting cell division and bacterial growth in insects (Sagisaka et al. 2001, Login et al. 2011) and involved in personal immunity in burying beetles involved in personal immunity (Jacobs et al. 2016; Parker et al. 2015), was lower in immune-challenged and infected females than in injured and control females. This was opposite to my prediction and surprising given prior evidence showing that immune-challenged and infected females upregulate personal immunity genes, such as *defensin* (Ziadie et al. 2019), in response to immune-challenges (Reavey et al. 2014). In contrast, the expression of *PGRP-SC2*, a gene active in the gut and providing protection against overactivation of the immune system in insects (Broderick et al. 2009, Paredes et al. 2011, Guo et al. 2014), which has a role in social immunity gene in burying beetles as it provides offspring with antimicrobial protection (Parker et al. 2015, Ziadie et al. 2019), was higher in infected females than in injured females. Given that there was no difference in immune gene expression between immune-challenged and infected females, it seems unlikely that the pathogen suppressed the immune system in the study species. Instead, these results might reflect immune responses to the presence of a pathogen or, in the case of immune-challenged females, to the presence of cues

from a potential pathogen. Thus, my finding that infected females had lower personal immunity and maintained normal levels of social immunity points towards a shift in investment towards current reproduction. This suggests that infected and immune-challenged females maintained their investment in social immunity that benefits larval survival, which would support the idea that infected females overall sought to maintain their allocation towards current reproduction.

My findings have important implications for our understanding of parental behaviour under the risk of infection by showing that infected females maintained a high level of care despite the fact that infections could expose their offspring to the pathogen. Thus, my results show that the level of care is remarkably stable in response to infection, notwithstanding evidence that parents often show a great amount of plasticity in response to other environmental factors, such as resource abundance and the presence of competitors and infanticidal conspecifics (Smiseth and Moore 2002, Hopwood et al. 2015, Georgiou Shippi et al. 2018). Furthermore, behavioural plasticity represents the first mechanism of immunity (Schaller 2006, Schaller and Park 2011, Kiesecker et al. 1999) and might allow infected individuals to reduce the risk of transmission to close kin, including offspring (Shakhar and Shakhar 2015, Shakhar 2019). My study found no evidence that females transmitted the pathogen to their offspring given that we found no indication that larvae of infected females had higher mortality than larvae of other females. Nevertheless, we urge future studies to consider the potential consequences of disease transmission by caring parents to their offspring (Chakarov et al. 2015). For example, infected parents might be expected to maintain their level of care in situations where the risk of females passing on the pathogen to their offspring is low. In contrast, infected parents might reduce their level of care in situations where the risk of females passing on the pathogen to their offspring is high and where the offspring are not completely dependent on their parents.

In summary, my study shows that infected females maintained their level of parental care and reproductive output despite showing changes in immune gene

expression and suffering from greater mortality. My results demonstrate that parental care, which is generally highly flexible, can remain robust and stable in response to pathogenic infections. The results also suggest that infected females maintain their current reproductive success over survival, which could ensure that offspring receive the necessary amount of care. My findings stress the need for more studies on infection in species where parents care for and interact with their offspring, as parental care is a fundamental social interaction in all birds and mammals as well as some amphibians, fishes and arthropods and as it can have contradicting effects by buffering against environmental hazards on the one hand and providing a potential route for disease transmission on the other hand.

5 Biparental responses to resource availability

Abstract

In species where both sexes provide care, each parent has to coordinate its behaviour with its partner to best respond to offspring needs. Given that the costs of care are shared between the two parents, whereas parents receive equal indirect benefits of care, each individual parent is expected to reduce its own care at the expense of the other parent. In addition, parents should adjust their care to environmental changes that affect the costs and benefits of care. One key environmental variable is resource availability as it influences both investment in the current brood and in future reproduction and survival. I investigated the impact of resource availability on biparental care using the burying beetle *Nicrophorus vespilloides* as a study species. Burying beetles breed on the carcass of a small vertebrate, which is also the sole food source for parents and offspring during breeding and can easily be manipulated. I provided breeding pairs with mouse carcasses from a broad range of different sizes and subsequently monitored parental care, parental food consumption, weight change over breeding, and larval traits related to offspring performance. I found that the duration of biparental care increased with carcass size, which was mainly the result of males adjusting care to carcass size, while females showed little response. Further, lower resource availability led to more pronounced sex differences in parental care. I also found that parents gained more weight and produced more offspring on larger carcasses. These findings highlight that resource availability may have contrasting effects on the balance between cooperation and conflict depending on whether it reduces or increases the benefits of biparental cooperation.

5.1 Introduction

Biparental care occurs when male and female parents cooperate to care for their joint offspring. It is the predominant pattern of care in birds (Cockburn 2006) but has also evolved in a small number of mammals, amphibians, fishes, and arthropods (Balshine 2012, Trumbo 2012). The evolution of biparental care has attracted much interest because it is associated with sexual conflict, arising because the benefits in terms of enhanced offspring fitness result from the combined effort of the two parents, whilst the costs in terms of reduced future survival and reproduction depend on each parent's personal effort (Trivers 1972, Chase 1980, Lessells 2012). Thus, biparental care involves a balance between cooperation and conflict, and any shift in this balance could be detected as a change in the frequency and/or duration of biparental care relative to uniparental care (Westneat and Sargent 1996, Lessells and McNamara 2012, Johnstone and Savage 2019). Such shifts between biparental and untiparental care are likely to be driven by changes in environmental conditions that alter the costs and/or benefits of care, including ambient temperature (e.g. Vincze et al. 2013), predation risk (Expósito Granados et al. 2016), density of interspecific competitors (Burdick and Siefferman 2020), or habitat structure and altitude (Lejeune et al. 2019).

Variation in resource availability is likely to be an important environmental condition in this respect. Greater availability of resources may have contrasting effects on the balance between conflict and cooperation between parents depending on whether it reduces or increases the benefits of biparental cooperation. The rationale for why this is the case can be explored in light of evolutionary game theoretic models for the evolution of male and female parental care (e.g., Maynard Smith 1977). On the one hand, greater availability of food may reduce the benefits of biparental cooperation in species where parents provision food to the offspring. When food is abundant, the female can provision more food to the brood on her own, thereby reducing the benefits to the male from assisting the female. In support of this argument, a comparative study on weaverbirds found that uniparental female care is more common in species that breed in en-

vironments where food is abundant (Crook 1963). On the other hand, greater availability of food may increase the benefits of biparental cooperation in species where parents protect the offspring from predators or conspecific intruders and where the risk of predation is correlated with food availability, amongst other environmental variables. For example, if greater availability of food increases the risk of nest predation or infanticide by conspecific intruders (e.g., Wilson and Fudge 1984, Robertson 1993), there may be an increase in the benefits to the male from assisting the female when food is more abundant. In order to advance our understanding of the effects of variation in resource availability, there is now a need for experiments that manipulate the amount of resources and then monitor effects on the frequency and/or duration of biparental care relative to uniparental care.

The way in which variation in resource availability shifts the balance between cooperation and conflict may depend critically on potential differences in the responses of male and female parents. For example, in Palestine sunbirds, females respond to food-supplementation by provisioning more food to the nestlings, whilst males increase their mobbing effort (Markman et al. 2002). Such sex-specific responses may reflect that biparental care often involves sex differences in the level of care with females usually making greater contributions than males (Kokko and Jennions 2012, West and Capellini 2016). For example, females provide more care than males in red-winged blackbirds (Whittingham 1989), house sparrows (Schwagmeyer et al. 2008), oldfield mice (Margulis 1998), convict cichlids (Lavery and Keenleyside 1990) and burying beetles of the genus *Nicrophorus* (Smiseth and Moore, 2004a, Trumbo 2007). Such sex differences in care may reflect sex differences in the costs and/or benefits of care. For example, in Kentish plovers, where females desert the brood earlier than males, females incur higher costs of care because they find a new partner quicker and thus lose more mating opportunities than males when not deserting (Székely and Williams 1995, Székely et al. 1999). Thus, variation in resource availability, by altering the costs and benefit of care, may lead to changes in the magnitude of any sex differences in

care, and such sex differences may in turn impact on the balance between cooperation and conflict if parents of one sex are more likely to desert the brood.

I used the burying beetle *Nicrophorus vespilloides* to investigate how availability of resources shifts the balance between cooperation and conflict and alters the magnitude of sex differences in care. Burying beetles of the genus *Nicrophorus* are ideal to address these issues because they breed on carcasses of small vertebrates that vary considerably in mass (Müller et al. 1990, Smiseth and Moore 2002). The vertebrate carcass used for breeding provides the sole source of food for both developing larvae and caring parents (Scott and Traniello 1990, Scott 1998, Pilakouta et al. 2016). Thus, it is straightforward to manipulate the availability of resources by simply providing parents with carcasses of variable masses (Smiseth et al. 2014). Furthermore, these species show facultative biparental care, whereby male and female parents cooperate to varying degrees by providing extended care to the developing larvae (Eggert et al. 1998, Scott 1998). Thus, a shift in the balance between cooperation and conflict could be detected as a change in the duration of biparental care relative to uniparental care. Both female and male parents provide care by provisioning pre-digested carrion to the larvae and by defending the carcass and the brood from conspecific intruders (Eggert et al. 1998, Scott 1998). Prior work on *N. vespilloides* shows that there are synergistic effects of biparental cooperation, and that that these often outweigh the detrimental effects of sexual conflict on offspring growth and survival (Pilakouta et al. 2018). However, it is unclear how variation in carcass mass would impact on the balance between cooperation and conflict. Females spend more time on parental care (e.g. Smiseth et al. 2005, Georgiou-Shippi et al. 2018) and care for longer than males (Bartlett 1988, Ford and Smiseth 2016), yet it is unclear what impact variation in carcass mass would have on the magnitude of sex differences in care. Although there is good evidence that parents produce larger broods on heavier carcasses (e.g. Müller et al. 1990) and that both males and females provide more care towards larger broods relative to small ones (e.g. Smiseth et al. 2007a,b, Ratz et al. 2020b), there is little understanding about how variation in

carcass mass might directly impact on parental care.

My aim was to test for effects of variation in carcass mass on the balance between cooperation (i.e. the amount and duration of care) and conflict (i.e. consumption of shared food resource, brood abandonment) and on sex differences in care. I provided breeding pairs with mouse carcasses of variable mass (3.65–26.15g) and monitored subsequent effects on the duration of biparental care relative to uniparental care. I also monitored effects on sex differences in the duration of male and female care and the amount of time spent providing care by males and females. I also monitored effects on resource consumption and weight change by males and females during breeding, as well as effects on brood size and mean larval mass at the time of larval dispersal. As argued above, an increase in carcass mass may lead to a shift towards either more conflict or more cooperation between parents. The latter prediction seems more likely in *N. vespilloides* given that larger carcasses are more valuable to conspecific intruders, and that two parents are more efficient at protecting their brood against intruders than single ones (Trumbo 1991). Meanwhile, I predicted that sex differences in parental care would be more pronounced as carcass mass decreased. The reason is that male contribution to parental care should be less necessary as carcass size decreases given than smaller carcasses are less valuable to conspecific intruders. I also predicted that females would respond less to an increase in carcass mass than males in terms of carrion consumption and weight gain given that caring parents have greater access to the carcass as food source for themselves because they spend more time at the carcass than non-caring parents (Pilakouta et al. 2016). This is because females are predicted to remain at the carcass for a similar amount of time regardless of carcass size, whereas males are predicted to provide care for longer on larger carcasses, thereby giving them more opportunities to consume from the carcass (Keppner et al. 2018).

5.2 Methods

I used virgin beetles from an outbred laboratory population maintained for at least four generations at the University of Edinburgh. The laboratory population descended from beetles that originally were collected in Hermitage of Braid and Blackford Hill Local Nature Reserve, Edinburgh, U.K. Non-breeding adult beetles were maintained in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil, under a constant temperature (20°C) and a 16:8h light:dark photoperiod, and fed a small piece organic beef twice a week.

5.2.1 Experimental design

To test for effects of variation in carcass mass on the balance between cooperation and conflict and the magnitude of sex differences in care, I designed a laboratory experiment where I manipulated the mass of the carcass that pairs were provided with at the start of breeding. I started the experiment by paring virgin females with a randomly assigned, unrelated, virgin male partner (270 pairs in total). I weighed all males and females at this stage to record their pre-breeding mass. To initiate breeding, each pair was moved into a larger, transparent container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and provided with a previously frozen mouse carcass (Livefoods Direct, Sheffield). I randomly assigned each pair with a mouse carcass that weighed between 3.65g and 26.15g (mean ± SE = 13.41g ± 0.396g). This mass range matches that used by my study species under natural (2–30g; Müller et al. 1990) and laboratory conditions (2–40g; Smiseth and Moore 2002).

From the day of mating onwards, I checked each container daily for the presence of eggs. I did this to record the day on which the first eggs were laid. Females lay their eggs in the soil surrounding the carcass, and most eggs are visible from the bottom of the transparent container in a thin layer of soil (Monteith et al. 2012), as used in my experiment. I counted the eggs two days after the onset of egg-laying (i.e. the day preceding the time of hatching of the first eggs in the

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clutch) and used the number of eggs as a measure of clutch size. On the following day, when the eggs started to hatch, I counted the number of newly hatched larvae, using this as a measure of brood size on the day of hatching. Given that females lay their eggs asynchronously over a mean period of 27 h (Müller 1987, Smiseth et al. 2006), the final brood size may be larger than brood size on the day of hatching.

I recorded shifts in the balance between cooperation and conflict by monitoring the duration of biparental care relative to uniparental care. I checked the containers daily from the time of mating until the time of dispersal, recording whether the male and the female were still present on the carcass or whether either of them had deserted the brood. I scored the male and the female as having deserted the brood if absent from the crypt (i.e., the depression in the soil surrounding the carcass) on two consecutive days. I removed any parent that had deserted the brood from the breeding container to prevent the deserting parent from posing a risk to the brood. Removing a deserting parent matches natural conditions given deserting parents leaves the carcass permanently (Scott and Traniello 1990) and tend kill larvae when artificially constrained to remain with the brood as the result of laboratory experiment (Ratz et al. personal observation). I weighed any deserting parent to record information on weight change during breeding (see below). I recorded the duration of biparental care as the number of days from mating until one of the parents deserted the brood. If both parents cared for the brood until the larvae dispersed from the carcass, I recorded the duration of biparental care as the number of days from mating until the larvae dispersed from the carcass (normally 7 days; Scott 1998, Grew et al. 2019).

To estimate the amount of time that each parent spent providing care and consuming resources, I monitored the behaviour of parents on the day after the first eggs had hatched. This time point corresponds to the peak of parental food provisioning towards the larvae in this species (Smiseth et al. 2003). I conducted behavioural observations for 30 min under red light, recording the behaviour of

both parents at 60 s intervals in line with established protocols (e.g. Smiseth and Moore 2002, 2004a, Pilakouta et al. 2018). I recorded whether each parent was provisioning food, defined as any mouth-to-mouth contact between a parent and at least one larva, maintaining the carcass, defined as excavation of the soil around the carcass or coating the carcass with exudates, or in near proximity to the brood, defined as whenever a parent was at a distance from larvae that was equal to or shorter than its pronotom length (e.g., Smiseth and Moore 2002, 2004a). I recorded time spent consuming carrion as any instances where a parent was feeding within the crater (i.e. the opening on the top of the carcass; e.g. Pilakouta et al. 2016). I also recorded the number of larvae that were begging to a parent at each scan. I then calculated the average proportion of time spent begging per larva in the brood as $B = (\Sigma b/n)/p$, where Σb is the cumulative number of begging events during the 30-min observation period, n is the brood size at the time of observation, and p is the number of scans during which a parent was in near proximity to the brood.

I then left experimental broods undisturbed until the larvae dispersed from the carcass. At the time of dispersal, I counted the number of larvae and weighed the whole brood to calculate mean larval mass as total brood mass divided by brood size. I also weighed each parent again at dispersal and calculated relative weight change during breeding as the difference in body mass measured at dispersal (or removal) and pre-breeding mass, divided by pre-breeding mass. In this species, parents feed from the carcass during breeding (Pilakouta et al. 2016), and parental weight change is used a proxy for investment in future reproduction (Creighton et al. 2009, Billman et al. 2014, Gray et al. 2018).

5.2.2 Data analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team 2019) loaded with the packages *car* (Fox et al. 2016), *MASS* (Ripley et al. 2017), and *glmmTMB* (Brooks et al. 2017). We analysed data on the shift between cooperation and conflict between the two parents as a number

of days of biparental care relative to uniparental care using a generalised linear models (GLM) assuming a Poisson error structure and including carcass mass as the only fixed effect. We analysed data on sex differences in the duration of care using GLMs assuming Poisson error structures. We verified the good fit of the models and the absence of over-dispersion using the âtestResidualsâ function of the *DHARMa* package in R (Hartig 2017). To analyse data on sex differences in parental behaviour on the day after hatching (i.e., the amount of time spent provisioning food to the brood, maintaining the carcass, and consuming carrion), we used GLMs with zero-adjusted binomial distributions to account for zero-inflation and over-dispersion. We used linear models to analyse data on parental weight change over breeding. In all other models, we included carcass mass, the sex of the focal parent and, to test for potential sex-specific response to resource availability, the interaction between carcass mass and sex. We also tested whether potential effects of carcass mass on parental behaviours on the day of hatching were fully or partially driven by clutch size or brood size. The reason for this is that parents adjust the amount of care that they provide to the number of offspring in the brood (Ratz and Smiseth 2018), and that brood size covaries with carcass size (Bartlett and Ashworth 1988, Smiseth et al. 2014). To determine whether any overall effect of carcass size was causally linked to variation in carcass mass or brood size, we first ran each model excluding clutch size and then compared this model to a full model that included clutch size as a fixed effect.

For our analyses on offspring behaviour and performance, we used a GLM assuming a binomial error structure to analyse data on the average time spent begging by individual larvae, a GLM assuming a negative binomial error structure to analyse data on brood size at dispersal, and a linear model to analyse data on mean larval mass at dispersal. All models included carcass mass as a fixed effect. We also tested the effect of biparental cooperation on offspring performance by including the duration of biparental care as a covariate in the models on brood size and mean larval mass at dispersal. As described above, we first excluded clutch size or brood size at the time of observation from the models and then ran

each model again including clutch size or brood size at the time of observation as an additional fixed effect.

5.3 Results

5.3.1 Duration of biparental care

The duration of biparental care increased by approximately 0.6 days for each additional 10g of carcass (Figure 5.1; estimate = 0.016, SE = 0.005, z = 3.27, P = 0.001), supporting the prediction that an increase in carcass mass was associated with a shift towards more cooperation between parents. Clutch size had a significant positive effect on the duration of biparental care (estimate = 0.007, SE = 0.003, z = 2.09, P = 0.037). Including clutch size in the model, however, did not change the direction and significance of the effect carcass mass on duration of biparental care.

5.3.2 Sex differences in duration of care

There was a significant effect of the interaction between the sex of the focal parent and carcass mass on the duration of care (Table 5.1). This interaction effect reflected that males provided care for longer as carcass mass increased, whilst females tended to provide care until the time of larval dispersal regardless of carcass mass (Figure 5.2a; sex × carcass mass: estimate = 0.016, SE = 0.006, z = 2.59, P = 0.010). Thus, as predicted, sex differences in parental care became more pronounced as carcass mass decreased. There was no significant main effect of carcass mass on the duration of female care (Table 5.1). However, males deserted the brood earlier, and thus provided care for a shorter period of time, than females (Table 5.1; mean ± SE duration of care from the day of mating: male = 4 days ± 0.15 day, female = 7 days ± 0.13 day; estimate (male versus female) = −0.61, SE = 0.103, z = −6.21, P <0.001).
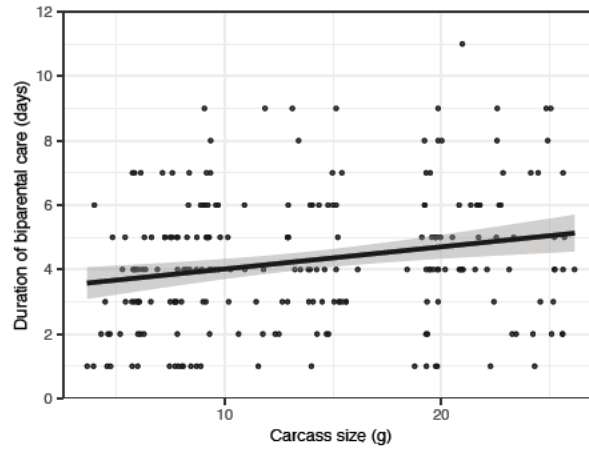
Figure 5.1: Effects of carcass size on the duration of biparental care. Filled circles represent individual data point, the line represents a linear regression line and shaded ribbons the 95% confidence intervals.

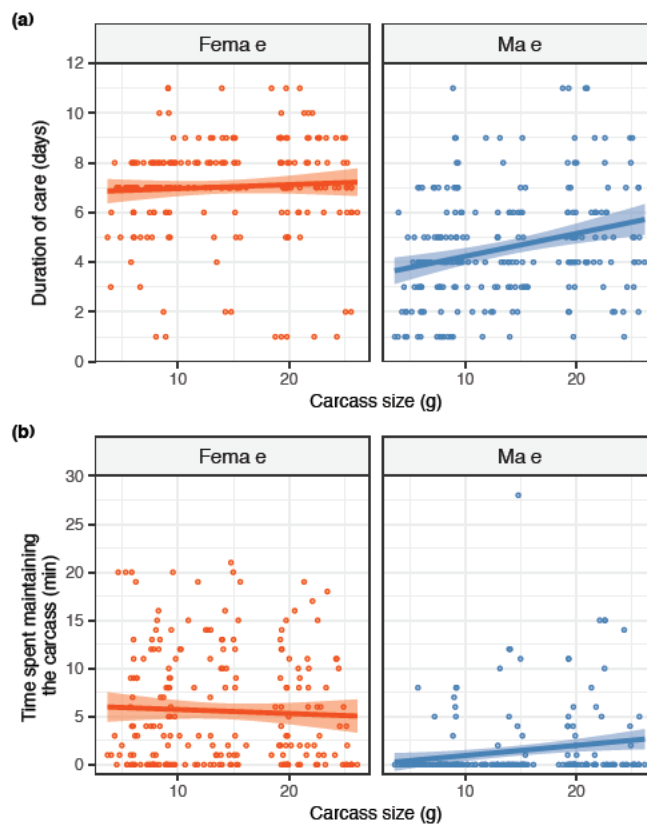Figure 5.2: Effects of carcass size on the duration of female and male parental care (a) and on the time each parent spent on carcass maintenance (b). Filled circles represent individual data, lines represent linear regression lines and shaded ribbons the 95% confidence intervals.

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Table 5.1: Effects of the interaction between parental sex and carcass size (and clutch size or brood size) on the duration of uniparental care, on time spent provisioning food to the brood, maintaining the carcass and consuming carrion when clutch size or brood size is excluded (a) and included (b) in the model. Values are obtained from GLMMs.

	Carcass size:Sex			Carcass size			Focal parent's sex			Clutch/Brood size		
	χ^2	df	P	χ^2	df	P	χ^2	df	P	χ^2	df	P
Duration of care												
(a)	8.12	1	**0.004**	0.340	1	0.562	48.8	1	**<0.001**			
(b)	6.70	1	**0.010**	0.005	1	0.943	38.6	1	**<0.001**	5.56	1	**0.018**
Food provisioning												
(a)	2.40	1	0.121	0.021	1	0.884	39.1	1	**<0.001**			
(b)	2.53	1	0.111	0.286	1	0.592	39.4	1	**<0.001**	4.61	1	**0.032**
Carcass maintenance												
(a)	9.56	1	**0.001**	0.176	1	0.674	48.2	1	**<0.001**			
(b)	10.0	1	**0.001**	1.18	1	0.275	48.7	1	**<0.001**	41.3	1	**<0.001**
Carrion consumption												
(a)	<0.001	1	0.998	3.78	1	0.051	15.9	1	**<0.001**			
(b)	0.194	1	0.659	3.642	1	0.064	20.0	1	**<0.001**	0.177	1	0.673

5.3.3 Sex differences in amount of care

There was no effect of the interaction between the sex of the focal parent and carcass mass on the amount of time parents spent provisioning food to the brood on the day after hatching (Table 5.1). There was no significant main effect of carcass mass on the amount of time spent provisioning food the brood (Table 5.1). Males spent significantly less time, on average, provisioning food to the larvae than females (mean $\pm$ SE time spent provisioning food out of 30 min: male = 0.74 min $\pm$ 0.18 min, Female = 4.4 min $\pm$ 0.3 min; estimate (male versus female) = −4.59, SE = 0.732, z = −6.27, P <0.001).

The interaction between the sex of the focal parent and carcass mass had a significant effect on the time spent maintaining the carcass (Table 5.1, Figure 5.2b), reflecting that males spent more time maintaining the carcass as carcass mass increased whereas carcass mass had no noticeable effect on the amount of time spent maintaining the carcass by females (sex $\times$ carcass mass: estimate = 0.148, SE = 0.046, z = 3.17, P = 0.001). There was no main effect of carcass mass on time spent maintaining the carcass (estimate = −0.031, SE = 0.028, z = −1.09, P = 0.275). However, females spent significantly more time maintaining the carcass than males (mean $\pm$ SE time spent on carcass maintenance out of 30 min: male = 1.4 min $\pm$ 0.23 min, female = 5.6 min $\pm$ 0.38 min; estimate (male versus female) = −5.34, SE = 0.764, z = −6.98, P <0.001).

To disentangle the causal effects of carcass mass and the number of offspring in the brood, which is positively correlated with carcass mass (r = 0.20, t = 3.0365, df = 204, P = 0.002), on parental behaviour, I compared models where I excluded and included clutch size (or brood size at hatching in models on the amount of care) at the time of observation as fixed effects. Excluding or including clutch size or brood size at the time of observation did not change the effect of carcass mass (Table 5.1), suggesting that the effects of carcass mass on the parents' behaviour were independent of any potential effects due to the number of offspring in the brood.

5.3.4   Sex differences in carrion consumption and weight change

There were no significant effects of the interaction between the sex of the focal parent and carcass mass and no significant main effects of carcass mass on the amount of time spent consuming carrion by the female or male parent measured on the day after hatching (Table 5.1). However, females spent significantly more time consuming carrion than males (mean $\pm$ SE time spent consuming out of 30 min: male = 0.87 min $\pm$ 0.21 min, female = 3.6 min $\pm$ 0.33 min; estimate (male versus female) = -3.69, SE = 0.825, z = -4.47, P <0.001).

There was a significant effect of the interaction between the sex of the focal parent and carcass mass on weight change over the breeding attempt ($F_{1,368}$ = 0.046, P = 0.027), reflecting that carcass mass had a stronger positive effect on female weight change than on male weight change (Figure 5.3a; mean $\pm$ SE weight change: male = 0.027g $\pm$ 0.006g, female = 0.068g $\pm$ 0.007g; sex $\times$ carcass mass: estimate = -0.004, SE = 0.002, t = -2.36, P = 0.019). Parents gained more mass as carcass mass increased (estimate = 0.005, SE = 0.001, t = 4.52, P <0.001). There was no significant difference between male and female parents in the average weight change ($F_{1,368}$ = 0.0009, P = 0.754). Excluding or including clutch size at the time of observation did not change the effect of carcass mass, suggesting that any effect of carcass mass on the parents' weight gain was independent of any potential effects due to the number of offspring in the brood.

5.3.5   Offspring behaviour and performance

There was no significant effect of carcass mass on the average time spent begging by individual larvae (Table 5.2). However, brood size at dispersal increased by approximately 2 larvae for each additional 10g of carcass (Table 5.2, Figure 5.3b; estimate = 0.016, SE = 0.006, z = 2.51, P = 0.012) and, when carcass mass is close to zero, mean larval mass at dispersal increased by approximately 0.026g
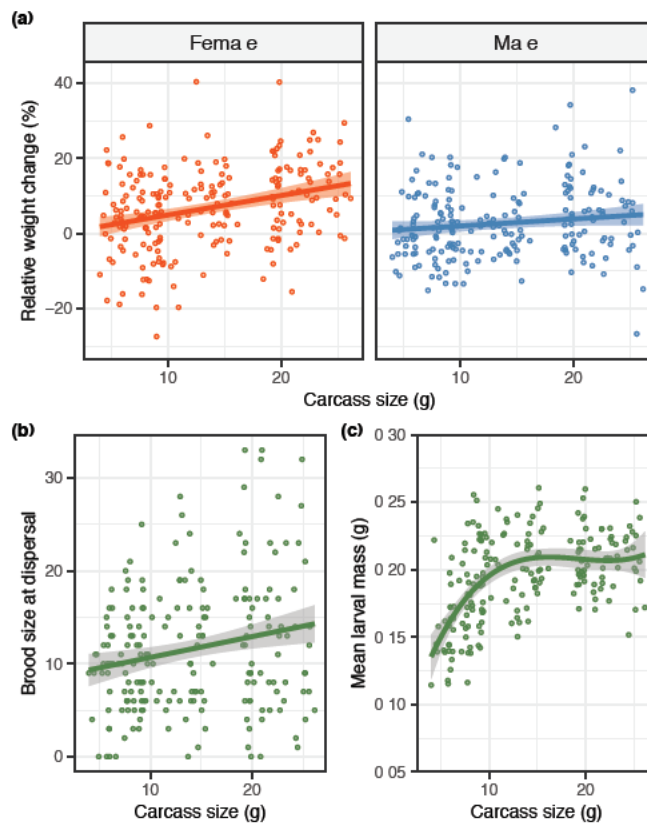
Figure 5.3: Effects of carcass size on the weight change of each parent (a), brood size at dispersal (b), and mean larval mass at dispersal (c). Filled circles represent individual data, lines represent linear regression lines in (a) and (b), a polynomial regression line in (c), and shaded ribbons the 95% confidence intervals.

for each additional 10g of carcass (Table 5.2, Figure 5.3c; estimate = 0.025, SE = 0.006, t = 4.00, P <0.001). There were significant effects of both the quadratic (χ^2 = 8.89, df = 1, P = 0.0028) and the cubic (χ^2 = 5.52, df = 1, P = 0.018) terms of carcass mass on mean larval mass at dispersal. Thus, mean larval mass increased with carcass mass when carcasses were relatively small and plateaued as carcass mass approached the upper end of the range of carcasses used in our experiment (Figure 5.3c). In addition, the duration of biparental care had a positive effect on brood size at dispersal (χ^2 = 5.91, df = 1, P = 0.015), increasing by approximately 0.8 larvae for each additional day of biparental care. The duration of biparental care had no effect on mean larval mass at dispersal (χ^2 = 0.324, df = 1, P = 0.568). Including clutch size in the model of brood size at dispersal removed the significant effect of carcass mass (Table 2), suggesting that the effect of carcass mass on brood size at dispersal was driven by differences in the number of eggs laid on carcasses of different masses. Including or excluding clutch size in the model on mean larval mass did not change the effect of carcass mass (Table 5.2), suggesting that the effects of carcass mass on mean larval mass was independent of any potential effects due to the number of offspring in the brood.

Table 5.2: Effects of carcass size (and clutch size) on larval begging, brood size at dispersal and mean larval mass at dispersal when clutch size is excluded (a) and included (b) in the model. Values are obtained from GLMs.

		Carcass size			Clutch size		
		χ^2	df	P	χ^2	df	P
Begging							
	(a)	0.082	1	0.774			
	(b)	0.187	1	0.665	0.666	1	0.414
Brood size at dispersal							
	(a)	6.08	1	**0.014**			
	(b)	3.42	1	0.064	5.07	1	**0.024**
Mean larval mass							
	(a)	16.0	1	<**0.001**			
	(b)	14.65	1	<**0.001**	16.4	1	<**0.001**

5.4 Discussion

Here I show that variation in food availability shifted the balance between cooperation and conflict, and altered the magnitude of sex differences in the duration of care, the amount of time spent providing care on the day after hatching, and parental weight change during breeding. I found that an increase in carcass mass was associated with an increase in the duration of biparental care, indicating a shift towards more cooperation between male and female parents. Meanwhile, a decrease in carcass mass was associated with more pronounced sex differences in both the duration of care and the time spent providing care, reflecting that males deserted the brood earlier and spent less time maintaining the carcass as carcass mass decreased. In contrast, females nearly always provided care until the larvae dispersed and spent a similar amount of time maintaining the carcass regardless of carcass mass. Furthermore, an increase in carcass mass was associated with a greater increase in weight gain by females than by males. Below I discuss the wider implications of my results for our understanding of how environmental conditions may drive the origin and maintenance of biparental care.

My first main result was that the duration of biparental care increased with carcass mass, supporting my prediction that there was a shift towards more cooperation when parents bred on larger carcasses. The rationale for this prediction was that the benefits of biparental cooperation would be greater on larger carcasses given that such carcasses are more valuable as a breeding resource to conspecific intruders, which may attempt to take over the carcass from the resident parents (Trumbo 1991). If successful, such intruders would eliminate the original brood and use what is left of the carcass as a resource to rear their own brood. Furthermore, a study on the closely related *N. orbicollis* found that two parents are better able to protect the brood against conspecific intruders than single parents (Trumbo 1991). Given that larger carcasses are subject to more intense competition than small ones (Wilson and Fudge 1984, Robertson 1993), it seems likely that the benefits to the male from assisting the female (and to the female from accepting assistance from the male) in terms of to offspring survival would be

greater as carcass mass increases. My results contrast with a comparative study on weaverbirds, which found that biparental cooperation was less common in species that breed in environments where there is greater availability of resources (Crook 1963). In altricial birds, greater availability of food may reduce the benefits of biparental cooperation given that the female is more likely to be able to provision sufficient food for the brood on her own when food is plentiful as compared to when it is scarce. Biparental cooperation over food provisioning may be particularly important in altricial birds because parents must provide a constant supply of food from the surrounding environment. Thus, in altricial birds, the benefits of the male assisting the female may be greater when food is scarce. In contrast, biparental cooperation over food provisioning may be less important in burying beetles of the genus *Nicrophorus*. The reason for this is that these beetles breed on a fixed resource (i.e., a vertebrate carcass), which means that the supply of food will be limited by the size of the carcass rather than by the number of caring parents.

My second main result was that there was a significant effect of the interaction between the sex of the focal parent and carcass mass on the duration of care and the amount of time spent maintaining the carcass on the day after hatching. These interaction effects reflected that males provided care for longer and spent more time maintaining the carcass as carcass mass increased, while carcass mass had no effect on the duration of care or time spent maintaining the carcass by females. These results chime with the findings on a related species of burying beetle (Kishida and Suzuki 2010) and support my prediction that sex differences in parental care would be more pronounced as carcass mass decreased, reflecting that males often adjust the amount of care they provide in response to variation in environmental conditions, whilst females tend to provide a similar amount of care regardless of such variation (Royle et al. 2014, Smiseth et al. 2015, Walling et al. 2008). In *N. vespilloides*, females spend more time provisioning food to the brood (e.g. Smiseth et al. 2005, Georgiou-Shippi et al. 2018) and care for longer than males (Bartlett 1988, Ford and Smiseth 2016). These sex differences

in parental care are thought to reflect that males can gain some reproductive success by mating away from a carcass whilst female require access to a carcass in order to reproduce (Müller et al. 2007). This is because males can simply mate with females away from a carcass. In contrast, females necessarily require the presence of a carcass in order to lay eggs. Thus, variation in the availability of resources may have a greater impact on the duration of male care because it increases their benefits of providing care relative to their benefits of deserting to mate with females away from a carcass (Ward et al. 2009).

I found that carcass mass had a greater positive effect on female weight gain than on male weight gain. This finding contrasts with my prediction that carcass mass would have a stronger impact on male weight change. This prediction was based on the assumption that, if males provided care for longer on larger carcasses, this would give them more opportunities to consume from the carcass. Thus, these results contradict my initial assumption that sex differences in weight change would be linked to sex differences in parental care. This assumption is also contradicted by the finding that females gained more weight as carcass mass increased, even though females nearly always provided care until the larvae dispersed. Females gaining more weight as carcass mass increased suggests that females balance the personal benefits of consuming food from the carcass in terms of enhancing their own condition at the end of breeding against the costs of consuming food to the larvae (Gray et al. 2018, Keppner et al. 2020). In this species, both the parents and the larvae feed from the carcass, and any increase in food consumption by a parent would therefore reduce the amount of food available to the other parent and the brood. In addition, food-deprived females consume more food resources during breeding, which seems is can have a negative impact on the brood (Keppner et al. 2018, but see Gray et al. 2018). Thus, females might restrict their own food consumption when breeding on smaller carcasses to avoid inflicting a cost to the larvae. On larger carcasses, where food is more plentiful, females may consume more food and put on more weight without inflicting such a cost to the larvae. However, it is unclear why this argument would only apply to female

weight change. One potential explanation for why males seem to gain a similar amount of weight regardless of carcass mass is that males have a lower optimal body weight compared to females. Females may have a higher optimal body weight than males given that females must secure a carcass to reproduce, which means that they must fly in search of a carcass and compete with rival females. Gaining more weight might be beneficial given that flight is energetically costly and that heavier females tend to win more fights than lighter ones (Richardson et al. 2020). In contrast, males can mate females at a carcass or attract and mate with females away from a carcass by emitting pheromones (Pukowski 1933). Thus, males might benefit less from putting on more weight than females. I encourage future research to investigate potential sex differences in optimal body mass and the potential reasons for such sex differences.

My final results were that parents produced larvae with a greater mean mass when breeding on larger carcasses, whilst carcass mass had no effect on larval begging or brood size when controlling for clutch size. This is consistent with previous findings reporting positive effects of carcass size on offspring growth and mass at dispersal (e.g. Xu and Suzuki 2001, Andrews et al. 2017, Gray et al. 2018) but no effect on larval begging (Smiseth and Moore 2002, Sieber et al. 2017). Such positive effects on offspring performance are likely to reflect that larvae simply have access to more food when self-feeding from the carcass, rather than an increase in the amount of care provided by the male. This is because the carcass represents the sole source of food for the larvae, and larvae may run out of food earlier on a smaller carcass than on a larger one. Moreover, prior work suggests that male care has no detectable effects on offspring growth and survival under laboratory conditions (Smiseth et al. 2005, Ratz et al. 2018), and may even have detrimental effects on females (Boncoraglio and Kilner 2012). We note that, however, male's presence in the wild is likely to be critical to deter potential conspecific intruders or predators that represent a risk to the larvae (Trumbo 1991).

In summary, I found that greater food availability shifted the balance towards more cooperation between parents and reduced sex differences in parental care. Overall, these findings stress the importance that environmental conditions, such as resource availability, play in determining the balance between cooperation and conflict over care, as well as determining the magnitude of any sex differences in parental behaviour. This is perhaps not surprising given that resource availability has long been recognised as a crucial environmental condition driving the emergence and maintenance of parental care in general (Tallamy and Wood 1986, Klug et al. 2012). However, less consideration has been given to the role that resource availability plays as an environmental driver of the evolution of biparental care. I highlight that resource availability may have contrasting effects on the balance between cooperation and conflict depending on whether it reduces or increases the benefits of biparental cooperation.

6   Offspring response to parental body size

This chapter has been published as:

Abstract

Offspring begging behaviours have evolved in many birds and mammals, as well as in some amphibians and insects, as a potential behavioural mechanism for resolving parent-offspring conflict by enabling offspring to communicate their needs and influence parental care. When begging is costly, offspring will be under selection to reduce such costs and maximise their returns on begging. For example, in species where multiple parents provide care (e.g., species with communal breeding or biparental care), offspring should beg towards the parent that provisions more food than the others. Here, I investigated whether larvae spend more time begging towards larger females in the burying beetle *Nicrophorus vespilloides*. Prior work on this species shows that larger females provision more food than smaller ones, suggesting that larvae would benefit by preferentially begging towards larger parents. To test for such a preference, I provided experimental broods of 10 larvae with a simultaneous choice between a smaller and a larger dead female parent. Larvae spent more time begging towards larger females. I next examined the behavioural mechanism for why larvae begged towards larger females. Larvae spent more time associating with larger females over smaller ones, whilst there no was no evidence that larvae begged more when associating with larger females. Thus, larvae begged more towards the larger female simply as a consequence of associating more with such females. My findings have important implications for our understanding of parent-offspring communication by showing that offspring can choose between parents based on parental attributes, such as body size, that

may reflect how much food parents are likely to provision.

6.1 Introduction

Offspring beg for food from their parents across many animal taxa, including birds (e.g. Budden and Wright 2001), mammals (e.g. Smiseth and Lorentsen 2001), amphibians (e.g. Yoshioka et al. 2016) and insects (e.g. Rauter and Moore 1999). Theoretical models propose that costly offspring begging behaviours evolved as a behavioural mechanism for resolving parent-offspring conflict over parental care (Godfray 1991, Parker et al. 2002). The reason for this is that costs of begging ensure that parents benefit by adjusting their food provisioning in response to begging because parents obtain honest information on the nutritional need of their offspring. There is empirical evidence that begging often incurs costs to offspring in terms of reduced growth (e.g. Kilner 2001, Takata et al. 2019), increased risk of predation (e.g., Haskell 1994, Redondo and Castro 1992), or increased mortality due to filial infanticide (e.g., Andrews and Smiseth 2013). When begging is costly, offspring should be under selection to reduce such costs to maximise their returns on begging (Bell 2008, Madden et al. 2009). For instance, in species where both parents provision food for the offspring, and where parents of one sex provision more food than the other, offspring may maximise their returns on begging by begging more towards parents of the sex that provisions the most food, as reported in studies on birds (e.g. Kölliker et al. 1998, Roulin and Bersier 2007, Dickens et al. 2008) and insects (Suzuki 2015, Paquet et al. 2018).

Although there is good evidence that offspring beg more towards parents of the sex that provisions the most food, it is currently unclear whether begging offspring also respond to other attributes of their parents that might reflect how much food parents are likely to provision, such as the body size, nutritional state, or age of parents (Paquet et al. 2018). For example, offspring may beg more towards larger parents if larger parents provision more food than smaller ones. Larger parents have been found to produce more milk than smaller parents in

mammals (Landete-Castillejos et al. 2003), provision food more often to the nest in insects (Bosch and Vicens 2006), and provision larger loads of food in birds (Tveera et al. 1998). Thus, in species where more than one parent provides care, such as in species with biparental care or where females breed communally, offspring might maximise their returns on begging by begging more towards larger parents. To my knowledge, no prior studies have investigated this issue and it remains unknown whether offspring beg more towards larger parents.

Here, I conducted an experiment on the burying beetle *Nicrophorus vespilloides* in which I tested whether larvae begged more towards larger parents than towards smaller ones. Burying beetles are an ideal study system to explore this issue because multiple parents provision their larvae with pre-digested regurgitated food in the contexts of communal breeding (Trumbo 1992, Eggert and Müller 1992) and biparental care (Smiseth and Moore 2002, Smiseth et al. 2003). Females can tolerate the presence of other females and breed communally on large carcasses (Eggert and Müller 1992, Komdeur et al. 2013, Richardson and Smiseth 2020). Females breeding communally provide care indiscriminately to a joint brood as they cannot recognise their own larvae. This is because females have a temporal kin discrimination mechanism and would accept any larvae that hatch at the expected time of hatching of their own larvae (MuÌller and Eggert 1990, Oldekop et al. 2007). In contrast to most birds, larvae show partial begging, whereby they obtain some food by begging from the parents and some by self-feeding directly from the carcass used for breeding (Smiseth et al. 2003). There is evidence that begging incurs costs to larvae in terms of increased mortality and reduced growth (Andrews and Smiseth 2013, Takata et al. 2019). Female parents provision more food than males (Eggert et al. 1998, Smiseth and Moore 2004a), and larvae spend more time begging towards females (Paquet et al. 2018). Prior work shows that larger females spend more time provisioning food to the brood than smaller females (Steiger 2013), and larger females may also process food more efficiently than smaller ones (Pilakouta et al. 2015b). Thus, larvae might maximise their returns on begging by begging more towards larger parents. Fi-

nally, prior work shows that larvae beg towards dead parents, thereby allowing for experimental designs that exclude any potential effects of parental behaviour on larval behaviour (Smiseth and Parker 2008, Mäenpää et al. 2015, Paquet et al. 2018).

The first aim of this study was to test whether larvae in *N. vespilloides* spend more time begging towards larger females than towards smaller ones. Given that larvae behave differently towards female and male parents, I focused on begging towards females only to ensure that my design specifically focused on how begging larvae respond to the body size of parents. I used a simultaneous choice design similar to that used in prior studied where larvae could choose between one larger and one smaller female (Paquet et al. 2018). I used simultaneous choice designs because such designs are better suited for detecting preferences than sequential choice designs (Dougherty and Shuker 2014). My second main aim was to test between two potential behavioural mechanisms for why larvae might beg towards the larger female: (1) larvae may spend more time associating with larger females and beg more towards larger females simply as a consequence of associating more with them, and (2) larvae may spend more time begging when they associate with larger females. Given that these two mechanisms are not mutually exclusive, larvae might beg more towards larger females by both associating more with them and begging more when associating with them.

6.2   Methods

The beetles used in the experiments descended from individuals collected in the wild in the Hermitage of Braid and Blackford Hill Local Nature Reserves, Edinburgh, UK. The beetles had been kept under laboratory conditions for at least three generations. Beetles were housed in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil, and kept at (20°C) and under a 16:8h light:dark photoperiod. Non-breeding adults were fed small pieces of organic beef twice a week.

6.2.1 Experimental design

At the start of the experiment, I generated the smaller and larger females that I later used as stimuli when investigating larval begging towards larger and smaller females. I generated these females following established protocols (Steiger 2013, Pilakouta et al. 2015b). To this end, I paired up 34 pairs of unrelated males and females from the stock population by provided them with a mouse carcass to initiate breeding. From each of these 34 broods, I removed some larvae from the carcass when they had reached a mass of 100–150 mg (mean mass $\pm$ SE measured at dispersal: 0.130 g $\pm$ 0.004), and some when they had reached a mass of 200–250 mg (mean $\pm$ SE mass measured at dispersal: 0.222 g $\pm$ 0.003). The former larvae were destined to become smaller females, whilst the latter were destined to become larger females. These treatments are effective in generating different-sized females in the study species because larvae obtain all the resources they put into growth from the carcass on which they are reared such that larval mass at the time of leaving the carcass determines adult body size (Lock et al. 2004).

Once removed from the carcass, larvae destined to become smaller or larger females were placed in individual containers (12 cm $\times$ 8 cm $\times$ 2 cm) filled with moist soil until eclosion. After eclosion, I sexed all individuals, keeping females only for use in my experiment. I kept all smaller and larger females in their individual containers for a minimum of 10 days after eclosion to allow them sufficient time to undergo sexual maturation. During this period, I fed all females small pieces of organic beef twice a week until they were used in my experiment.

Once the females reached sexual maturity, I selected 32 larger and 32 smaller females for use in my experiments. These females were used to produce the experimental broods of larvae that I later used to test whether larvae begged more towards larger or smaller females. To initiate breeding, I paired each female with

an unrelated male from the stock population, placed each pair in a larger container (17 cm × 12 cm × 6 cm) with 2 cm of moist soil, and provided them with a previously frozen mouse carcass of a standardized size (20.01–23.64 g) (Livefoods Direct, Sheffield). I checked the containers daily for the presence of eggs, defining the day on which the first eggs were laid as the onset of egg-laying. Two days after the outset of egg-laying (corresponding to the day preceding the expected time of hatching; Smiseth et al. 2006), I separated each female from her eggs by transferring females and their carcasses into fresh containers lined with moist soil. I did this to ensure that no larvae were present when I later allocated females with an experimental donor brood (see below). At this time, I also removed the male to exclude any potential effects of the presence of males on the preferences of begging larvae. There is no evidence that the removal of the male has any detrimental effects on offspring growth or survival under laboratory conditions (Smiseth et al. 2005).

I generated experimental donor broods by pooling newly hatched larvae from across multiple broods. All such broods were comprised of 10 same-aged larvae of mixed maternity. Once assembled, experimental donor broods were allocated at random to a smaller or a larger female foster parent. Given that experimental donor broods were composed of larvae of mixed maternity, most larvae would be genetically unrelated to their foster female. I used experimental broods in my experiment to exclude any potential confounding effects due to variation in brood size (Ratz and Smiseth 2018), larval age (Smiseth et al. 2007a,b), or age composition within the brood (Smiseth and Moore 2007) on larval behaviour.

6.2.2   Larval behaviour

To test whether larvae begged more towards larger females, I conducted behavioural observations in which larvae were given a simultaneous choice between one larger and one smaller female. I used a simultaneous choice design to test whether larvae showed a preference between two different-sized females (Paquet

et al. 2018). This design is biologically realistic given that females will breed communally on large carcasses (Eggert and Müller 1992, Komdeur et al. 2013, Richardson and Smiseth 2020). Females have a temporal kin discrimination mechanism, selectively culling any larvae that hatch earlier than the expected time of hatching of their own larvae. However, females cannot recognise their own larvae after hatching (Müller and Eggert 1990, Oldekop et al. 2007), and cobreeding females provide care indiscriminately to any larva in a joint brood. Cobreeding females may differ in size when breeding on a larger carcass (Eggert and Müller 2000), in which case larvae would be in a position to choose between different-sized females. To beg, larvae move near a parent, raise their body towards the parent and wave their legs at the parent's mouthparts (Rauter and Moore 1999, Smiseth and Moore 2002).

I conducted the behavioural observations 24 h (± 15 min) after I had allocated an experimental donor brood to a foster female. Approximately 1 h before the start of each behavioural observation session, I killed the larger and smaller females used as stimuli to trigger larval begging by freezing them at −20°C for 30 min. I then thawed the females at ambient temperature for a minimum of 10 min to ensure that I could position their body and legs (see below). To ensure that there was no difference between broods in whether larvae had prior experience with a female of a particular size, I ensured that all experimental broods used in the trials comprised of 5 larvae derived from a brood that had been reared by a larger female and 5 larvae derived from a brood that had been reared by a smaller female. I placed the 10 larvae in a small container (11 cm × 11 cm × 3 cm) lined with moist paper towel 30 min prior observation to ensure that the larvae were not fully satiated and therefore motivated to beg at the start of the observation. Just before the start of the observation sessions, I pinned the larger and smaller females in the centre of the box (Figure 6.1a), and positioned them such that they mimicked a parent provisioning food (Mäenpää et al. 2015, Paquet et al. 2018; Figure 6.1b). I then placed all larvae in front of the two females such that they were equidistant from them (Figure 6.1a). Larvae can presumably detect an
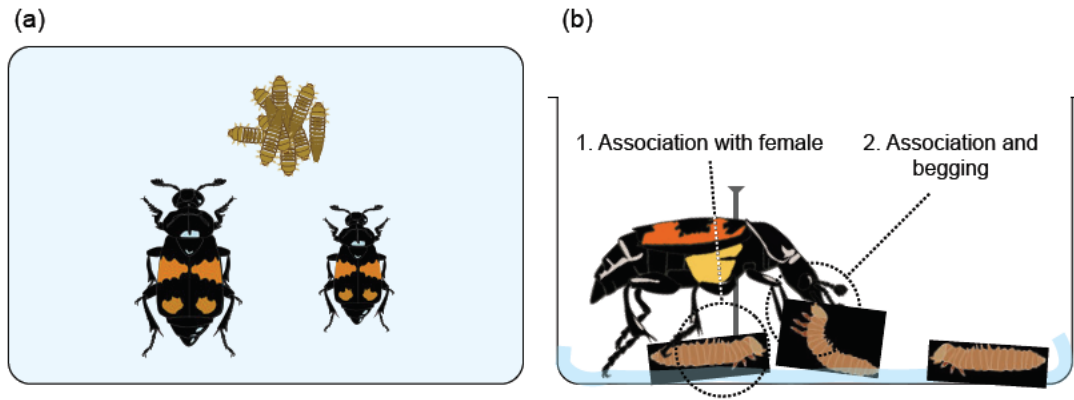
Figure 6.1: Experimental design used to test whether larvae spend more time begging towards a larger female over a smaller female. At the beginning of the behavioural observation, I placed the brood of 10 larvae equidistant from the two females (a). I then recorded the number of larvae that begged towards each female and that associated with each female (i.e. at a distance less than or equal to the female's pronotum length) (b).

adult at a distance using olfactory cues (Takata et al. 2019). I allowed the larvae to settle for 5 min before I started the observation session.

I observed larval behaviour using instantaneous recording, noting the number of larvae that were begging towards each female and the number of larvae that were associating with each female every 60 s for 15 min. I defined larval begging as larvae touching any body part of a female with their legs (Smiseth et al. 2003; Figure 6.1b). Based on these data, I calculated the proportion of time spent begging per larva in the brood towards each female (B) as the number of begging events towards each female across the 15 scans (Σb) divided by the number of sampling scans (15) and the number of larvae in the brood (10), as $B = (\Sigma b/15)/10$. This score represents the average proportion of time spent begging by an individual larva and gives a dimensionless index with no units of measurement (Martin and Bateson 2007). I next investigated two potential behavioural mechanisms for why larvae might beg more towards larger females. First, larvae might beg more towards larger females simply by associating more with them. I defined larval association with each female as the number of larvae that were within reaching distance from the female (i.e., distance approximately equals to or less than an

adult pronotom length; Figure 6.1b). Given that the proportion of larvae that were close to the larger female is inversely related to the proportion of larvae that were close to the smaller one, I focused on larvae associating with the larger female only. I calculated the proportion of larvae associating with the larger female (A) as the number of larvae near the larger female across the 15 scans (Σa) divided by the total number of larvae that associated with either female (n), as $A = \Sigma a / n$. Second, larvae might beg more towards larger females by begging more when associating with them. I therefore calculated the proportion of time spent begging per larva when larvae were associating with each female. I calculated this metric (B') as the number of begging events towards each female across the 15 scans (Σb) divided by the total number of larvae associating with the female in question (n'), as $B = \Sigma b / n'$.

6.2.3 Data analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team 2019) loaded with the packages car (Fox et al. 2016), $MASS$ (Ripley et al. 2017), and $lme4$ (Bates et al. 2014). I first investigated whether larvae begged more towards the larger female. To do so, I tested whether there was a difference in proportion of time spent begging per larva between larger and smaller females. I used generalized linear mixed models with a Poisson distribution, including the brood ID and female ID as random effects to account for the fact that larvae from a single brood could beg towards both females at the same time. I then tested whether larvae spent more time associating with the larger female. To this end, I used a Wilcoxon signed-rank test comparing the observed proportion of larvae associating with the larger female against the null expectation of 0.5 as expected if larvae associated equally with both females (Crawley 2005). Finally, I tested whether larvae begged more when associating with the larger female. I used generalized linear mixed models with a binomial distribution and including the brood ID and female ID as random effects for the same reasons as detailed above.

I excluded 10 broods from my analyses because at least one of the two females was not observed at the carcass at the time females were removed from their original container. I did this because such females might have deserted their brood and because prior work shows that larvae behave differently towards breeding and non-breeding females (Smiseth et al. 2010). The final sample size in my study was 22 broods.

6.3 Results

Larvae begged around three times more towards the larger female as towards the smaller one ($\chi^2 = 12.7$, df = 1, P < 0.001; Figure 6.2a), confirming that begging larvae discriminate between larger and smaller females. I next tested between two potential behavioural mechanisms underpinning this preference; that is, whether larvae associated more with the larger female or whether larvae begging more when associating with the larger female. I found that a larger proportion of larvae associated with the larger female than expected due to chance (Wilcoxon signed-rank test: V = 180, P = 0.04; Figure 6.2b), whilst there was no evidence that larvae begged more when associating with the larger female ($\chi^2 = 0.01$, df = 1, P = 0.942; Figure 6.2c). Thus, my results show that larvae begged more towards the larger female because they associated more with this female.

6.4 Discussion

Here I show that larvae in the burying beetle *Nicrophorus vespilloides* begged more towards the larger female when given a choice between a larger and a smaller female. This finding provides support for the prediction that larvae beg more towards the parent that is likely to provision more food, thereby allowing larvae to maximise their returns on begging. I also show that larvae begged more towards the larger female by associating more with the larger female, whilst there was no
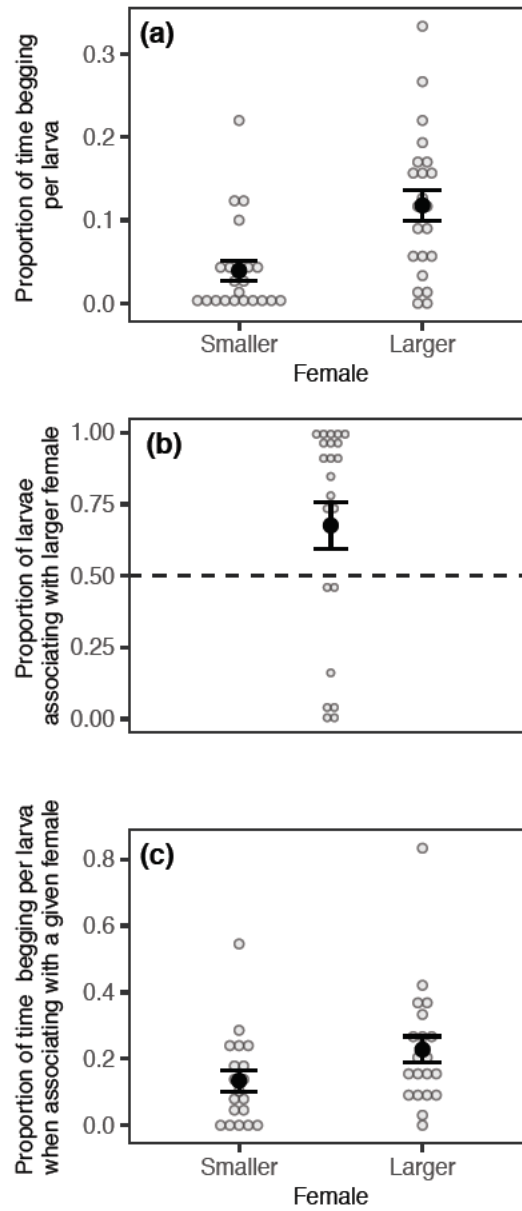
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Figure 6.2: Proportion of time spent begging per larva towards the larger and smaller female (a), proportion of larvae associating with the larger female (b), and proportion of time spent begging when associating with the larger female and smaller female (c). The dash line in (b) represents the null expectation when larvae associated as much with the larger female as with the smaller one. Black dots and error bars represent mean ± SE.

evidence that larvae begged more when associating with the larger female. This finding provides insights into the behavioural mechanisms of larval preferences for begging towards larger females. My results have important implications for our understanding of offspring begging behaviour by showing that offspring adjust their begging behaviour not just in response to their own nutritional needs, as shown in prior work (Kilner and Johnstone 1997), but also in response to attributes of their parents, in this case parental body size, that reflect the amount of food that parents are likely to provision to offspring. Below, I first discuss the wider implications of my results to our understanding of the evolution of offspring begging behaviour.

My main finding was that larvae spent more time begging towards the larger female when given a simultaneous choice between a larger and a smaller female. I predicted that begging larvae should have such a preference given that begging incurs costs to larvae (Andrews and Smiseth 2013, Takata et al. 2019) and that larger females provision more food than smaller females (Steiger 2013). My results are consistent with prior work showing that offspring beg more towards parents of the sex that provisions the most food in species with biparental care (Kölliker et al. 1998, Roulin and Bersier 2007, Dickens et al. 2008, Suzuki 2015, Paquet et al. 2018). My study adds to this work by showing that offspring have preferences based on other attributes of their parents, in this case body size, that also reflect the amount of food that parents are likely to provision to offspring. I note that I used a simultaneous choice design because such designs are more likely to detect preferences than sequential choice designs (Dougherty and Shuker 2014). Prior work on my study species have reported a preference for begging towards female parents only when using a simultaneous choice design (Paquet et al. 2018), and no such preferences were found when using a sequential choice design (Mäenpää et al. 2015). In light of this, I urge caution in extrapolating the evidence for larval preferences for larger parents to contexts where larvae cannot compare two different-sized parents, such as when larvae are cared for by a single parent.

My study provides valuable insights into the behavioural mechanisms for why larvae beg more towards the larger female. I found that larvae associated more with the larger female, whilst there was no evidence that larvae begged more when associating with the larger female. Thus, larvae begged more towards the larger female simply by associating more with this female. As argued above, larvae may associate more with the larger female because larger females provision more food in this species (Steiger 2013). However, there are alternative explanations for this finding. For example, larvae might associate more with the larger female if more larvae can fit under the body of larger females. This explanation seems unlikely given that the brood sizes used in my experiment were small enough for all larvae to fit under the smaller female. Alternatively, larvae might associate more with the larger female if larger females are better at protecting larvae from potential threats. I cannot rule out this explanation. For example, in this study species, conspecific intruders pose a threat to the larvae as they may commit infanticide in order to attempt to takeover the carcass (Trumbo 2007, Trumbo and Valetta 2007, Georgiou Shippi et al. 2018). Prior work shows that larger females are stronger competitors than smaller females (Otronen 1988, Trumbo 2007). Thus, a potential alternative explanation for my results is that larvae preferentially associate with the larger female for protection, and that larvae spend more time begging towards larger females as a consequence of this preference.

My finding that larvae begged more towards larger females, and associated more with them, implies that larvae somehow assessed the body size of the two females. As my study species normally breeds underground in complete darkness (Scott 1998), it is unlikely that larvae did so based on visual cues. Instead, larvae might assess differences in parent's body size based on behavioural, acoustic, tactile or vibrational cues that might reflect the body size of a parent. Although I cannot rule out that such cues play a role when larvae interact with live parents, it seems unlikely that they could explain my results given that I used dead females as stimuli to trigger larval begging. Potentially, the preference to associate more

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with larger parents could simply reflect that larger females carried a stronger scent of carcass due to their larger surface area. However, it seems unlikely that larvae would use such a cue to discriminate between females, given that larvae would not be able to identify the females from the carcass based on such scents. Instead, I suggest that larvae may assess the body size of parents based on chemical cues that reflect the body size of a parent. A recent study on the closely related *Nicrophorus quadripunctatus* shows that begging larvae respond to a provisioning pheromone (2-phenoxyethanol) produced by caring parents (Takata et al. 2019). This pheromone provides larvae with a reliable signal that the parent is about to provision food to the brood, thereby reducing the cost of larval begging and enhancing the efficiency of parental food provisioning (Takata et al. 2019). However, it does not seem plausible that this pheromone can explain my results given the dead females used in my experiment could not produce this pheromone. Alternatively, larvae might assess the body size of parents based on more persistent chemicals, such as cuticular hydrocarbons and methyl geranate (Steiger et al. 2007, Smiseth et al. 2010, Steiger et al. 2011, Engel et al. 2016). Thus, there is now a need for studies that compare cuticular chemical profiles of different-sized parents.

My study adds to our understanding of begging behaviour by showing that begging offspring respond to cues from their parents that are likely to provide information on the expected returns on begging. Prior work on my study species and birds shows that offspring adjust their behaviour in response to changes in their own state, such as their hunger state (Kilner and Johnstone 1997, Smiseth and Moore 2004a, 2007), their long-term need (Price et al. 1996) or their inbreeding status (Mattey et al. 2018). My results show that offspring are sensitive not just to their own needs but also to attributes of their parents. Furthermore, my results shed new light on findings from prior work showing that larger parents provision more food to their offspring in insects (e.g. Bosch and Vicens 2006), birds (e.g. Tveera et al. 1998), and mammals (Landete-Castillejos et al. 2003). These findings are thought to reflect that larger parents are better able to forage, carry and

deliver food resources to the offspring than smaller ones (Creighton et al. 2009). My finding that offspring begged more towards larger parents suggests that a positive correlation between a parent's body size and parental food provisioning may be reinforced by parental responses to the offspring's begging behaviour (Kilner and Johnstone 1997). Traditionally, parent-offspring communication is described as a process where begging offspring signal their needs, thereby playing the role as senders, and parents respond to the offspring's signals, thereby playing the role as receivers. My results suggest that parent-offspring communication is more complex as begging offspring also act as receivers by responding to cues from their parents. Thus, it seems more appropriate to describe parent-offspring communication as a two-way process where both parents and offspring act as senders and receivers, adjusting their behaviour based on signals or cues produced by each other.

To conclude, this study adds to our understanding of offspring behaviour by showing that offspring adjust their begging behaviour both to their own state (e.g. hunger, long-term conditions) and to cues from their parents that provide offspring with information about their likely returns on begging. My study demonstrates that offspring show a preference for larger females, reflecting that larger females provision more food than smaller ones. There is now a need for future work investigating whether begging offspring also respond to other parental attributes that might indicate the offspring's likely returns on begging, such as the age, nutritional state, inbreeding state, or infection status of the parent.

7 Effects of inbreeding on parent and offspring plasticity

This chapter has been published as:

Abstract

Inbreeding depression is defined as a fitness decline in progeny resulting from mating between related individuals, the severity of which may vary across environmental conditions. Such inbreeding-by-environment interactions might reflect that inbred individuals have a lower capacity for adjusting their phenotype to match different environmental conditions better, as shown in prior studies on developmental plasticity. Behavioural plasticity is more flexible than developmental plasticity because it is reversible and relatively quick, but little is known about its sensitivity to inbreeding. Here I investigate effects of inbreeding on behavioural plasticity in the context of parent-offspring interactions in the burying beetle *Nicrophorus vespilloides*. Larvae increase begging with the level of hunger, and parents increase their level of care when brood sizes increase. Here I find that inbreeding increased behavioural plasticity in larvae: inbred larvae reduced their time spent associating with a parent in response to the length of food-deprivation more than outbred larvae. However, inbreeding had no effect on the behavioural plasticity of offspring begging or any parental behaviour. Overall, my results show that inbreeding can *increase* behavioural plasticity. I suggest that inbreeding-by-environment interactions might arise when inbreeding is associated with too little or too much plasticity in response to changing environmental conditions. In this case, the plastic response would no longer be adaptive and the extent to which inbred individuals suffer from reduced fitness would depend on the environment.

7.1 Introduction

Inbreeding, or mating between related individuals, is a key issue in ecology and evolution because of its impact on the persistence of populations and their ability to evolve in response to changing environments (Keller and Waller 2002, Charlesworth 2003). Inbreeding is often associated with a decline in fitness of any resulting progeny, a phenomenon known as inbreeding depression (Davenport 1908, East 1908). Inbreeding depression is caused by greater homozygosity associated with inbreeding, which reduces fitness by increasing the risk that rare, deleterious and recessive alleles are expressed and exposed to selection (dominance hypothesis; Davenport 1908) or by reducing any potential benefits due to heterozygote advantage (overdominance hypothesis; East 1908). The severity of inbreeding depression can vary across environments (Armbruster and Reed 2005, Cheptou and Donohue 2011, Fox and Reed 2011), and sources of environmental stress, such as intense intraspecific competition (Meagher et al. 2000, Haag et al. 2002), extreme temperatures (Bijlsma et al. 1999, Fox et al. 2011), parasitic infection (Haag et al. 2003) and nutrient deprivation (Auld and Henkel 2014, Schou et al. 2015), are known to exacerbate inbreeding depression. However, little is known about the mechanisms for these inbreeding-by-environmental stress interactions (Reed et al. 2012). Potentially, environmental stress might exacerbate inbreeding depression by increasing the intensity of selection acting against deleterious alleles (Laffafian et al. 2010) or by increasing the amount of phenotypic variation induced by stress, and thereby fitness differences, between inbred and outbred individuals (Waller et al. 2008). A plausible underlying mechanism is that inbreeding is associated with reduced phenotypic plasticity (Fowler and Whitlock 1999, Reed et al. 2003, Reed et al. 2012, Bijlsma and Loeschcke 2012). This mechanism requires that inbred individuals have a lower capacity for adjusting their phenotype to match different environmental conditions than outbred ones.

There is good empirical evidence that inbreeding alters developmental plasticity. For example, inbreeding reduces the duration of developmental growth in response to changing temperatures in *Drosophila subobscura* (e.g. Maynard Smith et al. 1955) and the development of morphological defences in response to the presence of predators in the freshwater snail *Physa acuta* (e.g. Auld and Relyea 2010). Inbreeding also reduces plasticity in life history traits, such as laying date in response to advancing spring temperatures in red-cockaded woodpeckers (Schiegg et al. 2002) and brood size in response to changes in resource availability in the burying beetle *Nicrophorus vespilloides* (Richardson et al. 2018). On the other hand, inbreeding increases plasticity in the development of wing shape in response to changing temperatures in Drosophila melanogaster (Schou et al. 2015), which is likely associated with negative effects (Frazier et al. 2008). However, little is known about the effects of inbreeding on behavioural plasticity; that is, how an individual adjusts its behaviour in response to changing environmental conditions. Unlike developmental traits, behaviours can change relatively quickly in response to variation in the social and physical environment. These changes are also reversible, allowing an individual to match its behavioural phenotype rapidly to environmental changes that occur within its lifetimes (Candolin and Wong 2012, Snell-Rood 2013, Piersma and Drent 2003). Behavioural plasticity is likely to be linked to an individual's reproductive success and survival given that many behaviours play a key role during mating (e.g., Rodríguez et al. 2013), parenting (e.g., Royle et al. 2014), foraging (e.g., Sol et al. 2002) and avoidance of predators or pathogens (e.g., Benard 2004). Understanding the interplay between behavioural plasticity and inbreeding is now an important challenge given that anthropogenic environmental change is expected to cause a reduction in population sizes, thereby increasing the risk of inbreeding, and induce changes in environmental conditions, such as resources required for breeding due to advancing spring temperatures (Schiegg et al. 2002). Thus, there is now a need for studies that investigate the effects of inbreeding on behavioural plasticity.

I investigate the effects of inbreeding on behavioural plasticity, focusing on be-

haviours expressed in social interactions between individuals. I examine these behaviours because the social environment is usually highly variable and social interactions often involve highly plastic behaviours (Foster 2013). This is because individuals often adjust their behaviour in response to characteristics of the conspecifics with which they interact, such as their behaviour, body size or state, as well as the number of individuals in the group or the population. For instance, individuals often adjust aggression to the competitive ability of competitors (Simmons 1986), mating behaviour to the availability or quality of mating partners (Kokko and Rankin 2006, Kvarnemo and Ahnesjo 1996), and parental behaviour to the presence of and/or the amount of care provided by their partner (Johnstone and Hinde 2006) or the offspring's begging behaviour (Kacelnik et al. 1995). Furthermore, there is evidence that inbreeding affects social interactions (e.g. Richardson and Smiseth 2017, Mattey et al. 2018), suggesting that inbreeding impacts how individuals respond to variation in their social environment. Inbreeding might alter behavioural plasticity in social interactions if inbred individuals invest less in costly mechanisms required for adaptive behavioural plasticity (Dingemanse and Wolf 2013, Snell-Rood 2013). These might include the necessary sensory and cognitive systems to perceive variation in the social environment, process the relevant information, and mount a plastic behavioural response (DeWitt et al. 1998, Auld et al. 2010, Coppens et al. 2010, Mathot et al. 2012). If so, I expect inbred individuals to adjust their behaviour to the social context (requiring high cognitive abilities; Humphrey, 1976) less well than outbred ones. Altogether, we might expect behaviours expressed in social interactions to be particularly sensitive to the effects of inbreeding due to the key role of behavioural plasticity in social interactions and the potential impact of inbreeding on the necessary sensory and cognitive systems of such behaviours.

In this study, I investigate whether inbreeding alters the behavioural plasticity of offspring and parental behaviours expressed in parent-offspring interactions in the burying beetle *Nicrophorus vespilloides*. I focus specifically on parent-offspring interactions because both offspring and parental behaviours are highly flexible

(Kilner and Johnstone 1997, Smiseth et al. 2008, Royle et al. 2014). Larvae beg to obtain food from their parents, and parents provision pre-digested food to larvae (Eggert et al. 1998, Smiseth et al. 2003). Larvae adjust begging behaviour to their hunger state, (which reflects the amount of food provisioned by parents in the recent past), spending more time begging when subject to food-deprivation (Smiseth and Moore 2004b, 2007). This plasticity in larval begging behaviour is likely to be adaptive given that begging is associated with both fitness benefits and fitness costs (Andrews and Smiseth 2016, Takata et al. 2019). Likewise, parents adjust their parental behaviour in response to brood size, providing more care towards larger broods (Smiseth et al. 2007a, Ratz and Smiseth 2018). This plasticity in parental behaviour is also likely to be adaptive given that parents caring for larger broods incur a fitness cost from providing more care (Ratz and Smiseth 2018). Thus, assuming that larval and parental responses are adaptive, any changes in plasticity in larval behaviour in response to food-deprivation and parental behaviour in response to brood size are likely to have detrimental fitness consequences. This change in plastic responses should remain irrespective of changes in the average behaviour due to inbreeding. For example, inbred larvae may be needier and overall beg more than outbred larvae. Such a difference between inbred and outbred larvae would be consistent regardless of their level of food deprivation. In addition, previous work shows that inbreeding affects larval begging behaviour (Mattey and Smiseth 2015, Mattey et al. 2018), and offspring inbreeding affects the amount of care provided by outbred parents (Mattey et al. 2013, Mattey et al. 2018, Ratz et al. 2018). Thus, inbreeding alters trait values of behaviours involved in parent-offspring interactions.

My aim was to test for effects of inbreeding on behavioural plasticity by focusing on the interactions between inbreeding status and larval and parental behaviours across two environmental gradients. In the first experiment, I manipulated the inbreeding status of larvae (inbred or outbred) and monitored larval responses to variable lengths of food deprivation. In the second experiment, I manipulated the inbreeding status of parents (inbred or outbred) and monitored parental responses

to variable brood sizes. If inbreeding reduced the ability of individuals to respond to variation in their environment, I predicted an effect of the interaction between the inbreeding status of larvae and food-deprivation on the amount of time spent begging and/or associating with the parent by larvae. Likewise, I predicted an effect of the interaction between the inbreeding status of the parent and brood size on time spent provisioning food and/or associating with the brood by parents.

7.2 Methods

I used beetles from the 7–9th generations of an outbred laboratory population descending from individuals collected in Corstorphine Hill, Edinburgh, UK. The population was maintained under 20°C and a 16:8h light:dark photoperiod. Non-breeding adult beetles were kept in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil and fed organic beef twice a week. Inbreeding was minimized in the stock population by avoiding breeding between closely-related individuals (defined as individuals sharing at least one common grandparent), by maintaining a large stock population comprised of 100–150 breeding pairs per generation (Mattey et al. 2018), and by supplementing the stock population annually with wild-caught beetles from the collection site in Blackford Hill, Edinburgh, UK. I produced inbred individuals by pairing full-sibling beetles from the stock population in the previous generation (Mattey et al. 2018). Given the negligible level of inbreeding in the stock population (see Mattey et al. 2018), inbred and outbred individuals had a coefficient of inbreeding of F ≈ 0.25 and 0, respectively, when referenced to the local wild population from the collection site.

7.2.1 Larval behaviour

In my first experiment, I manipulated the inbreeding status of larvae and monitored their response to three different levels of food deprivation. I manipulated the inbreeding status of larvae by assembling experimental broods where all lar-

vae in the brood were either outbred (N = 26) or inbred (N = 28). To this end, I set up pairs of virgin outbred parents at the start of the experiment by placing a male and a female in a large plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass weighing 20.1–25.0g. I generated inbred offspring by mating females to their full sibling brothers and I produced outbred offspring by mating other females to unrelated males. On the day before I anticipated the eggs to hatch (i.e. two days after the onset of egg-laying; Smiseth et al. 2006), I moved females and their carcasses to new containers lined with fresh soil (the males were discarded; Figure 7.1a) while leaving their eggs behind in the old container. These separations were done so that I could allocate an experimental brood made up of 15 same-aged larvae of mixed maternal origin to each female (Smiseth et al. 2007a). I standardized brood sizes in order to avoid potential confounding effects due to variation in brood size and larval age on larval behaviour (Smiseth et al. 2003, Smiseth et al. 2007a, Paquet and Smiseth 2017). I only allocated experimental broods to each female once her own eggs had hatched because parents will kill any larvae that emerge on the carcass before their own eggs have hatched (Müller and Eggert 1990).

For each brood, I collected data on larval behaviour at three different lengths of food-deprivation: 0 min, 90 min, and 180 min. I selected these durations based on information from preliminary tests confirming that these treatment were significantly increasing food deprivation without being lethal to larvae (Ratz et al. unpublished data). To this end, I performed three consecutive 15-min observation sessions on each brood over a 195-min period, starting 24h (±15 min) after a given brood was placed on a carcass. I recorded larval behaviour away from the mouse carcasses using a dead female parent as a stimulus. I did so to ensure that larvae had no access to food during the experiment, which otherwise would have interfered with the food-deprivation treatment. Using a dead female as a stimulus also allowed me to exclude any potential effect of variation in female behaviour on larval behaviour (Smiseth and Parker 2008, Smiseth et al. 2010),
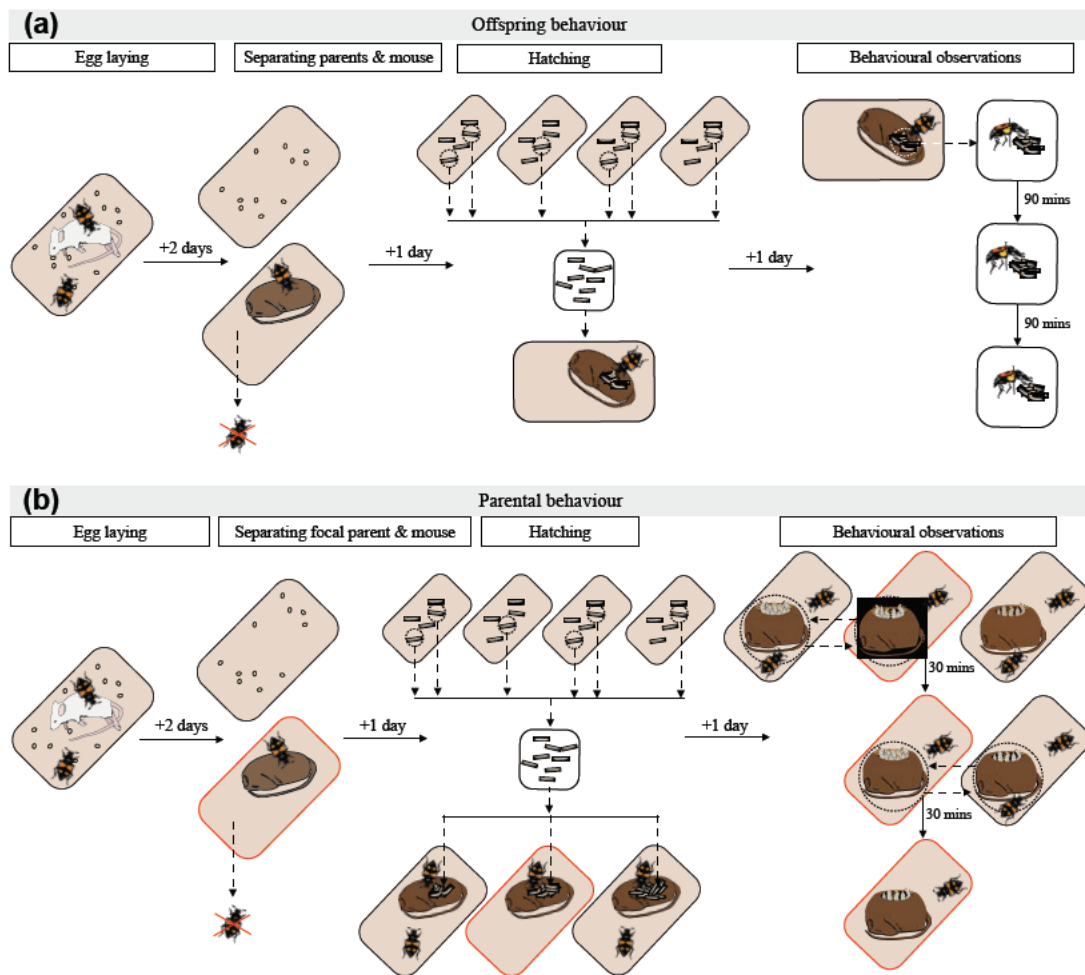
Figure 7.1: Diagram of the experimental design to investigate offspring response to the length of food deprivation (a) and parental response to variation in brood size (b).

and larvae beg towards a dead female in a similar way as towards a live female (Smiseth and Parker 2008, Smiseth et al. 2010). I used dead female parents that had bred and produced a brood to ensure that larvae perceive them as caring parents (Smiseth et al. 2010).

I killed females used as a stimulus approximately 1h before the start of each behavioural session by freezing them for 30 min and then thawing them for another 30 min. Once thawed, I pinned each dead female to the centre of a small container (12 cm × 8 cm × 2 cm) lined with moist paper and in a position mimicking that of a parent provisioning food to the brood (Mäenpää et al. 2015). I placed the

experimental brood away from the female and left the larvae to acclimatise for 5 min before starting the first observation (see details below). Thus, in order to beg for food from the female, larvae first had to move towards the female to associate with her. Larvae might later move away from the female to search for other sources of food given that the female was dead and that larvae would receive no returns on their begging effort. Larvae were often observed to remain cohesive as a group, regardless of whether they were associating with the female or away from her. When away from the female, larvae would sometimes split into multiple groups and move around the container at a slow pace either individually or as a group. Note that each brood was placed with its caring female, and that larvae therefore always were exposed to a familiar female during the observation. After the first observation, the female was removed, and the larvae were kept in the container for another 75 min to give a total of 90 min of food deprivation. For the second observation, I again pinned the female in the centre of the container and returned the experimental brood to where it was placed at the start of the first observation. I repeated this procedure once more by removing the female at the end of the second observation and keeping the larvae in the container for another 75 min for a total of 180 min of food deprivation. Although larvae may not experience this level of food deprivation in natural situations, there will be natural variation in hunger level due to the time elapsed since they were last provisioned food by a parent (Smiseth et al. 2003). Larvae beg more and are hungrier when they cannot receive food from a parent, suggesting that larvae are less efficient at obtaining food by self-feeding and have greater benefits when they obtain food from their parents (Smiseth and Moore 2004b). Furthermore, larvae may have limited access to those parts of the carcass that are most easily processed, especially when larvae are young and have relatively small mandibles (Eggert et al. 1998, Jarrett et al. 2018). I used these food-deprivation treatments for pragmatic reasons, because it provides a straightforward procedure for generating variation in larval hunger levels (Smiseth and Moore 2004b, 2007).

During each observation session, I monitored larval behaviour every 60 s over

a 15-min period. I recorded larval begging as the number of larvae that were touching any part of the female's body with their legs (Smiseth et al. 2003). I also recorded larval association with the female as the number of larvae that were within reaching distance from the female (i.e., a distance equal to or less than the pronotom length of the female). Based on these measures, I calculated the average time spent begging per larva in the brood (B) as the number of begging events cumulated across the 15 scans (Σb) divided by the cumulated number of larvae near the female (n), or $B = \Sigma b / n$. I also calculated the average time per larva in the brood spent associating with the female (A) as the number of larvae that were near the female across the 15 scans (Σa) divided by the total number of larvae in the brood (n), or $A = \Sigma a / n$.

7.2.2 Parental behaviour

In my second experiment, I manipulated the inbreeding status of parents and monitored their response to small and large broods. In the previous generation, I generated inbred parents by mating their mother to her full sibling brother, and I generated outbred parents by mating their mother to an unrelated male. I used both male and female parents in this experiment, allowing me to detect potential sex differences in behavioural plasticity of parents (Royle et al. 2014, Royle and Hopwood 2017). Thus, I used a 2×2 factorial design in which I recorded the behaviour of 313 adult beetles. As I was interested in how parents adjust care in response to brood size, I excluded 175 individuals that were not observed providing care at least once to any one of the two broods. The final sample included 36 inbred males, 31 outbred males, 36 inbred females and 35 outbred females. To initiate breeding, I paired each experimental parent to an unrelated outbred partner. I placed the breeding pair into a larger plastic container (17 cm $\times$ 12 cm $\times$ 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass of a standardized size (20.3–23.9 g) (Livefoods Direct, Sheffield). I separated the parents from their eggs two days after the first egg was laid by moving the parents and their carcass to a new container containing fresh soil (Figure

7.1b). I discarded the partner at the same time to ensure that any effect of brood size on parental behaviour was not confounded by the presence of the partner. Once the eggs had started hatching, each experimental parent was allocated a brood of ten larvae (hereafter referred to as the baseline brood) to whom they provided care until being allocated the first experimental broods 24h later (see below). To avoid filial cannibalism, I allocated baseline broods to parents only once their own larvae had hatched.

In parallel with setting up the experimental parents, I set up additional pairs of unrelated males and females. I did this to produce additional larvae that were used to generate both baseline and experimental broods. The additional pairs also functioned as foster parents for the small and large experimental broods until they were allocated to an experimental parent 24h after it had been allocated its initial baseline brood. As described for the experimental parents above, I separated foster parents from their eggs two days after the first egg was laid by moving the parents and their carcass to a new container containing fresh soil. However, I left both foster parents with the broods to ensure that all experimental broods had encountered both a male and a female parent. Once eggs had started hatching, I allocated each foster pair either a small brood of 5 larvae or a large brood of 20 larvae, which fall well within the range of natural brood sizes for this species (Smiseth and Moore 2002). I used these brood sizes because prior studies have shown that parents provide double the amount of time spent caring towards a brood of 20 compared to a brood of 5 larvae (Smiseth et al. 2007a, Ratz and Smiseth 2018).

For each parent, I collected data on their parental behaviour towards two different brood sizes: 5 and 20 larvae. I performed two consecutive 30-min observation sessions for each parent, starting 24h ($\pm$15 min) after the parent had been provided with the initial baseline brood. I randomised the order in which experimental parents were provided with broods of different sizes. I first removed the original mouse carcass containing the baseline brood of 10 larvae and immediately

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replaced it with a carcass from a foster pair containing an experimental brood of either 5 or 20 larvae. I allowed the larvae to settle for 30 min before starting the first observation. Immediately after the first observation was completed, I replaced this carcass with a carcass from a different foster pair containing an experimental brood of the opposite treatment (5 larvae if the first experimental brood had 20 larvae and vice versa). I again allowed the larvae to settle 30 min before starting the second observation.

During each observation session, I monitored the behaviour of experimental parents every 60 s over a 30-min period. I recorded parental provisioning of food to the brood as a mouth-to-mouth contact between the parent and at least one larva. I also recorded parental association with the brood as the parent being present on the carcass or within the crypt (the depression in the soil immediately surrounding the carcass). I calculated the percentage of time spent provisioning food to the brood and associating with the brood as the total number of scans the parents was performing the behaviour of interest (i.e., 0–30) divided by the number of scans in the observation session (i.e., 30).

7.2.3   Data analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team, 2019) with the packages *car* (Fox et al., 2016) and *lme4* (Bates et al. 2014). I quantified differences in behavioural plasticity between inbred and outbred larvae by estimating the effect of the interaction between the inbreeding status of larvae and the length of food deprivation on larval behaviour. I used general linear mixed models that assumed a binomial error structure to analyse larval behaviours (i.e., time spent begging towards and associating with the female). These models included the length of food-deprivation (0 min, 90 min and 180 min) as a continuous fixed effect and inbreeding status of larvae (inbred or outbred) as a categorical fixed effect, as well as the interaction between the two. I included brood size at the time of observation as covariate in the models to ac-

count for potential effects of brood size on larval behaviour. I also included brood ID and observation level as random effects to account for repeated observations on each brood and overdispersion of the data (Harrison 2015), respectively.

To quantify differences in behavioural plasticity between inbred and outbred parents, I estimated the effect of the interaction between the inbreeding status of parents and brood size on parental behaviour. I used generalised linear mixed models that assumed a binomial error structure to analyse parental behaviours (i.e., time spent provisioning food and associating with the brood). These models included brood size (5 and 20 larvae) as a continuous fixed effect, inbreeding status of the parent (inbred or outbred) as a categorical fixed effect, and an effect of the interaction between the two. I also included sex of the parent as covariate to test for potential sex differences in the behavioural plasticity of parental behaviour. To account for repeated observations on the same focal individuals, I included parental ID as random effects in both models. To account for overdispersion, I also included observation level as additional random effects in the model testing for effects on time spent associating with the brood.

7.3 Results

7.3.1 Larval behaviour

My main aim was to test for differences in behavioural plasticity between inbred and outbred individuals, and I therefore focused first on the interaction between the inbreeding status of larvae and the length of food-deprivation on larval behaviour. There was no effect of this interaction on time spent begging (Table 7.1). Thus, for larval begging, there was no difference between inbred and outbred larvae with respect to behavioural plasticity in response to a change in hunger state (Figure 7.2a). However, there was a significant effect of this interaction on the amount of time spent associating with the female (Table 7.1), indicating that inbred larvae spent less time associating with the female as they became hungrier
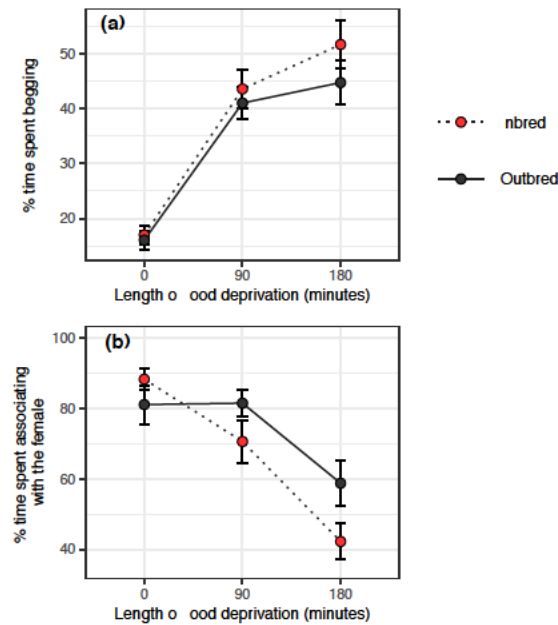
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Figure 7.2: Effects of increasing length of food deprivation on the amount of time (percentage) larvae spent begging towards (a) and associating with (b) a female parent. Mean ±SE.

compared to outbred ones (Figure 7.2b). Thus, for time spent associating with the female, inbreeding increased behavioural plasticity exhibited by larvae in response to a change in their hunger state.

The length of food-deprivation had a significant positive main effect on time spent begging and a negative main effect on time spent associating with the female (Figure 7.2a, Table 7.1). There was no main effect of the inbreeding status on time spent begging or associating with the female (Table 7.1). Finally, there was a negative main effect of brood size at the time of observation on time spent begging (estimate $= -0.117$, SE $= 0.047$, z $= -2.47$, P $= 0.014$), but brood size had no effect on time spent associating with the female (estimate $= 0.012$, SE $= 0.120$, z-value $= 1.01$, P $= 0.314$).

7.3.2 Parental behaviour

For reasons explained above, I first focused on the interaction between the inbreeding status of the parent (inbred versus outbred) and brood size (5 and 20
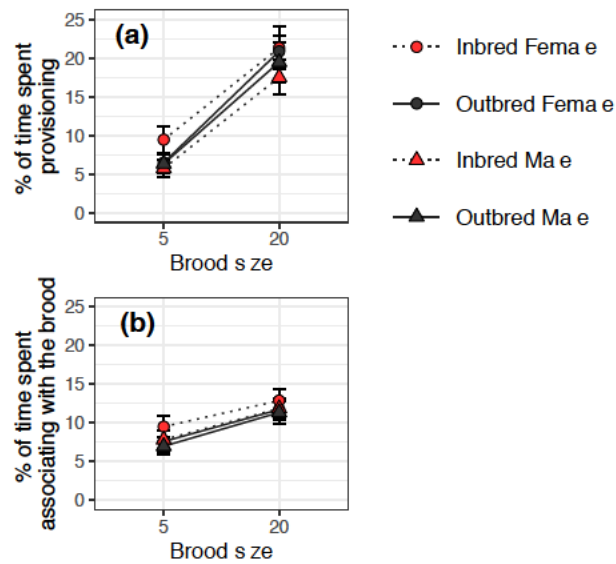
Figure 7.3: Effects of brood size on the amount of time (percentage) male and female parents spent provisioning food (a) and associating with (b) the brood. Mean ±SE.

larvae) to test for potential differences in behavioural plasticity between inbred and outbred parents. There was no effect of this interaction on time spent provisioning food or associating with the brood (Figure 7.3a-b, Table 7.1). Thus, inbreeding did not appear to change behavioural plasticity exhibited by parents in response to changes in brood size.

As expected, brood size had a significant positive main effect on time spent provisioning food and associating with the brood (Table 1), confirming that parents spent more time provisioning food and associating with the brood when brood size increased. Finally, there were no main effects of parental inbreeding status (Table 7.1) or sex (estimate $= -0.229$, SE $= 0.153$, z-value $= -1.50$, P $= 0.133$) on time spent provisioning food. Likewise, there were no main effects of parental inbreeding status (Table 7.1) or sex (estimate $= -0.255$, SE $= 0.244$, z-value $= -1.05$, P $= 0.296$) on time spent associating with the brood.

Table 7.1: Effects of the level of food deprivation, inbreeding status (outbred vs. inbred), and the interaction between food deprivation and inbreeding on average larval begging and average time spent associating with the female by the larvae. Effects of brood size, inbreeding status, and the interaction between brood size and inbreeding on the time spent provisioning and associating with the brood by the carrying parent. Values are obtained from GLMMs.

	Environmental variable				Inbreeding status				Environment:Inbreeding			
	Estimate	SE	z-value	P	Estimate	SE	z-value	P	Estimate	SE	z-value	P
Food-deprivation (0, 90 or 180 min)												
Offspring Begging	0.850	0.095	8.99	**<0.0001**	0.031	0.298	0.103	0.918	0.046	0.131	0.351	0.725
Offspring association with the parent	−0.891	0.236	−3.99	**<0.0001**	1.70	0.762	2.23	**0.026**	−0.891	0.330	−2.70	**0.007**
Brood size (5 or 20 larvae)												
Time spent provisioning	1.37	0.109	12.6	**<0.0001**	0.032	0.295	1.07	0.284	−0.215	0.148	−1.46	0.146
Time spent associating with the brood	1.00	0.261	3.85	**0.0001**	0.394	0.607	0.650	0.516	−0.127	0.367	−0.346	0.730

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7.4 Discussion

I show that inbreeding in larvae of *N. vespilloides* was associated with increased behavioural plasticity for time spent associating with the female parent, as inbred larvae showed a greater response to food deprivation than outbred ones. However, inbreeding was not associated with a change in behavioural plasticity in time spent begging or in the time that parents of either sex spent provisioning food or associating with the larvae. My results derive from two experiments, in which I monitored behavioural plasticity in larvae in response to experimental variation in the length of food deprivation and behavioural plasticity in parents in response to experimental variation in brood size. I generated variation across two environmental stress gradients experimentally in order to remove confounding effects on plasticity in larval and parental behaviours. Furthermore, my study focused on behavioural plasticity in environmental gradients that larvae and parents are exposed to and respond to under natural conditions. Below, I discuss the wider implications of my results for our understanding of the effects of inbreeding on behavioural plasticity and how such effects may provide a mechanism for inbreeding-by-environment interactions affecting fitness.

My study shows that larvae spent less time associating with the female as they became hungrier and that this decline was more pronounced in inbred larvae than in outbred ones. Currently, little is known about the potential adaptive value of behavioural plasticity in larval association with the female. Larvae associate with parents because they need to be in close proximity to them in order to beg for food (Smiseth and Moore 2002). In my experimental design, larvae had to move towards the female in order to be in close proximity to her. Larvae may later move away from the female because they would receive no returns on their begging given that I used a dead female as a standardised stimulus. My results suggest that inbred and outbred larvae spent a similar amount of time associating with the female at the start of the experiment, but inbred larvae spent more time away from the female as the length of food deprivation increased (Figure 7.2b). Thus, my results show that inbred larvae had a greater degree of behavioural plasticity

than outbred ones. Nevertheless, I urge caution when interpreting my results given that I monitored larval behaviour towards a dead parent in the absence of a carcass. I used a dead parent as a stimulus to ensure that larvae had no access to food during the experiment (which would otherwise interfere with my experimental treatment) and to control for confounding effects caused by parental behaviour (e.g., Smiseth and Parker 2008, Smiseth et al. 2010). Prior work shows that the presence of a dead parent stimulates high levels of larval begging for at least 180 min (Smiseth and Parker 2008). Yet, a consequence of this design is that larvae were exposed to an unresponsive parent for a considerable amount of time, which might explain why hungrier larvae spent less time associating with the female. In natural situations, where larvae interact with a live female on a carcass, I would expect hungrier larvae to spend more time associating with the female because larvae must stay in close proximity to her in order to have the opportunity to beg for food (Smiseth and Moore 2002). In such situations, larvae face a choice between self-feeding from within the crater of the carcass (i.e. the cavity prepared by the parents) and leaving the crater to associate with a caring parent (Smiseth et al. 2003). Given that the larvae in my experiment could not get access to food from the dead female, and that there was no carcass from which to self-feed, larvae may have responded to food deprivation by associating less with the female and by searching for opportunities to obtain food by self-feeding (Smiseth et al. 2003). In light of this, I would not necessarily expect larvae to respond in a similar way to food deprivation when interacting with a live parent (Smiseth et al. 2003).

One potential explanation for my finding that inbreeding was associated with increased behavioural plasticity in larvae is that inbred larvae have higher nutritional needs than outbred ones. Offspring begging is thought to be an honest signal that reliably reflects the offspring's nutritional needs (Godfray 1995), and there is good evidence that begging reflects larval hunger in my study species (Smiseth and Moore 2004b). Thus, if inbred larvae did have higher nutritional needs than outbred ones, I would expect inbred larvae to spend more time begging

and to show greater plasticity in this behaviour. However, I found no evidence that this was the case as there was no effect of the interaction between larval inbreeding status and length of food-deprivation on time spent begging. Furthermore, prior work on this species shows that inbred larvae spend less time begging to a live parent than outbred ones (Mattey et al. 2018). An alternative explanation is that inbred larvae were less able to sustain the costs of begging with an increase in the length of food deprivation than outbred ones. This explanation, however, seems unlikely given that I found that inbred and outbred larvae increased their level of begging to similar degrees in response to an increase in the length of food deprivation. Thus, there is no evidence that my results can be explained as a consequence of inbred larvae having higher nutritional needs or greater costs of begging. A final explanation is that inbreeding constrains an individual's ability to invest in costly cognitive and/or sensory mechanisms required for adaptive behavioural plasticity (Dingemanse and Wolf 2013, Snell-Rood 2013). In this case, inbred individuals may not be able to adjust their behaviour as effectively to match changing conditions (e.g. Schiegg et al. 2002). For example, a recent study on my study species found that inbred females are less able than outbred females to adjust brood size when the size of the carcass is changed experimentally just prior to hatching (Richardson et al. 2018). Thus, inbreeding undermines the ability of burying beetles to make sensible life decisions, suggesting that my results may reflect that inbred larvae were less able to make an appropriate decision between staying near the female and searching for opportunities to self-feed.

My finding that inbred larvae showed greater behavioural plasticity has important implications for our understanding of the mechanism for inbreeding-by-environment interactions. Inbreeding is often associated with an increased sensitivity to environmental stress (Armbruster and Reed 2005, Cheptou and Donohue 2011, Fox and Reed 2011), and prior work suggests that such inbreeding-by-environment interactions may arise if inbreeding is associated with reduced phenotypic plasticity (Fowler and Whitlock 1999, Reed et al. 2003, Reed et al.

2012, Bijlsma and Loeschcke 2012). The rationale for this explanation is that inbred individuals are less able to adjust their phenotype to cope with stressful environmental conditions than outbred individuals. However, my results show that inbreeding can be associated with increased phenotypic plasticity. Increased behavioural plasticity may cause inbreeding-by-environment interactions for traits that are canalised because, for some traits, there may be selection that favours resistance to phenotypic plasticity (Schou et al. 2015). For example, Schou et al. (2015) found that inbred lines of Drosophila melanogaster had higher plasticity in the developmental response of wing size in response to high temperatures. This may come at a fitness cost as small wings may reduce flight performance in warmer environments more in inbred individuals (Frazier et al. 2008). Just as there can be detrimental effects from too much developmental plasticity, stabilizing selection may also favour the evolution of intermediate levels of behavioural plasticity. Thus, if there is an optimal behavioural response, we might expect inbreeding-by-environment interactions if inbred individuals show either too much or too little behavioural plasticity. Furthermore, inbreeding-by-environment interactions could occur under stabilizing selection if inbred individuals show greater variance in behavioural plasticity, even if there is no difference in mean plasticity between inbred and outbred individuals. This would be the case if some inbred individuals show reduced behavioural plasticity whilst others show increased behavioural plasticity compared to outbred individuals. Thus, there is a need for further work focusing on how selection works on behavioural plasticity.

In summary, I found that inbreeding affects behavioural plasticity of some larval behaviours (time spent associating with a parent), whereas inbreeding had no effect on behavioural plasticity of other larval behaviours (time spent begging) or any parental behaviours (time spent provisioning food and associating with the brood). To my knowledge, this is the first study investigating how inbreeding affects plasticity of social behaviours. My findings suggest that effects of inbreeding on behavioural plasticity may be one of the potential mechanisms underlying the effects of inbreeding on social interactions among individuals (e.g. Richard-

son and Smiseth 2017, Mattey et al. 2018). More generally, my findings have important implications for our knowledge about inbreeding depression by showing that inbred individuals can show greater behavioural plasticity in response to environmental variation than outbred ones. I suggest that effects of inbreeding on behavioural plasticity may cause inbreeding-by-environment interactions for traits where there are negative fitness consequences of showing either too much or too little plasticity in response to changing environmental conditions. I encourage more work on the interplay between inbreeding and adaptive behavioural plasticity given that inbreeding and stress due to environmental change are growing conservation concerns in many natural populations (e.g. Reed et al. 2012, Hamilton and Miller 2015). Understanding the interplay between them will now be critical in our understanding of how natural populations respond to environmental change, such as climate change and population decline.

8 Effects of biparental care and inbreeding on offspring performance

This chapter has been published as:

Abstract

The severity of inbreeding depression often varies across environments and recent work suggests that social interactions can aggravate or reduce inbreeding depression. For example, stressful interactions such as competition can exacerbate inbreeding depression, whereas benign interactions such as parental care can buffer against inbreeding depression in offspring. Here, I test whether male assistance in parental care can buffer against the detrimental effects of maternal inbreeding on offspring fitness in the burying beetle *Nicrophorus vespilloides*. My results confirm that maternal inbreeding had detrimental effects on offspring survival. However, I found no evidence that male assistance in parental care buffered against those effects on offspring fitness. Outbred females benefited from male assistance, gaining more weight over the breeding attempt when assisted by a male. In contrast, inbred females did not benefit from male assistance, gaining as much weight regardless of whether they were assisted by a male or not. Surprisingly, I find that males gained more weight during the breeding attempt when mated to an inbred female, suggesting that males benefited from assisting an inbred female partner in terms of their weight gain. Overall, my findings suggest that parental care or other benign social interactions may not always reduce indirect detrimental effects of inbreeding depression.

8.1 Introduction

Inbreeding depression, defined as the reduction in fitness of progeny produced as a consequence of mating between relatives, has been reported in a broad diversity of animals and plants (reviewed in Charlesworth and Charlesworth 1987, Keller and Waller 2002, Charlesworth and Willis 2009). There is ample evidence for inbreeding depression in fitness-related traits, including fecundity, offspring growth and survival, and longevity from studies conducted under both laboratory and natural conditions (Keller 1998, Slate et al. 2000, Keller and Waller 2002). There is growing awareness that the magnitude of inbreeding depression often varies between species or studies on the same species (e.g. Fox and Scheibly 2006). This may reflect that inbreeding depression is often more severe under more stressful environmental conditions (Hoffmann and Parsons 1991, Armbruster and Reed 2005, Cheptou and Donohue 2011). The social environment may play an important role in this context because social interactions can amplify or alleviate stress, thereby exacerbating or buffering against inbreeding depression. For example, direct competition between inbred and outbred males exacerbates inbreeding depression in house mice (*Mus domesticus*) (Meagher et al. 2000). Meanwhile, parental care buffers against inbreeding depression in offspring in the burying beetle *Nicrophorus vespilloides* (Pilakouta et al. 2015a).

The examples provided above illustrate that social interactions with other individuals can have an important impact on the fitness of those individuals that are themselves inbred (e.g., Meagher et al. 2000, Pilakouta et al. 2015a). However, there is mounting evidence for indirect genetic effects associated with inbreeding whereby outbred individuals suffer fitness costs as a result of interacting with or depending upon inbred ones (Mattey et al. 2013, Richardson and Smiseth 2017). For example, in species where parents care for their offspring, parental inbreeding can have detrimental effects on the offspring's fitness. Recent studies on the burying beetle *Nicrophorus vespilloides* and red deer (*Cervus elaphus*) show that maternal inbreeding is associated with lower offspring survival (Mattey et al. 2013, Huisman et al. 2016). Such effects of maternal inbreeding on offspring

fitness may result from inbred females providing less or lower-quality care than outbred ones (Mattey et al. 2013). Currently, it is unclear whether interactions with third-party individuals may buffer against the detrimental effects of maternal inbreeding on offspring fitness. For example, in species with biparental care, the presence of a male partner may offset some of the detrimental effects of maternal inbreeding on offspring. In support for this hypothesis, a study on zebra finches (*Taeniopygia guttata*) found that maternal inbreeding had no detectable effect on offspring fitness, independently of male care and even though inbred mothers spent less time incubating their eggs (Pooley et al. 2014). In this study, males always assisted with parental care. Thus, there is now a need for studies that examine whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness by manipulating the presence and absence of male assistance.

The burying beetle *Nicrophorus vespilloides* is well suited to test whether male assistance in care buffers against the detrimental effects of maternal inbreeding on offspring fitness. In this species, both parents cooperate to bury, maintain and guard the vertebrate carcass, which serves as the sole food source for both larvae and parents during breeding. Both parents also care for the larvae after hatching, though females spend more time provisioning food than males and males desert the brood earlier than females (Bartlett and Ashworth 1988, Smiseth et al. 2005). Males respond to the removal or desertion of the female or the reduced amount of care of handicapped females by increasing their time spent on paternal care (Smiseth et al. 2005, Royle et al. 2014, Creighton et al. 2015). Furthermore, males spend more time providing care when paired with an inbred female, suggesting that males respond to inbreeding status of their female partner (Mattey and Smiseth 2014). There is good evidence that maternal inbreeding has a detrimental impact on the fitness of outbred offspring, reducing larval survival (Mattey et al. 2013, 2018, Ford et al. 2018; but see Mattey and Smiseth 2014). However, there is no information as to whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness.

Here, I use a 2×3 factorial design to test whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness in the burying beetle *Nicrophorus vespilloides*. I paired an inbred or outbred female with an unrelated inbred or outbred male that assisted the female with parental care during larval development. I also added additional treatments where the male was experimentally removed before larval hatching such that the inbred or outbred female received no assistance in parental care. My first aim was to test whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness. If so, I predicted effects of the interaction between maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) on offspring fitness (i.e., mean offspring survival and/or weight), reflecting that maternal inbreeding had a greater negative impact on offspring fitness when the male is absent than when the female receives assistance from a male. Furthermore, if inbred males have a reduced capacity to buffer against the detrimental effects of maternal inbreeding, I predicted that maternal inbreeding would have a greater negative impact on offspring fitness when the female was assisted by an inbred male rather than an outbred male. I next tested whether male assistance in parental care had an impact on female and male weight change whilst providing care. In this species, the amount of carrion consumed by a parent reflects parental allocation towards future reproduction (Creighton et al. 2009). Thus, if male assistance in parental care buffers against the detrimental effects of maternal inbreeding, thereby allowing females to save more resources for investment in future reproduction, I predicted females to gain more weight when assisted by a male than when the male was absent. If outbred males were better able to buffer for the effects of maternal inbreeding than inbred ones, I predicted that females assisted by an outbred male would gain more weight than those assisted by an inbred male. Finally, as inbred females are expected to provide lower quality care than outbred ones, I predicted that males paired with an inbred female would gain less weight than males paired with an outbred female, reflecting that the former increase their investment in current

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reproduction (Mattey and Smiseth 2014).

8.2 Methods

The beetles used in these experiments originated from wild beetles originally collected in Corstorphine Hill, Edinburgh, U.K. In order to avoid unintended inbreeding, a large outbred laboratory population was maintained (Mattey et al. 2018). To this end, 200–300 individuals each generation were bred, while three offspring from each brood were recruited to the next generation. Non-breeding adult beetles were kept in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil, and fed small pieces of organic beef twice a week. The beetles were kept under constant temperature (20°C) and photoperiod (16:8 hours light:dark).

8.2.1 Experimental design

To test whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness, I used a 2×3 factorial design in which an inbred or an outbred female was mated with an inbred or outbred male that later assisted the female in parental care (inbred female mated to an inbred male: N = 51; inbred female mated to an outbred male: N = 36; outbred female mated to an inbred male: N = 48; outbred female mated to an outbred male: N = 35). This design also included two additional treatments where an inbred or an outbred female was mated with a male that was removed before the larvae hatched (N = 40 and N = 38 for inbred and outbred females, respectively).

I generated inbred females and males for use as parents in this experiment by paring their mother with her full-sibling brother in the previous generation (Mattey et al. 2018). Meanwhile, I generated outbred females and males by paring their mother with an unrelated male (i.e., a male with which the mother did not share an ancestor for at least two generations; Mattey et al. 2018). Once the inbred

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and outbred females and males had reached sexual maturity, I randomly assigned each individual to one of the six treatments. At the start of the experiment, I weighed each female and male. I then paired inbred and outbred females with an unrelated inbred or outbred male partner depending on the treatment to which they had been assigned, and transferred them into a larger transparent plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil. I provided each pair with a previously frozen mouse carcass of a standardized size (22.33–26.89 g) (supplied by Livefoods Direct, Sheffield, UK) to initiate breeding. I checked each container for the presence of eggs daily and recorded the date at which the first eggs appeared as the start of egg laying. Two days after the start of egg laying, I recorded clutch size as the number of eggs that were visible through the bottom of the transparent containers (Monteith et al. 2012). In the limited amount of soil that I used, the number of eggs visible at the bottom of the container is strongly correlated with the actual clutch size (Monteith et al. 2012).

In those treatments where the male was absent during larval development, I removed the male from the container two days after the outset of egg laying as this corresponds to the day before hatching (Smiseth et al. 2006). In the remaining treatments, I left the inbred or outbred male within the container thereby allowing him to assist the female in providing parental care until the larvae completed development and dispersed from the mouse carcass (hereafter referred to as larval dispersal). At larval dispersal, I recorded brood size by counting the number of larvae in the brood and weighed the total mass of the brood. I estimated the proportion of offspring surviving until dispersal as the brood size at dispersal divided by the clutch size. I calculated mean larval mass as the total mass of the brood divided by the brood size. At larval dispersal, I also weighed each female and male parent. I then estimated the percentage of weight gain of females and males during breeding as the relative difference in body mass measured at mating (W_m) and the body mass at larval dispersal (W_d): $\frac{W_d - W_m}{W_m} \times 100$. I used information on the relative weight gain rather than the absolute weight gain in females in order to control for potential differences in body size across individuals given

that inbred females were significantly lighter than outbred females at the start of the experiment (LRχ^2 = 4.43, df = 1, P = 0.035).

8.2.2 Data analysis

All statistical analyses were conducted using R v 3.3.3 (R Development Core Team, 2019) loaded with the package *car* (Fox et al., 2016). To analyse data on offspring survival until dispersal, I used a Poisson generalized linear model. I analysed data on mean larval mass at dispersal and weight gains by females and males using general linear models fitted with a Gaussian error structure. These models always included maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) as fixed factors. I included female relative weight gain as an additional fixed factor in the model on male relative weight gain as male carrion consumption and weight change has been shown to depend on female carrion consumption and weight gain (Pilakouta et al. 2016). I also controlled for potential differences in body size by including mating weight as a fixed factor in the model on female absolute weight gain. I excluded carcass size from the analyses given that I used mouse carcasses of a narrow, standardized size in my experiment (22.33–26.89 g). Furthermore, there was no significant effect of carcass size in any of my analyses when I included it as a fixed factor. I assessed and evaluated whether the structure of all models was appropriate for each variable by plotting the residuals from the models. Whenever there was a significant effect of male status (inbred, outbred or absent), I tested for differences between each treatment using Tukey contrasts reporting p-values based on the Bonferroni correction for multiple testing.

8.3 Results

8.3.1 Offspring fitness

There was no significant effect of the interaction between maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) on the proportion of offspring surviving until dispersal (Maternal inbreeding:Male status, Table 8.1, Figure 8.1a). Thus, there was no evidence that male assistance in parental care buffered against the detrimental effects of maternal inbreeding on offspring fitness.

I next explored potential main effects of maternal inbreeding and male status on offspring fitness. As expected, broods reared by outbred females had a larger proportion of offspring surviving until dispersal than broods reared by inbred females (Table 8.1, Figure 8.1a), thus confirming that maternal inbreeding had detrimental effects on offspring survival. There was no difference in the proportion of offspring surviving until dispersal depending on whether the male was inbred, outbred or absent (Table 8.1, Figure 8.1a). There was no difference in mean larval mass at dispersal between inbred and outbred females (Table 8.1, Figure 8.1b). Likewise, there were no significant main effects of male status mean larval mass at dispersal (Table 8.1, Figure 8.b). These findings suggest that there was no detrimental effect of maternal or paternal inbreeding on mean offspring weight and that male assistance had no positive main effects on mean offspring weight.

Table 8.1: Effects of maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) on offspring survival until dispersal and mean larval mass at larval dispersal. Values are obtained from GLMs.

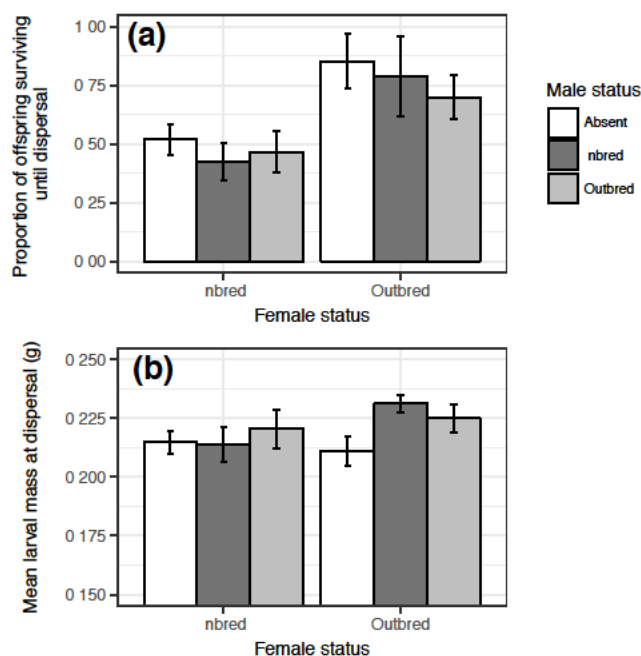|	Offspring survival			Mean larval mass		
	LRχ^2	df	P	LRχ^2	df	P
Maternal inbreeding:Male status	0.275	2	0.871	2.85	2	0.239
Maternal inbreeding	9.55	1	**0.002**	1.21	1	0.270
Male status	0.711	2	0.700	3.52	2	0.172

Figure 8.1: Effects of maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) on the proportion of offspring surviving until dispersal (a), and on mean larval mass at dispersal (b). Mean ± SE.

8.3.2 Female and male weight gain

There was a significant effect of the interaction between maternal inbreeding and male status on female relative and absolute weight gain (Table 8.2). This interaction effect reflected that outbred females gained more weight when a male assisted in parental care than when the male was removed (Figure 8.2a), while inbred females gained a similar amount of mass regardless of whether the male assisted with parental care or not. There was no difference in female relative or absolute weight gain depending on whether the male was inbred or outbred (Female inbred, Male outbred vs. Male inbred: Estimate $= -0.010 \pm 0.007$, $Z = -1.34$, $P > 0.999$; Female outbred, Male outbred vs. Male inbred: Estimate $= 0.007 \pm 0.008$, $Z = 0.891$, $P > 0.999$). Thus, there was no evidence that females gained more weight when assisted by an outbred rather than an inbred male.

There was no significant effect of the interaction between maternal inbreeding and male status on male relative weight gain (Table 8.2, Figure 8.2b). There were no significant differences in relative weight gain between inbred and outbred
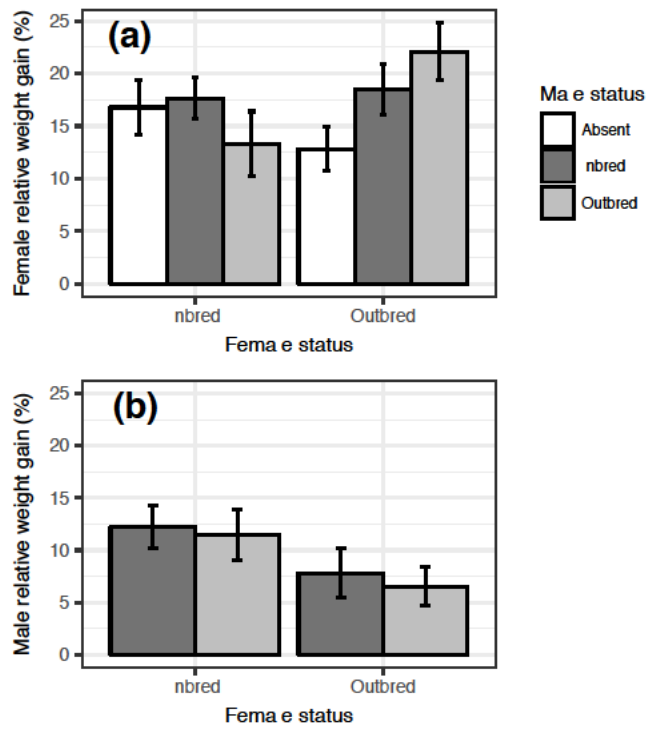
Figure 8.2: Effects of maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) on female relative weight gain (a), and male relative weight gain (b) over the breeding attempt. Mean ± SE.

males (Table 8.2, Figure 8.2a). However, males gained less weight when paired with an outbred female than when paired with an inbred female (Figure 8.2a), suggesting that males benefited from having an inbred partner in terms of gaining more weight.

Table 8.2: Effects of maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) on female and male relative and absolute weight gains over the breeding attempt. Values are obtained from GLMs.

	Female relative weight gain			Female absolute weight gain			Male relative weight gain			Male absolute weight gain		
	LRχ^2	df	P	LRχ^2	df	P	LRχ^2	df	P	LRχ^2	df	P
Maternal inbreeding:Male status	6.59	2	**0.037**	7.80	2	**0.020**	0.067	1	0.795	0.064	1	0.799
Maternal inbreeding	0.737	1	0.390	1.61	1	0.203	5.11	1	**0.023**	3.91	1	**0.047**
Male status	2.16	2	0.339	3.46	2	0.176	0.131	1	0.717	0.039	1	0.842
Parent's initial weight	–	–	–	0.006	1	0.938	–	–	–	1.26	1	0.261
Female relative weight gain	–	–	–	–	–	–	2.31	1	0.127	–	–	–
Female absolute weight gain	–	–	–	–	–	–	–	–	–	2.31	1	0.127

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8.4 Discussion

Here, I tested whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness in *N. vespilloides*. I found that maternal inbreeding had detrimental effects on offspring fitness in terms of reduced offspring survival, indicating that inbred females provide a less favourable environment compared with outbred females. This confirms the results of prior work on this species (Mattey et al. 2013, Ford et al. 2018; but see Mattey and Smiseth 2014) and evidence from studies on vertebrate systems (Keller 1998, Huisman et al. 2016, Bérénos et al. 2016). However, I found no evidence that male assistance in parental care buffered against these detrimental effects. Male assistance in care had a positive effect on female weight gain during breeding, showing that male assistance in parental care was beneficial to females. However, this was only the case when females were outbred, suggesting that outbred females benefitted more from male assistance than inbred ones. Finally, males paired with an inbred female gained more weight than those paired with an outbred female. This finding is opposite to what I predicted if males paired with an inbred female increased their investment in current reproduction. Instead, this result may reflect that males paired with an inbred female spent more time provisioning food to the larvae, thereby gaining better access to consume food from the carcass (Pilakouta et al. 2016). Overall, my results provide no support that male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness. Below, I provide a more detailed discussion of my results and their implications for our understanding of the consequences of inbreeding in populations where social interactions are prevalent.

My first key finding was that maternal inbreeding had detrimental effects on offspring fitness in terms of reduced larval survival from egg laying until dispersal, but this effect was independent of whether the male was absent or present, and when the male was present, whether the male was inbred or outbred. Thus, my results provide no evidence that male assistance in parental care buffered against the detrimental effects of maternal inbreeding on offspring fitness. This finding

contrasts with experimental evidence from a recent study on zebra finches, suggesting that male assistance in parental care buffers against the detrimental effects of maternal inbreeding. In zebra finches, inbred females spend less time incubating eggs, and buffering is thought to reflect that males compensate for the reduced incubation by inbred females (Pooley et al. 2014). Prior work on *N. vespilloides* shows that maternal inbreeding can reduce both offspring hatching (i.e., at the egg stage; Ford et al. 2018, Mattey and Smiseth 2014) and offspring survival after hatching (i.e., at the larval stage; Mattey et al. 2013, Ford et al. 2018). Thus, one potential explanation for why male assistance did not buffer against the detrimental effects of maternal inbreeding on offspring is that post-hatching male care cannot buffer against effects on hatching success. This explanation may also apply to other systems as detrimental effects of maternal inbreeding on hatching success have also been reported for example in song sparrows (Keller 1998). An alternative explanation for why male assistance in parental care did not buffer against the detrimental effects of maternal inbreeding is that male assistance in care in this species does not increase larval survival from hatching until dispersal under laboratory conditions (Smiseth et al. 2005). This presumably reflects that males contribute far less towards parental care than females in this species (Smiseth et al. 2005). This sex difference in parental care may also explain why there were detrimental effects of maternal inbreeding on offspring fitness, whilst there were no such detrimental effects of paternal inbreeding. I note that male assistance in guarding and defending the brood against predators or conspecific intruders plays an important role under natural conditions in burying beetles (Scott 1990). Thus, it is possible that male assistance in parental care could buffer against detrimental effects of maternal inbreeding on offspring fitness under natural conditions where competitors or conspecific intruders may reduce offspring survival. Further studies are now needed to investigate whether male assistance in care might buffer against detrimental effects of maternal inbreeding when there is a risk of predation or takeovers by conspecific intruders.

My second main finding was that there was an effect of the interaction between

maternal inbreeding and male status on female weight gain with male assistance in parental care having a positive effect on relative mass gain of outbred females only. I anticipated that females would benefit from male assistance leading to an increase in their mass gain, which indicates a shift towards investment in future reproduction (Creighton et al. 2009), regardless of their own inbreeding status. Thus, this finding suggests that outbred females benefitted more from male assistance than inbred ones. One potential explanation for why this might be the case is that inbreeding is associated with terminal investment and that inbred females therefore always invest more effort into current reproduction. There is some evidence from previous studies on *N. vespilloides* suggesting that inbreeding is associated with terminal investment (Mattey and Smiseth 2014, Richardson and Smiseth 2017, Mattey et al. 2018). In light of the finding that the presence of a male had a positive effect on the weight gain of outbred females only, future work should now test for a differential effect of male assistance in care on the subsequence breeding performance by outbred and inbred females. Presumably, outbred females would perform better in subsequent breeding attempts when assisted by a male during a first breeding attempt, while inbred females would perform equally well regardless of whether they were assisted by a male or not.

I found no evidence that females gained more mass when assisted by an outbred male, suggesting that females did not benefit more from assistance by outbred males as compared to inbred ones. I predicted that females would gain more mass when assisted by an outbred male if outbred males are better parents than inbred ones. My results show that that this was not case, which might explain why outbred males were not more able to buffer against the detrimental effects of maternal inbreeding on offspring fitness. My finding echoes previous work in this species showing that male weight gain has little influence on carrion consumption or weight gain of the female, whereas females adjust their consumption and weight gain to match their male partner's own weight gain (Pilakouta et al. 2016). In light of this evidence, and keeping in mind that there was no difference in weight gain by inbred and outbred males in my experiment (see discussion be-

low), it seems unlikely that females based their carrion consumption and weight change according to male status.

The final main result of my study was that males paired with inbred females gained more weight over the breeding attempt than males paired with outbred females. I predicted that males paired with inbred females would gain less weight over the breeding attempt. The reason for this is that males paired with inbred females should be expected to increase their allocation to current reproduction to compensate for the detrimental effects of maternal inbreeding. Thus, my finding suggests that males instead might increase their investment into future reproduction when their partner is inbred. However, this seems unlikely given that, a previous study on *N. vespilloides* found that males paired with inbred females provided more care than males paired with outbred females (Mattey and Smiseth 2014). An alternative explanation is that males paired with an inbred female gained more weight over the breeding attempt because they provided more care than males paired with an outbred female. In this species, parents feed from the carcass whilst breeding and males might gain better access to the carcass if they provide more care (Pilakouta et al. 2016). If so, we might expect a positive correlation between male food provisioning and male weight gain in this species. Altogether my findings suggest that males benefitted in terms of gaining more weight during the breeding attempt when assisting an inbred partner. Given that male weight gain serves as a proxy for investment in future reproduction (Creighton et al. 2009), one avenue for future work is to compare the subsequent reproductive performance of males paired with an outbred or inbred female during a previous breeding attempt.

These results have broader implications for understanding how social interactions shape the severity of inbreeding depression. There is increasing evidence that social interactions can alter the severity of inbreeding depression, with stressful interactions aggravating the severity of inbreeding depression (e.g. Meagher et al. 2000) and benign interactions buffering against inbreeding depression (e.g.

Pilakouta et al. 2015a). It is well documented that maternal care enhances larval survival and growth in burying beetle (e.g. Eggert et al. 1998, Trumbo 2007, Arce et al. 2012). Thus, maternal care may buffer against inbreeding depression in offspring by reducing environmental stresses to offspring, such as the risk of death due to starvation, infanticide by conspecific intruders and predation. In contrast, as discussed above, there is mixed evidence as to whether male assistance in care enhances offspring fitness (Pooley et al. 2014, this study). Thus, my results suggest that parental care or other benign social interactions will not always reduce the severity of inbreeding depression. There is now a need for further work on the buffering effects of male assistance in parental care against the detrimental effects of maternal inbreeding on offspring fitness in systems where males contribute more towards care. For example, such experiments could be conducted on bird species where males and females contribute more equally towards parental care (Clutton-Brock 1991).

9   General Discussion

I first presented evidence that behavioural plasticity plays an important role in parental and offspring responses to environmental variation in the burying beetle *Nicrophorus vespilloides* (Chapters 2–6). In Chapter 2, I showed that females increase their time spent caring for the brood in response to increasing energetic costs. This suggests that females do not only adjust their level of care to the direct energetic costs, but also to their perceived chance to survive and reproduce again. In Chapter 3, I furthered showed that females respond independently to variation in the energetic costs and in brood size. In Chapter 4, I found that infected females maintain their level of care and reproductive output, which suggests that females facing infection prioritise their current reproductive success over survival and future reproduction. I then showed in Chapter 5 that males provide care for longer with increasing resource availability, whereas females tend to always provide care for the same duration irrespective of resource availability. This finding supports the idea that initial differences in parental care between males and females shape plastic responses to variation in resource availability, and that overall more abundant resources favour more parental cooperation over care. I next focused on offspring behaviour and tested whether larvae preferentially beg towards larger females (Chapter 6). I found that larvae beg more towards larger females over smaller ones as a result of spending more time in close contact with larger females. This suggests that larvae might seek to maximise their returns on begging by associating with the parent that is susceptible to provide more care.

I next investigated the consequences of inbreeding on behavioural plasticity in parent-offspring interactions (Chapter 7) and whether male parental care can buffer against indirect effects of maternal inbreeding on offspring (Chapter 8). In Chapter 7, I showed that inbreeding increases plasticity in offspring association with a parent, while inbreeding had no effect on behavioural plasticity in parental care or offspring begging. This provides some evidence that inbreeding

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can cause too much plasticity, which could have negative fitness consequences on canalised behaviours. In Chapter 8, I first showed that maternal inbreeding has detrimental effects on offspring fitness, reducing larval survival. I then showed that these negative effects of maternal inbreeding on offspring remain regardless of male presence or inbreeding status. Overall, these findings indicate that the consequences of inbreeding are not limited to inbred individuals, but can be more widespread in a population and affect outbred individuals via parent-offspring interactions. They also highlight the importance of considering the type of social interactions (i.e. mother-offspring versus father-offspring) in mediating indirect effects of inbreeding between interacting individuals.

9.1   Behavioural plasticity and parent-offspring interactions

Behavioural plasticity allows quick and reversible responses to environmental variation (Gabriel et al. 2005, Mery and Burns 2010). As such, behavioural plasticity is a widespread mechanism that allows balancing the costs and benefits of behaviours. In the case of parent-offspring interactions, parents are expected to adjust their behaviour to obtain the highest benefits from caring at the lowest costs, while offspring are expected to adjust their behaviour to obtain the highest amount of care from associating with and begging towards parents at the lowest costs. Behavioural adjustments during parent-offspring interactions should thus reflect that individuals are trying to maximise their returns from the interaction. Behavioural plasticity is particularly important in this context because the costs and benefits of parental and offspring behaviour are likely to vary with changes in the environment. For example, a given amount of food provisioned by parents might be more beneficial to offspring in poor nutritional state compared with well-fed offspring. The benefits of begging to the offspring would then increase with diminishing nutritional state. Similarly, a given amount of care might be more costly to parents that incur higher risk of predation or infection when caring than parents in a safer environment. The costs of care to parents would then increase with increasing environmental threat.

In this thesis, I first explored how parents and offspring adjust plastically their behaviour to maximize their returns from parent-offspring interactions (Chapters 2–6). When parents perceive their chance to survive and reproduce again to be very low, they often shift their reproductive investment towards current reproduction as a terminal investment response, and provide more care towards the current brood. In Chapters 2 and 3, I presented evidence that handicapping, by increasing the energetic costs, can trigger such terminal investment response in burying beetles. Shifting reproductive investment and increasing care towards the current brood should be advantageous when the prospects for future reproduction are low. This is because, in such situation, the current brood is likely to be the last (or only) progenies parents will successfully produce and hence parents expect more benefits by producing more and/or larger offspring without additional costs. In Chapter 3, I also provided additional evidence showing that females spend more time providing care towards larger broods. Taken together, the two studies demonstrate that parents adjust their level of care to variation in the costs and benefits of care, which occurs for instance with changes in the energetic costs or brood size.

Environmental conditions, such as pathogen exposure and resource availability, are likely to alter the costs and/or benefits associated with parental and offspring behaviour. Yet in Chapter 4 I found that infected females maintain their level of care and reproductive output, despite incurring high mortality. This result shows that understanding how an environmental variable should in theory alter the costs and benefits of care is not enough to predict how parents will adjust care. In fact, it strongly suggests that parents also base their decision to provide care on other factors, such as the probability of survival and future reproduction (see discussion below). In Chapter 5, I show that the degree of behavioural plasticity in the duration of parental care in response to variation in resource availability differs between sexes. This suggests that behavioural plasticity provides a mechanism to resolve sexual conflict over care when environmental variation affects the costs

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and benefits of care to each sex differently. Behavioural plasticity might thus be a key mechanism that allows balancing biparental cooperation and conflict over care. Behavioural plasticity might also allow transitions between different forms of care, such as between uniparental and biparental care (e.g. Ringler et al. 2015). Furthermore, in Chapter 6 I showed that behavioural plasticity provides a mechanism enabling larvae to beg towards larger females. When larvae are in presence of multiple parents, such as in biparental or communal breeding situations, behavioural plasticity would allow larvae to target the parents that are more likely to provision food and obtain higher returns from a given time or effort spent on begging. Behavioural plasticity should thus be a key mechanism that allows balancing the costs and benefits of offspring behaviour.

9.2   Implications for parental and offspring behaviour

The findings of Chapters 2–6 have important implications for our understanding of how parents and offspring base their flexible decisions regarding how much care or begging they engage in when interacting with one another. The first implication is that parents do not only respond to changes in the costs/benefits of care, but also to other key components that also depend on parental investment in care: the probability of survival and the expected success at future reproduction. Indeed, parental care is one aspect of a parent's reproductive investment. In turn, current reproduction is one component among multiple functions in which individuals allocate limited resources. In addition to current reproduction and parental care, parents are expected to allocate resources to somatic maintenance, immunity, and future reproduction. Thus, when there is a change in the environment, parents should balance their allocation to parental care as well as other functions to maximise their overall fitness. Parental decision might ultimately not maximise the cost/benefit of parental care, but would rather reflect the best strategy when considering balancing allocation across different functions (Figure 9.1). This is the case for example in the studies presented in Chapters 2 and 3, where I initially predicted females to reduce care in response to higher energetic

costs, whereas I found the opposite results. This finding would have appeared surprising if only considering the costs and benefits of providing care. It is, however, obvious that altering energetic costs can affect how parents perceive their future reproductive success and survival, and that parents should also base their parental care decision on these aspects.

The fact that parental care reflects a strategy to balance multiple aspects of an individual's resource allocation implies that different individuals are likely to show contrasting parental care responses. In other words, although environmental conditions influence the costs and benefits of care, and thus the optimal level of care, genetic and life-history differences across individuals should influence the realised level of care (Figure 9.1). Hence, initial differences in intrinsic conditions (e.g. age, sex, permanent environment) and micro-environmental conditions (e.g. nest of development, individual niche), combined with differences in how individuals balance their resource allocations between functions, should contribute to individual differences in parental behaviour plasticity. I suggest that these complex responses, involving a decision based on multiple functions to which individuals allocate resources, are a major cause of individual differences in parental care (e.g. Nakagawa et al. 2007, Westneat et al. 2011). More generally, we should expect individual differences in behavioural plasticity to be an important, yet understudied, source of phenotypic variation and raw material for natural selection to act upon (Dingemanse et al. 2010). This is because the process of natural selection relies on (genetically determined) individual differences in the phenotype and that these differences, rather than being between average trait values, would also exist in trait responsiveness to environmental change.

The second implication is that maintaining parental care, regardless of environmental variation, might sometimes be essential for offspring survival and growth. This ought to be the case when, for example, parental care is required for the survival of the brood and when variation in the environment would only have minor effects on offspring growth and survival. In such a situation, parents are better off
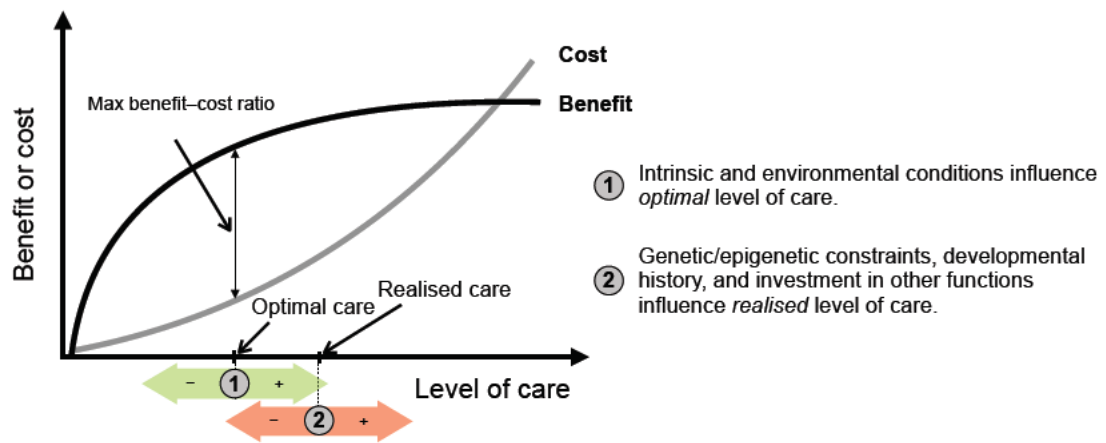
Figure 9.1: Hypothetical cost and benefit functions associated with parental care, from the perspective of parents. The benefit function of care is expected to increase and reach a plateau at high levels of care, as offspring cannot receive more care. In contrast, the cost function is expected to increase linearly or at an accelerating rate. The optimal level of care corresponds to the level of care that maximises the difference between the benefits and costs of care (1). The optimal level of care might change with conditions because the benefits and costs depend on intrinsic and environmental variables. In addition, genetic, epigenetic and environmental constraints, as well as trade-offs with other functions will influence the realised level of care (2).

maintaining parental care to successfully raise a brood, rather than reducing care and taking the risk of fully failing to produce a brood. This is suggested by the findings in Chapter 4 showing that females always maintain their level of care to the brood despite being infected, immune-challenged or injured. I am not aware of any study specifically focusing on how the dependence of offspring survival on parental care influences parents in their decision to maintain or reduce care in response to detrimental environmental conditions. However, a useful parallel can be drawn between the conditions favouring parental care responses and conditions favouring the evolution of parental care. For instance, theoretical studies show that parental care is generally most likely to be favoured when offspring survival in the absence of care is low and adult survival is high (Klug and Bonsall 2010, 2014). In situations where both parents provide care and when the probability of offspring survival in the absence of care is low, we should expect offspring to always receive care from at least one parent (McNamara et al. 2000). Yet, as discussed in Chapter 4, a caring parent receiving help from a partner might be

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more likely to reduce its own care if its condition deteriorates. This is because the other parent would still provide a minimum level of care or even compensate for a reduction in care by its partner.

A third important implication is that parental and offspring responses and interactions can have a more widespread impact in a population by spreading potential effects of a focal individual's conditions on social partners. In Chapter 8, I show that maternal inbreeding has detrimental effects on offspring survival. In burying beetles, it is well established that inbreeding has strong negative effects on hatching success, larval and adult survival (Mattey et al. 2013, Pilakouta et al. 2015a). There is now evidence that the inbreeding status of a female can impact indirectly on her offspring via parental care, regardless of the offspring inbreeding status (Mattey et al. 2013, Mattey et al. 2018, Ratz et al. 2018). Furthermore, although maternal care can buffer against inbreeding depression on offspring, male parental care does not seem to buffer against the negative effects of maternal inbreeding on offspring survival (Ratz et al. 2018, Chapter 8). Altogether, these studies suggest that inbreeding can affect outbred individuals via parental care and parent-offspring interactions. This idea is fundamental to our understanding of the role of parent-offspring interactions at a broader scale as it suggests that the effects of inbreeding are not confined to inbred individuals, but can be spread out in the population via social interactions. The effects of inbreeding might thus often be greater than expected. Moreover, the role of social interactions as a mechanism to spread an individual's condition to social partners might not be limited to inbreeding and we should expect similar patterns occurring with other attributes that can affect how animals interact with each other, such as age, nutritional state and infection status. This issue has received little attention and would deserve more detailed scrutiny, as it would greatly contribute to our understanding of the consequences of social interactions at the population level. Social interactions can have an impact on evolutionary responses as they might accelerate, slow down or reverse the response to selection (Moore et al. 1997, Wolf et al. 1998, Bijma and Wade 2008, McGlothlin et al. 2010). For example, McAdam

and Boutin (2004) found that maternal genetic effects, presumably resulting from maternal care, increase the evolutionary response to current selection on offspring growth rate in red squirrels. As a result of mother-offspring interactions, offspring growth rate is expected to change more rapidly across generations than expected when only considering selection operating on offspring. Parent-offspring conflict over resource allocation to the offspring can limit the evolution of offspring body size in other systems, such as in fruit flies (Rollinson and Rowe 2015) and blue tits (Thomson et al. 2017). Parent-offspring interactions, and more generally social interactions, can thus have major impacts on the ecological and evolutionary processes via their expected consequences on population dynamics and evolutionary responses to selection.

9.3 Implication for the study of behavioural plasticity

The findings presented in Chapter 5 and 7 have important implications for our understanding of factors promoting and limiting behavioural plasticity. In Chapter 5, I showed that males adjust the duration of their care to resource availability, providing care for longer on larger carcasses, whereas females always provide care for a similar duration irrespective of carcass size. Given that on average females provide care for longer than males (e.g. Smiseth et al. 2005), this finding suggests that initial sex differences in parental care influence plasticity in parental care. In general, the average degree to which organisms express a behaviour might, in part, determine the degree of plasticity in the behaviour. Thus, sex differences in behavioural plasticity might be linked to sexual dimorphism in parental care (i.e. females providing on average more care than males), as it is the case for morphological sexual dimorphism in developmental plasticity (Stillwell et al. 2010). Overall, these findings provide further support to the idea that behavioural plasticity is an important mechanism for the resolution of sexual conflict by allowing organisms to adjust their behaviour to match the variable social and ecological context (McLeod and Day 2017).

In Chapter 7, I showed that inbreeding increases behavioural plasticity in the time larvae spend associating with a parent in response to food deprivation. Although in this study I did not assess the potential fitness consequences of the greater plastic response, this result suggests that negative effects of inbreeding could arise from too much behavioural plasticity if canalisation in the behaviour is adaptive. This finding is particularly intriguing in the context of behavioural plasticity because it indicates that, whilst behavioural traits mediating parental and offspring behaviour are highly plastic, these traits might well be canalised to some degree. Therefore, over-responding to a change in the environment (i.e. showing too much plasticity) is likely to be a bad strategy. Limited plasticity in a trait, i.e. phenotypic canalisation, is thought to be a fundamental aspect of most developmental traits (reviewed in Flatt 2005). There is currently little knowledge about the role of canalisation in behavioural traits, the extent to which canalisation limits behavioural responses and how overly plastic behaviour might impact fitness. Yet this is an interesting perspective given the prominence of plasticity in behavioural traits (which are in essence flexible) and the importance of behaviours to fitness via their role in key life history processes such as foraging, mating, or parenting.

Some authors have proposed that phenotypic plasticity works as a mechanism allowing the emergence of new adaptations, under the "plasticity first" hypothesis (Levis and Pfennig 2016). This hypothesis states that phenotypic plasticity allows organisms to adjust their phenotype to new environments, whereby facilitating genetic accommodation and adaptation to new environmental conditions. I would argue that behavioural plasticity could also be a key mechanism allowing the emergence and evolution of complex behavioural traits, such as parent-offspring interactions (e.g. Stein and Bell 2019). It is tempting to speculate, for instance, that plasticity in feeding behaviour represents a first step in the evolution of both parental provisioning and offspring begging. This is because parental food regurgitation and offspring begging signals might involve pathways close to the feeding pathways (Fischer and O'Connell 2017). Parental feeding

and offspring begging behaviours could possibly derive from pathways that are involved in feeding behaviour. Nevertheless, this remains speculative and I would encourage future studies in the field to explore the role of behavioural plasticity as a potential driver of behavioural interactions.

9.4   Concluding remarks

Here, I present evidence that behavioural plasticity is a fundamental determinant of parent-offspring social interactions in *N. vespilloides*. Adults and larvae adjust their behaviour to variation in the social and ecological environment, such as offspring number, partner's presence, parental body size, resource availability, and own or social partner's inbreeding status. The different studies constituting this thesis highlight the fact that these behavioural adjustments are not made in isolation from other key aspects of an individual's life histories, but should rather reflect a general strategy balancing investment towards reproduction, growth and survival. The evidence reported in this thesis contributes to our understanding of the role of behavioural plasticity in parental and offspring behaviour by showing that behavioural plasticity is a mechanism that allows balancing the costs and benefits of a behaviour, and adjusting investment in current reproduction versus survival and future reproduction. The findings of this thesis also suggest that an individual behaviour can have a widespread impact in a population via parent-offspring interactions. Moreover, my findings suggest that initial differences and constraints on behaviours might be a source of variation in behavioural plasticity across individuals. Finally, these findings highlight the fact that behavioural responses, even if they are adaptive, might have negative consequences if they are overexpressed and that plastic behaviours should be canalised to some degree. In general, behavioural plasticity might be a mechanism allowing transitions between different forms of care and a source of behavioural diversity within species and across individuals, and a potential driver of evolution.

References

Adamo SA, Gomez-Juliano A, LeDue EE, Little SN, Sullivan K (2015) Effect of immune challenge on aggressive behaviour: how to fight two battles at once. Animal Behaviour 105:153–161.

Adelman JS, Martin LB (2009) Vertebrate sickness behaviors: adaptive and integrated neuroendocrine immune responses. Integrative and Comparative Biology 49:202–214.

Alonso-alvarez C, Velando A (2012) Benefits and costs of parental care. In: The Evolution of Parental Care (N.J. Royle et al., eds), pp. 40–61. Oxford University Press, Oxford.

Andrews CP, Kruuk LE, Smiseth PT (2017) Evolution of elaborate parental care: phenotypic and genetic correlations between parent and offspring traits. Behavioral Ecology 28:39–48.

Andrews CP, Smiseth PT (2013) Differentiating among alternative models for the resolution of parent-offspring conflict. Behavioral Ecology 24:1185–1191.

Arce AN, Johnston PR, Smiseth PT, Rozen DE (2012) Mechanisms and fitness effects of antibacterial defences in a carrion beetle. Journal of Evolutionary Biology 25:930–937.

Armbruster P, Reed DH (2005) Inbreeding depression in benign and stressful environments. Heredity 95:235–242.

Arundell KL, Wedell N, Dunn AM (2014) The impact of predation risk and of parasitic infection on parental care in brooding crustaceans. Animal Behaviour 96:97–105.

Aubert A, Goodall G, Dantzer R, Gheusi G (1997) Differential effects of lipopolysaccharide on pup retrieving and nest building in lactating mice. Brain Behavior and Immunity 11:107–118.

Auld JR, Agrawal AA, Relyea RA (2010) Re-evaluating the costs and limits of adaptive phenotypic plasticity. Proceedings of the Royal Society of London B: Biological Sciences 277:503–511.

Auld JR, Henkel JF (2014) Diet alters delayed selfing, inbreeding depression, and reproductive senescence in a freshwater snail. Ecology and Evolution 4:2968–2977.

Auld JR, Relyea RA (2010) Inbreeding depression in adaptive plasticity under predation in a freshwater snail. Biology Letters 6:222–224.

Balshine S (2012) Patterns of parental care in vertebrates. In: The Evolution of Parental Care (N.J. Royle et al., eds), pp. 62–80. Oxford University Press, Oxford.

Bartlett, J., 1987. Filial cannibalism in burying beetles. Behavioral Ecology and Sociobiology 21:179–183.

Bartlett J (1988) Male mating success and paternal care in *Nicrophorus vespilloides* (Coleoptera: Silphidae). Behavioral Ecology and Sociobiology 23:297–303.

Bartlett J, Ashworth CM (1988) Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera: Silphidae). Behavioral Ecology and Sociobiology 22:429–434.

Bates D, Mächler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using *lme4*. Journal of Statistical Software 67:1–48.

Bell MB (2008) Strategic adjustment of begging effort by banded mongoose pups. Proceedings of the Royal Society of London B: Biological Sciences 275:1313–1319.

Benard MF (2004) Predator-induced phenotypic plasticity in organisms with complex life histories. Annual Review of Ecology, Evolution, and Systematics 35:651–673.

Bérénos C, Ellis PA, Pilkington JG, Pemberton JM (2016) Genomic analysis reveals depression due to both individual and maternal inbreeding in a free-living mammal population. Molecular Ecology 25:3152–3168.

Bijlsma R, Bundgaard J, van Putten WF (1999) Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. Journal of Evolutionary Biology 12:1125–1137.

Bijlsma R, Loeschcke V (2012) Genetic erosion impedes adaptive responses to stressful environments. Evolutionary Applications 5:117–129.

Bijma P, Wade MJ (2008) The joint effects of kin, multilevel selection and indirect genetic effects on response to genetic selection. Journal of evolutionary biology 21:1175–1188.

Billman EJ, Creighton JC, Belk MC (2014) Prior experience affects allocation to current reproduction in a burying beetle. Behavioral Ecology 25:813–818.

Boncoraglio G, Kilner RM (2012) Female burying beetles benefit from male desertion: sexual conflict and counter-adaptation over parental investment. PLoS One 7:e31713.

Bonneaud C, Mazuc J, Gonzalez G, Haussy C, Chastel O, Faivre B, Sorci G (2003) Assessing the cost of mounting an immune response. American Naturalist 161:367–379.

Bos N, Lefèvre T, Jensen AB, d'Ettorre P (2012) Sick ants become unsociable. Journal of evolutionary biology 25:342–351.

Bosch J, Vicens N (2006) Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. Behavioral Ecology and Sociobiology 60:26–33.

Bradley CA, Altizer S (2005). Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts. Ecology Letters 8:290–300.

Broderick NA, Welchman DP, Lemaitre B (2009) Recognition and response to microbial infection in *Drosophila*, pp. 13–33. Oxford University Press, New York.

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker B (2017) Modeling zero-inflated count data with *glmmTMB*. bioRxiv 132753.

Budden AE, Wright J (2001) Begging in nestling birds. In: Current ornithology (pp. 83–118). Springer, Boston, MA.

Burdick C, Siefferman LM (2020) Interspecific density influences the adaptive significance of provisioning coordination between breeding partners. Frontiers in Ecology and Evolution 8:29.

Cameron PG, Semlitsch RD, Bernasconi MV (1993) Effects of body size and parasite infection on the locomotory performance of juvenile toads, *Bufo bufo*. Oikos 66:129–136.

Candolin U, Wong BBM (eds) 2012. Behavioural responses to a changing world: mechanisms and consequences. Oxford University Press, Oxford.

Capodeanu-Nägler A, Keppner EM, Vogel H, Ayasse M, Eggert AK, Sakaluk SK, Steiger S (2016) From facultative to obligatory parental care: interspecific variation in offspring dependency on post-hatching care in burying beetles. Scientific Reports 6:29323.

Chakarov N, Linke B, Boerner M, Goesmann A, Krüger O, Hoffman JI (2015) Apparent vector-mediated parent-to-offspring transmission in an avian malaria-like parasite. Molecular Ecology 24:1355–1363.

Chappell M, Bachman G (2002) Energetic costs of begging behaviour. In: The evolution of begging: competition, cooperation and communication (J Wright, ML Leonard, eds), pp. 62–80. Dordrecht, Kluwer Academic Publishers.

Charlesworth D (2003) Effects of inbreeding on the genetic diversity of populations. Philosophical Transactions of the Royal Society London 358:1051–1070.

Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. Annual Review of Ecology, Evolution, and Systematics 18:237–268.

Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. Annual Review of Ecology, Evolution, and Systematics 10:783–796.

Charmantier A, McCleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC (2008) Adaptive phenotypic plasticity in response to climate change in a wild bird population. Science 320:800–803.

Chase ID (1980) Cooperative and noncooperative behavior in animals. American Naturalist 115:827–857.

Cheptou PO, Donohue K (2011) Environment-dependent inbreeding depression: its ecological and evolutionary significance. New Phytologist 189:395–407.

Clutton-Brock TH (1991) The Evolution of Parental Care. Princeton University Press, Princeton.

Coppens CM, De Boer SF, Koolhaas JM (2010) Coping styles and behavioural flexibility: towards underlying mechanisms. Philosophical Transactions of the Royal Society B 365:4021–4028.

Cotter SC, Kilner R (2010a) Personal immunity versus social immunity. Behavioral Ecology 21:663–668.

Cotter SC, Kilner R (2010b) Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*. Journal of Animal Ecology 79:35–43.

Cotter SC, Topham E, Price AJP, Kilner RM (2010) Fitness costs associated with mounting a social immune response. Ecology Letters 13:1114–1123.

Cotter SC, Ward RJ, Kilner RM (2011) Age-specific reproductive investment in female burying beetles: independent effects of state and risk of death. Functional Ecology 25:652–660.

Clutton-Brock TH (1984) Reproductive effort and terminal investment in iteroparous animals. American Naturalist 123:212–219.

Clutton-Brock TH (1991) The evolution of parental care. Princeton University Press, Princeton.

Cockburn A (2006) Prevalence of different modes of parental care in birds. Proceedings of the Royal Society of London B 273:1375–1383.

Crawley MJ (2005) Statistics: An introduction using R. Wiley, Chichester.

Creighton CJ, Heflin ND, Belk MC (2009) Cost of reproduction, resource quality, and terminal investment in a burying beetle. American Naturalist 174:673–684.

Creighton CJ, Smith AN, Komendat A, Belk MC (2015) Dynamics of biparental care in a burying beetle: experimental handicapping results in partner compensation. Behavioral Ecology Sociobiology 69:265–271.

Crook JH (1963) Monogamy, polygamy, and food supply. Discovery 24:35–41

Curtis VA (2014) Infection-avoidance behaviour in humans and other animals. Trends in Immunology 35:457–464.

Davenport B (1908) Degeneration, albinism and inbreeding. Science 28:454–455.

DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. Trends Ecology and Evolution 13:77–81

Dickens M, Berridge D, Hartley IR (2008) Biparental care and offspring begging strategies: Hungry nestling blue tits move towards the father. Animal Behaviour 75:167–174.

Dingemanse NJ, Kazem AJ, Réale D, Wright J (2010) Behavioural reaction norms: animal personality meets individual plasticity. Trends in Ecology and Evolution, 25:81–89.

Dingemanse NJ, Wolf M (2013) Between-individual differences in behavioural plasticity within populations: causes and consequences. Animal Behaviour 85:1031–1039.

Dougherty LR, Shuker DM (2014) The effect of experimental design on the measurement of mate choice: A meta-analysis. Behavioral Ecology 26:311–319.

Duffield KR, Bowers EK, Sakaluk SK, Sadd BM (2017) A dynamic threshold model for terminal investment. Behavioral Ecology and Sociobiology 71:185.

East EM (1908) Inbreeding in corn. In: Report of the Connecticut Agricultural Experiment Station for the Year 1907, pp. 419–428.

Eggert A-K, Müller JK (1992) Joint breeding in female burying beetles. Behavioral Ecology and Sociobiology 31:237–242.

Eggert A-K, Müller JK (2000) Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*). Behav-

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ioral Ecology 11:357–366.

Eggert A-K, Reinking M, Müller JK (1998) Parental care improves offspring survival and growth in burying beetles. Animal Behaviour 55:97–107.

El Sanousi SM, El Sarag MSA, Mohamed SE (1987) Properties of *Serratia marcescens* isolated from diseased honeybee (*Apis mellifera*) larvae. Microbiology 133:215–219.

Engel KC, Stökl J, Schweizer R, Vogel H, Ayasse M, Ruther J, Steiger S (2016) A hormone-related female anti-aphrodisiac signals temporary infertility and causes sexual abstinence to synchronize parental care. Nature Communications 7:11035.

Escobar JS, Auld JR, Correa AC, Alonso JM, Bony YK, Coutellec MA, Koene JM, Pointier JP, Jarne P, David P (2011) Patterns of mating-system evolution in hermaphroditic animals: Correlations among selfing rate, inbreeding depression, and the timing of reproduction. Evolution: International Journal of Organic Evolution 65:1233–1253.

Expósito-Granados M, Parejo D, Avilés JM (2016) Sex-specific parental care in response to predation risk in the European roller, *Coracias garrulus*. Ethology 122:72–79.

Exton MS (1997) Infection-induced anorexia: active host defence strategy. Appetite 29:369–383.

Falconer DS, Mackay TFC (1996) An introduction to quantitative genetics. Longman, London.

Falk J, Wong JWY, Kölliker M, Meunier J (2014) Sibling cooperation in earwig families provides insights into the early evolution of social life. American Naturalist 183:547–557.

Farchmin PA, Eggert A-K, Duffield KR, Sakaluk SK (2020) Dynamic terminal investment in male burying beetles. Animal Behaviour 163:1–7.

Fischer EK, O'Connell LA (2017) Modification of feeding circuits in the evolution of social behavior. Journal of Experimental Biology 220:92–102.

Flatt T (2005) The evolutionary genetics of canalization. The Quarterly review of biology 80:287–316.

Forbes S (2007) Sibling symbiosis in nestling birds. Auk 124:1–10.

Ford LE, Henderson KJ, Smiseth PT (2018) Differential effects of offspring and maternal inbreeding on egg laying and offspring performance in the burying beetle *Nicrophorus vespilloides*. Journal Evolutionary Biology 31:1047–1057.

Ford LE, Smiseth PT (2016) Asynchronous hatching provides females with a means for increasing male care but incurs a cost by reducing offspring fitness. Journal of Evolutionary Biology 29:428–437.

Ford LE, Smiseth PT (2017) Asynchronous hatching in a nonavian species: a test of the hurry-up hypothesis. Behavioral Ecology 28:899–907.

Foster SA (2013) Evolution of behavioural phenotypes: influences of ancestry and expression. Animal Behaviour 85:1061–1075.

Fowler K, Whitlock MC (1999) The distribution of phenotypic variance with inbreeding. Evolution 53:1143–1156.

Fox CW, Reed DH (2011) Inbreeding depression increases with environmental stress: And experimental study and metaâanalysis. Evolution 65:246–258.

Fox CW, Scheibly KL (2006) Variation in inbreeding depression among populations of seed beetle, *Stator limbatus*. Entomologia experimentalis et applicata 121:137–144.

Fox CW, Stillwell RC, Wallin WG, Curtis CL, Reed DH (2011) Inbreeding-environment interactions for fitness: complex relationships between inbreeding depression and temperature stress in a seed-feeding beetle. Evolutionary Ecology 25:25–43.

Fox J, Weisberg S (2017) *car*: An R companion to applied regression. R package version 2.1-6.

Frazier MR, Harrison JF, Kirkton SD, Roberts SP (2008) Cold rearing improves cold-flight performance in *Drosophila* via changes in wing morphology. Journal of Experimental Biology 211:2116–2122.

Gabriel W, Luttbeg B, Sih A, Tollrian R (2005) Environmental tolerance, heterogeneity, and the evolution of reversible plastic responses. American Naturalist 166:339–353.

Georgiou Shippi AG, Paquet M, Smiseth PT (2018) Sex differences in parental defence against conspecific intruders in the burying beetle *Nicrophorus vespilloides*. Animal Behaviour 136:21–29.

Godfray HCJ (1991) Signalling of need by offspring to their parents. Nature 352:328.

Godfray CHJ (1995) Signaling of need between parents and offspring: Parent-offspring conflict and sibling rivalry. American Naturalist 146:1–24.

Gray F, Richardson J, Ratz T, Smiseth PT (2018) No evidence for parent-offspring competition in the burying beetle *Nicrophorus vespilloides*. Behavioral Ecology 29:1142–1149.

Grew R, Ratz T, Richardson J, Smiseth PT (2019) Parental care buffers against effects of ambient temperature on offspring performance in an insect. Behavioral Ecology 30:1443–1450.

Grodzinski U, Johnstone RA (2012) Parents and offspring in an evolutionary game: the effect of supply on demand when costs of care vary. Proceedings of the Royal Society of London B 279:109–115.

Guo L, Karpac J, Tran SL, Jasper H (2014) PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell 156:109–122.

Haag CR, Hottinger JW, Riek M, Ebert D (2002) Strong inbreeding depression in a *Daphnia* metapopulation. Evolution 56:518–526.

Haag CR, Sakwinska O, Ebert D (2003) Test of synergistic interaction between infection and inbreeding in *Daphnia magna*. Evolution 57:777–783.

Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the *MCMCglmm* R package. Journal of Statistical Software 33:1–22.

Haldane JBS (1927) A mathematical theory of natural and artificial selection, part V: selection and mutation. Proceedings of the Cambridge Philosophical Society 28:838–844.

Hamilton JA, Miller JM (2016) Adaptive introgression as a resource for management and genetic conservation in a changing climate. Conservation Biology 30:33–41.

Harrison XA (2015) A comparison of observation-level random effect and beta-binomial models for modelling overdispersion in binomial data in ecology and evolution. PeerJ 3:e1114.

Harrison F, Barta Z, Cuthill I, Székely T (2009) How is sexual conflict over parental care resolved? A meta-analysis. Journal of Evolutionary Biology 22:1800–1812.

Hart B (1988) Biological basis of the behavior of sick animals. Neuroscience and Biobehavioral Reviews 12:123–137.

Hartig F (2017) *DHARMa*: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.1.

Haskell DG (1994) Experimental evidence that nestling begging behavior incurs a cost due to nest predation. Proceedings of the Royal Society of London B 257:161–164

Haskell D (1999) The effect of predation on begging-call evolution in nestling Wood warblers. Animal Behaviour 57:893–901.

Hegner RE, Wingfield JC (1987a) Effects of brood-size manipulations on parental investment, breeding success, and reproductive endocrinology of house sparrows. Auk 104:470–480.

Hegner RE, Wingfield JC (1987b) Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. Auk 104:462–469.

Heinze J, Walter B (2010) Moribund ants leave their nests to die in social isolation. Current Biology 20:249–252.

Hejazi A, Falkiner FR (1997) *Serratia marcescens*. Journal of Medical Microbiology 46:903–912.

Hinde CA (2006) Negotiation over offspring care?–a positive response to partner-provisioning rate in great tits. Behavioral Ecology, 17:6–12.

Hinde CA, Kilner RM (2007) Negotiations within the family over the supply of parental care. Proceedings of the Royal Society B: biological sciences 274:53–60.

Hinde CA, Johnstone RA, Kilner RM (2010) Parent-offspring conflict and coadaptation. Science 327:1373–1376.

Hirshfield MF, Tinkle DW (1975) Natural selection and the evolution of reproductive effort. Proceedings of the National Academy of Sciences 72:2227–2231.

Hoback WW, Bishop AA, Kroemer J, Scalzitti J, Shaffer JJ (2004) Differences among antimicrobial properties of carrion beetle secretions reflect phylogeny and ecology. Journal of Chemical Ecology 30:719–729.

Hoffmann AA, Parsons PA (1991). Evolutionary genetics and environmental stress. Oxford University Press, Oxford.

Hopwood PE, Moore AJ, Tregenza T, Royle NJ (2015) Male burying beetles extend, not reduce, parental care duration when reproductive competition is high. Journal of Evolutionary Biology 28:1394–1402.

Houston AI, Székely T, McNamara JM (2005) Conflict between parents over care. Trends in Ecology and Evolution 20:33–38.

Huang WS, Lin SM, Dubey S, Pike DA (2013) Predation drives interpopulation differences in parental care expression. Journal of Animal Ecology 82:429–437.

Hughes DP, Brodeur J, Thomas F (2012) Host manipulation by parasites. Oxford University Press, Oxford.

Huisman J, Kruuk LEB, Ellis PA, Clutton-Brock TH, Pemberton JM (2016) Inbreeding depression across the lifespan in a wild mammal population. Proceedings of the National Academy of Sciences 113:3585–3590.

Humphrey NK (1976) The social function of intellect. In: Growing Points in Ethology (P.P.G. Bateson R.A. Hinde, eds), pp. 303–317. Cambridge University Press, Cambridge.

Imler JL, Bulet P (2005) Antimicrobial peptides in *Drosophila*: structures, activities and gene regulation. In Mechanisms of epithelial defense, pp. 1–21. Karger Publishers.

Ingleby FC, Hunt J, Hosken DJ (2010) The role of genotype-by-environment interactions in sexual selection. Journal of Evolutionary Biology, 23, 2031–2045.

Jacobs CG, Steiger S, Heckel DG, Wielsch N, Vilcinskas A, Vogel H (2016) Sex, offspring and carcass determine antimicrobial peptide expression in the burying beetle. Scientific reports 6:1–8.

Jacobs CG, Wang Y, Vogel H, Vilcinskas A, van Der Zee M., Rozen DE (2014) Egg survival is reduced by grave-soil microbes in the carrion beetle, *Nicrophorus vespilloides*. BMC Evolutionary Biology, 14:208.

Jarrett BJ, Evans E, Haynes HB, Leaf MR, Rebar D, Duarte A, Schrader M, Kilner RM (2018) A sustained change in the supply of parental care causes adaptive evolution of offspring morphology. Nature Communications 9:1–8.

Jennions MD, Petrie M (1997) Variation in mate choice and mating preferences: a review of causes and consequences. Biological Reviews 72:283–327.

Johnson RW (2002) The concept of sickness behavior: a brief chronological account of four key discoveries. Veterinary Immunology and Immunopathology 87:443–450.

Johnstone RA (2004) Begging and sibling competition: how should offspring respond to their rivals? American Naturalist 163:388–406.

Johnstone RA, Hinde CA (2006) Negotiation over offspring care–how should parents respond to each other's effort? Behavioral Ecology 17:818–827.

Johnstone RA, Savage JL (2019) Conditional cooperation and turn-taking in parental care. Frontiers in Ecology and Evolution 7:335.

Kacelnik A, Cotton PA, Stirling L, Wright J (1995) Food allocation among nestling starlings–sibling competition and the scope of parental choice. Proceedings of the Royal Society of London B: Biological Sciences 259:259–

263.

Keller LF (1998) Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). Evolution 52:240–250.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends in Ecology and Evolution 17:230–241.

Kelley K, Bluthe R, Dantzer R, Zhou J, Shen W, Johnson R, Broussard S (2003) Cytokine-induced sickness behavior. Brain, Behavior, and Immunolgy, 17:S112–S118.

Keppner EM, Ayasse M, Steiger S (2018) Manipulation of parental nutritional condition reveals competition among family members. Journal of Evolutionary Biology, 31:822–832.

Keppner EM, Ayasse M, Steiger S (2020) Contribution of males to brood care can compensate for their food consumption from a shared resource. Ecology and Evolution 10:3535–3543.

van Kerckhove K, Hens N, Edmunds WJ, Eames KT (2013) The impact of illness on social networks: implications for transmission and control of influenza. American Journal of Epidemiology 178:1655–1662.

Kiesecker JM, Skelly DK, Beard KH, Preisser E (1999) Behavioral reduction of infection risk. Proceedings of the National Academy of Sciences 96:9165–9168.

Kilner R (1997) Mouth colour is an honest signal of need in begging Canary nestlings. Proceedings of the Royal Society B: Biological Sciences 264:963–968.

Kilner RM (2001) A growth cost of begging in captive canary chicks. Proceedings of the National Academy of Sciences 98:11394–11398.

Kilner RM, Hinde CA (2012) Parent-offspring conflict. In: The Evolution of Parental Care (N.J. Royle et al., eds), pp. 119–132. Oxford University Press, Oxford.

Kilner R, Johnstone RA (1997) Begging the question: are offspring solicitation behaviours signals of need? Trends in Ecology and Evolution 12:11–15.

Kishida R, Suzuki N (2010) Effect of carcass size on feeding modes of larvae of *Nicrophorus quadripunctatus* Kraatz (Coleoptera: Silphidae). Psyche.

Klug H, Alonzo SH, Bonsall MB (2012) Theoretical foundations of parental care. In: The evolution of parental care (NJ Royle et al., eds), pp. 21–39. Oxford University Press, Oxford.

Klug H, Bonsall MB (2010) Life history and the evolution of parental care. Evolution 64:823–835.

Klug H, Bonsall MB (2014) What are the benefits of parental care? The importance of parental effects on developmental rate. Ecology and Evolution 4:2330–2351.

Knight RL, Temple SA (1986) Nest defence in the American goldfinch. Animal Behaviour 34:887–897.

Kokko H, Jennions MD (2012) Sex differences in parental care. In: The evolution of parental care (NJ Royle et al., eds), pp. 101–116. Oxford University Press, Oxford.

Kokko H, Rankin DJ (2006) Lonely hearts or sex in the city? Density-dependent effects in mating systems. Philosophical Transactions of the Royal Society B: Biological Sciences 361:319–334.

Kölliker M, Richner H, Werner I, Heeb P (1998) Begging signals and bi-parental care: Nestling choice between parental feeding locations. Animal Behaviour 55:215–222.

Kölliker M, Ridenhour BJ, Gaba S (2010) Antagonistic parent-offspring co-adaptation. PLoS One 5:e8606.

Komdeur J, Schrama MJ, Meijer K, Moore AJ, Beukeboom LW (2013) Cobreeding in the burying beetle, *Nicrophorus vespilloides*: tolerance rather than cooperation. Ethology. 119:1138–1148.

Komers PE (1997) Behavioural plasticity in variable environments. Canadian Journal of Zoology, 75:161–169.

Kvarnemo C, Ahnesjo I (1996) The dynamics of operational sex ratios and competition for mates. Trends in Ecology Evolution 11:404–408.

Laffafian A, King JD, Agrawal AF (2010) Variation in the strength and softness of selection on deleterious mutations. Evolution 64: 3232–3241.

Landete-Castillejos T, Garcia AJ, Gomez JA, Molina A, Gallego L (2003) Subspecies and body size allometry affect milk production and composition, and calf growth in red deer: comparison of *Cervus elaphus hispanicus* and *Cervus elaphus scoticus*. Physiological and Biochemical Zoology 76:594–602.

Lavery RJ, Keenleyside MH (1990) Parental investment of a biparental cichlid fish, *Cichlasoma nigrofasciatum*, in relation to brood size and past investment. Animal behaviour 40:1128–1137.

Lejeune LA, Savage JL, Bründl AC, Thiney A, Russell AF, Chaine AS (2019) Environmental effects on parental care visitation patterns in blue tits *Cyanistes caeruleus*. Frontiers in Ecology and Evolution 7:356.

Lendvai AZ, Barta Z, Chastel O (2009) Conflict over parental care in house sparrows: do females use a negotiation rule?. Behavioral Ecology 20:651–656.

Lesnoff M, Lancelot R (2012) *aod*: Analysis of overdispersed data. R package, version 1.3.

Lessells CM (2012) Sexual conflict. In: The evolution of parental care (NJ Royle et al., eds), pp. 150–170. Oxford University Press, Oxford.

Lessells CM, McNamara JM (2012) Sexual conflict over parental investment in repeated bouts: negotiation reduces overall care. Proceedings of the Royal Society B: Biological Sciences 279:1506–1514.

Levis NA, Pfennig DW (2016) Evaluating 'plasticity-first' evolution in nature: key criteria and empirical approaches. Trends in Ecology and Evolution 31:563–574.

Levri EP, Lively CM (1996) The effects of size, reproductive condition, and parasitism on foraging behaviour in a freshwater snail, *Potamopyrus antipodarum*. Animal Behaviour 51:891–901.

Liu XH, Yue LF, Li N, Cong L (2013) Inbreeding avoidance drives consistent variation of fine-scale genetic structure caused by dispersal in the

seasonal mating system of Brandt's voles. PloS One 8:e58101.

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-$\Delta\Delta$CT method. Methods 25:402–408.

Lock JE, Smiseth PT, Moore AJ (2004) Selection, inheritance, and the evolution of parent-offspring interactions. American Naturalist 164:13–24.

Login FH, Balmand S, Vallier A, Vincent-Monégat C, Vigneron A, Weiss-Gayet M, Rochat D, Heddi A (2011) Antimicrobial peptides keep insect endosymbionts under control. Science 334:362-365.

Lopes PC (2014) When is it socially acceptable to feel sick?. Proceedings of the Royal Society B: Biological Sciences 281:20140218.

Lopes PC, Adelman J, Wingfield JC, Bentley GE (2012) Social context modulates sickness behavior. Behavioral Ecology and Sociobiology, 66:1421–1428.

Lopes PC, Block P, König B (2016) Infection-induced behavioural changes reduce connectivity and the potential for disease spread in wild mice contact networks. Scientific reports 6:31790.

Lopes PC, Block P, Pontiggia A, Lindholm AK, König B (2018) No evidence for kin protection in the expression of sickness behaviors in house mice. Scientific reports 8:1–9.

Lynch M, Walsh B (1998) Genetics and Analysis of Quantitative Traits. Sinauer, Sunderland, MA.

Madden JR, Kunc HJP, English S, Manser MB, Clutton-Brock TH (2009) Do meerkat (*Suricata suricatta*) pups exhibit strategic begging behaviour and so exploit adults that feed at relatively high rates? Behavioral Ecology and Sociobiology 63:1259–1268.

Mäenpää MI, Andrews CP, Collette D, Leigh D, Smiseth PT (2015) Burying beetle larvae discriminate between individual parents and between some classes of adults. Ethology 121:395–402.

Margulis SW (1998) Relationships among parental inbreeding, parental behaviour and offspring viability in oldfield mice. Animal Behaviour 55:427–

438.

Markman S, Pinshow B, Wright J (2002) The manipulation of food resources reveals sex-specific trade-offs between parental self-feeding and offspring care. Proceedings of the Royal Society B: Biological Sciences 269:1931–1938.

Markman S, Yom-Tov Y, Wright J (1995) Male parental care in the orange-tufted sunbird: behavioural adjustments in provisioning and nest guarding effort. Animal Behaviour 50:655–669.

Marshall VT, Humfeld SC, Bee MA (2003) Plasticity of aggressive signalling and its evolution in male spring peepers, *Pseudacris crucifer*. Animal behaviour 65:1223–1234.

Martin P, Bateson P (2007) Recording methods. In Measuring Behaviour: An Introductory Guide. Cambridge University Press, Cambridge.

Mathot KJ, Wright J, Kempenaers B, Dingemanse NJ (2012) Adaptive strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. Oikos 121:1009–1020.

Mattey SN, Richardson J, Ratz T, Smiseth PT (2018) Effects of offspring and parental inbreeding on parent-offspring communication. American Naturalist 191:716–725.

Mattey SN, Smiseth PT (2015) Complex effects of inbreeding on biparental cooperation. American Naturalist 185:1–12.

Mattey SN, Strutt L, Smiseth PT (2013) Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: Offspring suffer fitness costs when either they or their parents are inbred. Journal of Evolutionary Biology 26:843–853.

Maynard Smith JM (1977) Parental investment: A prospective analysis. Animal Behaviour 25:1–9.

Maynard Smith J, Clarke JM, Hollingsworth MJ (1955) The expression of hybrid vigour in *Drosophila subobscura*. Proceedings of the Royal Society London B: Biological Sciences 144:159–171.

McAdam AG, Boutin S (2004) Maternal effects and the response to selection in red squirrels. Proceedings of the Royal Society B: Biological Sciences 271:75–79.

McGlothlin JW, Moore AJ, Wolf JB, Brodie ED III (2010) Interacting phenotypes and the evolutionary process. III. Social evolution. Evolution 64:2558–2574.

McLeod DV, Day T (2017) Female plasticity tends to reduce sexual conflict. Nature Ecology and Evolution, 1:1–4.

McNamara JM, Székely T, Webb JN, Houston AI (2000) A dynamic game-theoretic model of parental care. Journal of Theoretical Biology 205:605–623.

Meagher S, Penn DJ, Potts WK (2000) Male-male competition magnifies inbreeding depression in wild house mice. Proceedings of the National Academy of Sciences 97:3324–3329.

Mery F, Burns JG (2010) Behavioural plasticity: an interaction between evolution and experience. Evolutionary Ecology 24:571–583.

Milne LJ, Milne M (1976) The social behavior of burying beetles. Scientific American 235:84–89.

Mock DW, Parker GA (1998) The evolution of sibling rivalry. Oxford University Press, New York.

Monteith KM, Andrews C, Smiseth PT (2012) Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. Journal of Evolutionary Biology 25:1815–1822.

Moore AJ, Brodie ED III, Wolf JB (1997) Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. Evolution 51:1352–1362.

Moore J (2002) Parasites and the Behavior of Animals. Oxford University Press, Oxford.

Müller JK (1987) Replacement of a lost clutch: a strategy for optimal resource utilization in *Necrophorus vespilloides* (Coleoptera: Silphidae). Ethology 76:74–80.

Müller JK, Braunisch V, Hwang W, Eggert A-K (2007) Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. Behavioral Ecology 18:196–203.

Müller JK, Eggert A-K (1990) Time-dependent shifts between infanticidal and parental behavior in female burying beetles: a mechanism of indirect mother-offspring recognition. Behavioral Ecology Sociobiology 27:11–16.

Müller JK, Eggert A-K, Furlkröger E (1990) Clutch size regulation in the burying beetle *Necrophorus vespilloides* Herbst (Coleoptera: Silphidae). Journal of Insect Behavior 3:265–270.

Nakagawa S, Gillespie DOS, Hatchwell BJ, Burke T (2007) Predictable males and unpredictable females: sex difference in repeatability of parental care in a wild bird population. Journal of Evolutionary Biology 20:1674–1681.

Oldekop JA, Smiseth PT, Piggins HD, Moore AJ (2007) Adaptive switch from infanticide to parental care: how do beetles time their behaviour? Journal of Evolutionary Biology 20:1998–2004.

Otronen M (1988) The effect of body size on the outcome of fights in burying beetles. Annales Zoologici Fennici 25:191–201.

Paquet M, Covas R, Doutrelant C (2015) A cross-fostering experiment reveals that prenatal environment affects begging behaviour in a cooperative breeder. Animal Behaviour 102:251–258.

Paquet M, Drummond H, Smiseth PT (2018) Offspring are predisposed to beg more towards females in the burying beetle *Nicrophorus vespilloides*. Animal Behaviour 141:195–201.

Paquet M, Parenteau C, Ford L, Ratz T, Richardson J, Angelier F, Smiseth PT (2020) Females adjust maternal hormone concentration in eggs according to male condition in a burying beetle. Hormones and Behavior 121:104708.

Paquet M, Smiseth PT (2017) Females manipulate behavior of caring males via prenatal maternal effects. Proceedings of the National Academy of

Sciences 114:6800–6805.

Paredes JC, Welchman DP, Poidevin M, Lemaitre B (2011) Negative regulation by amidase PGRPs shapes the *Drosophila* antibacterial response and protects the fly from innocuous infection. Immunity 35:770–779.

Parker DJ, Cunningham CB, Walling CA, Stamper CE, Head ML, Roy-Zokan EM, McKinney EC, Ritchie MG, Moore AJ (2015) Transcriptomes of parents identify parenting strategies and sexual conflict in a subsocial beetle. Nature Communications 6:1–12.

Parker GA, Royle NJ, Hartley IR (2002) Intrafamilial conflict and parental investment: a synthesis. Philosophical Transactions of the Royal Society B: Biological Sciences 357:295–307.

Peck SB (1982) The Life History of the Japanese Carrion Beetle *Ptomascopus morio* and the Origins of Parental Care in *Nicrophorus* (Coleoptera, Silphidae, Nicrophini). Psyche 89:107–111.

Piersma T, Drent J (2003) Phenotypic flexibility and the evolution of organismal design. Trends in Ecology and Evolution 18:228–233.

Pilakouta N, Hanlon EJ, Smiseth PT (2018) Biparental care is more than the sum of its parts: experimental evidence for synergistic effects on offspring fitness. Proceedings of the Royal Society B: Biological Sciences 285:p.20180875.

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

Pilakouta N, Richardson J, Smiseth PT (2015b) 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.

Pilakouta N, Richardson J, Smiseth PT (2016) If you eat, I eat: resolution of sexual conflict over consumption from a shared resource. Animal Behaviour 111:175–180.

Podmokła E, Dubiec A, Drobniak SM, Arct A, Gustafsson CM (2014) Avian malaria is associated with increased reproductive investment in the blue tit. Journal of Avian Biology 45:219–224.

Pooley EL, Kennedy MW, Nager RG (2014) Maternal inbreeding reduces parental care in the zebra finch, *Taeniopygia guttata*. Animal Behaviour 97:153–163.

Porlier M, Charmantier A, Bourgault P, Perret P, Blondel J, Garant D (2012) Variation in phenotypic plasticity and selection patterns in blue tit breeding time: between- and within-population comparisons. Journal of Animal Ecology 81:1041–1051.

Portevin G (1926) Les grands Nécrophages du globe. Paul Lechevalier, Paris.

Price K, Harvey H, Ydenberg R (1996) Begging tactics of yellow-headed blackbirds, *Xanthocephalus xanthocephalus*, in relation to need. Animal Behaviour 51:421–435.

Pukowski E (1933) Ökologische Untersuchungen an *Necrophorus* F. Zeitschrift für Morphologie und Ökologie der Tiere 27:518–586.

R Development Core Team 2019. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Ratz T, Castel E, Smiseth PT (2018) Male assistance in parental care does not buffer against detrimental effects of maternal inbreeding on offspring. Frontiers in Ecology and Evolution 6:196.

Ratz T, Smiseth PT (2018) Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*. Journal of Evolutionary Biology 31:646–656.

Ratz T, Nichol TW, Smiseth PT (2020a) Parental responses to increasing levels of handicapping in a burying beetle. Behavioral Ecology 31:73–80.

Ratz T, Perodaskalaki A, Moorad J, Smiseth PT (2020b) Effects of inbreeding on behavioural plasticity of parent-offspring interactions in a burying beetle. Journal of Evolutionary Biology 33:1006–1016.

Ratz T, Stenson S, Smiseth PT (2020c) Offspring preferentially beg towards and associate with larger parents in the burying beetle *Nicrophorus vespilloides*. Behavioral Ecology 31:1250–1256.

Ratz T, Kramer J, Veuille M, Meunier J (2016) The population determines whether and how life-history traits vary between reproductive events in an insect with maternal care. Oecologia 182:443–452.

Rauter CM, Moore AJ (1999) Do honest signalling models of offspring solicitation apply to insects? Proceedings of the Royal Society B: Biological Sciences 266:1691.

Rauter CM, Moore AJ (2004) Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. Animal Behaviour 68:695–702.

Rebar D, Greenfield MD (2017) When do acoustic cues matter? Perceived competition and reproductive plasticity over lifespan in a bushcricket. Animal Behaviour 128:41–49.

Reavey CE., Silva FW, Cotter SC (2015) Bacterial infection increases reproductive investment in burying beetles. Insects 6:926–942.

Reavey CE, Warnock ND, Garbett AP, Cotter SC (2015) Aging in personal and social immunity: do immune traits senesce at the same rate? Ecology and Evolution 5:4365–4375.

Reavey CE, Warnock ND, Vogel H, Cotter SC (2014) Trade-offs between personal immunity and reproduction in the burying beetle, *Nicrophorus vespilloides*. Behavioral Ecology 25:415–423.

Redondo T, Castro F (1992) The increase in risk of predation with begging activity in broods of Magpies, *Pica pica*. Ibis 134:180–187.

Reed DH, Fox CW, Enders LS, Kristensen TN (2012) Inbreeding-stress interactions: evolutionary and conservation consequences. Annals of the New York Academy of Sciences 1256:33–48.

Reed DH, Lowe EH, Briscoe DA, Frankham R (2003) Fitness and adaptation in a novel environment: effect of inbreeding, prior environment, and lineage. Evolution 57:1822–1828.

Richardson J, Comin P, Smiseth PT (2018) Inbred burying beetles suffer fitness costs from making poor decisions. Proceedings of the Royal Society of London B: Biological Sciences 285:20180419.

Richardson J, Smiseth PT (2017) Intraspecific competition and inbreeding depression: increased competitive effort by inbred males is costly to outbred opponents. American Naturalist 189:539–548.

Richardson J, Smiseth PT (2020) Maternity uncertainty in cobreeding beetles: females lay more and larger eggs and provide less care. Behavioral Ecology 31:641–650.

Richardson J, Stephens J, Smiseth PT (2020) Increased allocation to reproduction reduces future competitive ability in a burying beetle. Journal of Animal Ecology 89:1918–1926.

Richner H, Christe P, Oppliger A (1995) Paternal investment affects prevalence of malaria. Proceedings of the National Academy of Sciences 92:1192–1194.

Ridgway MS (1989) The parental response to brood size manipulation in smallmouth bass (*Micropterus dolomieui*). Ethology 80: 47–54.

Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D (2017) *MASS*: Modern applied statistics with S. R package, version 7.3-48.

Robertson IC (1993) Nest intrusions, infanticide, and parental care in the burying beetle, *Nicrophorus orbicollis* (Coleoptera: Silphidae). Journal of Zoology 231:583–593.

Rodríguez RL, Rebar D, Fowlere-Finn KD (2013) The evolution and evolutionary consequences of social plasticity in mate preferences. Animal Behaviour 85:1041–1047.

Rollinson N, Rowe L (2015) Persistent directional selection on body size and a resolution to the paradox of stasis. Evolution 69:2441–2451.

Roulin A, Bersier L-F (2007) Nestling barn owls beg more intensely in the presence of their mother than in the presence of their father. Animal Behaviour 74:1099–1106.

Royle NJ, Hopwood PE (2017) Covetable corpses and plastic beetles–The socioecological behavior of burying beetles. In: Advances in the study of behavior (M. Naguib et al., eds), pp. 101–146. Academic Press, Cambridge.

Royle NJ, Russell AF, Wilson AJ (2014) The evolution of flexible parenting. Science 345:776–781.

Royle NJ, Smiseth PT, Kölliker M (2012) The evolution of parental care. Oxford University Press, Oxford.

Rozen DE, Engelmoer DJP, Smiseth PT (2008) Antimicrobial strategies in burying beetles breeding on carrion. Proceedings of the National Academy of Sciences 105:17890–17895

Safryn SA, Scott MP (2000) Sizing up the competition: do burying beetles weigh or measure their opponents? Journal of Insect Behavior 13:291–297.

Sagisaka A, Miyanoshita A, Ishibashi J, Yamakawa M (2001) Purification, characterization and gene expression of a glycine and proline-rich antibacterial protein family from larvae of a beetle, *Allomyrina dichotoma*. Insect molecular biology 10:293–302.

Sah P, Mann J, Bansal S (2018) Disease implications of animal social network structure: a synthesis across social systems. Journal of Animal Ecology 87:546–558.

Sanz JJ (1997) Clutch size manipulation in the pied flycatcher: effects on nestling growth, parental care and moult. Journal of Avian Biology 28:157–162.

Sanz JJ, Kranenbarg S, Tinbergen JM (2000) Differential response by males and females to manipulation of partner contribution in the great tit (*Parus major*). Journal Animal Ecology 69:74–84.

Sarkar A, Harty S, Johnson KVA, Moeller AH, Archie EA, Schell LD, Carmody RN, Clutton-Brock TH, Dunbar RI, Burnet PW (2020) Microbial transmission in animal social networks and the social microbiome. Nature Ecology and Evolution 4:1020–1035.

Schaller M (2006) Parasites, behavioral defenses, and the social psychological mechanisms through which cultures are evoked. Psychological Inquiry 17:96–101.

Schaller M, Park JH (2011) The behavioral immune system (and why it matters). Current Directions in Psychological Science 20:99–103.

Schiegg K, Pasinelli G, Walters JR, Daniels SJ (2002) Inbreeding and experience affect response to climate change by endangered woodpeckers. Proceedings of the Royal Society of London B: Biological Sciences 269:1153–1159.

Schou MF, Bechsgaard J, Muñoz J, Kristensen TN (2018) Genome-wide regulatory deterioration impedes adaptive responses to stress in inbred populations of *Drosophila melanogaster*. Evolution 72:1614–1628.

Schou MF, Kristensen TN, Loeschcke V (2015) Trait-specific consequences of inbreeding on adaptive phenotypic plasticity. Ecology and Evolution 5:1–6.

Schou MF, Loeschcke V, Kristensen TN (2015) Inbreeding depression across a nutritional stress continuum. Heredity 115:56–62.

Schrader M, Jarrett BJM, Kilner RM (2015) Parental care masks a density-dependent shift from cooperation to competition among burying beetle larvae. Evolution 69:1077–1084.

Schwagmeyer PL, Bartlett TL, Schwabl HG (2008) Dynamics of house sparrow biparental care: what contexts trigger partial compensation?. Ethology 114:459–468.

Schwagmeyer PL, Mock DW (2003) How consistently are good parents good parents? Repeatability of parental care in the house sparrow, *Passer domesticus*. Ethology 109:303–313.

Schwagmeyer PL, Schwabl HG, Mock DW (2005) Dynamics of biparental care in house sparrows: hormonal manipulations of paternal contributions. Animal Behaviour, 69:481–488.

Scott MP (1990) Brood guarding and the evolution of male parental care in burying beetles. Behavioral Ecology and Sociobiology 26:31–39

193

Scott MP (1994) The benefit of paternal assistance in intra-and interspecific competition for the burying beetle, *Nicrophorus defodiens*. Ethology Ecology and Evolution 6:537–543.

Scott MP (1998) The ecology and behavior of burying beetles. Annual Review of Entomology 43:595–618.

Scott MP, Traniello JFA (1990) Behavioural and ecological correlates of male and female parental and reproductive success in the burying beetle *Nicrophorus orbicollis*. Animal Behaviour 39:274–283.

Seyfarth, RM, Cheney DL (2015) Social cognition. Animal Behaviour 103:191–202.

Shakhar K (2019) The inclusive behavioral immune system. Frontiers in Psychology 10:1004.

Shakhar K, Shakhar G (2015) Why do we feel sick when infected–can altruism play a role?. PLoS Biology 13:e1002276.

Sieber DJ, Paquet M, Smiseth PT (2017) Joint effects of brood size and resource availability on sibling competition. Animal Behaviour, 129:25–30.

Sih A, Bell A, Johnson JC (2004) Behavioral syndromes: An ecological and evolutionary overview. Trends in Ecology and Evolution 19:372–378.

Sikes DS, Madge RB, Newton AF (2002) A catalog of the Nicrophorinae (Coleoptera: Silphidae) of the world. Zootaxa 65:1–304.

Simmons LW (1986) Inter-male competition and mating success in the field cricket, *Gryllus bimaculatus* (De Geer). Animal Behaviour 34:567–579.

Siva-Jothy JA, Prakash A, Vasanthakrishnan RB, Monteith KM, Vale PF (2018) Oral bacterial infection and shedding in *Drosophila melanogaster*. Journal of Visualized Experiments 135:e57676.

Slagsvold T, Lifjeld JT (1988) Ultimate adjustment of clutch size to parental feeding capacity in a passerine bird. Ecology 69:1918–1922.

Slagsvold T, Lifjeld JT (1990) Influence of male and female quality on clutch size in tits (*Parus* spp.). Ecology 71:1258–1266.

Slate J, Kruuk LEB, Marshall TC, Pemberton JM, Clutton-Brock TH (2000) Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). Proceedings of the Royal Society of London B: Biological Sciences 267:1657–1662.

Smiseth PT (2017) Parental care. In: Reference Module in Life Sciences (BD Roitberg, JC Perry, eds). Elsevier, Amsterdam.

Smiseth PT, Andrews C, Brown E, Prentice PM (2010) Chemical stimuli from parents trigger larval begging in burying beetles. Behavioral Ecology 21:526–531.

Smiseth PT, Andrews CP, Mattey SN, Mooney R (2014) Phenotypic variation in resource acquisition influences tradeâoff between number and mass of offspring in a burying beetle. Journal of Zoology 293:80–83.

Smiseth PT, Darwell CT, Moore AJ (2003) Partial begging: an empirical model for the early evolution of offspring signalling. Proceedings of the Royal Society of London B: Biological Sciences 270:1773–1777.

Smiseth PT, Dawson C, Varley E, Moore AJ (2005) How do caring parents respond to mate loss? Differential response by males and females. Animal Behaviour 69:551–559.

Smiseth PT, Kölliker M, Royle NJ (2012) What is parental care? In: The Evolution of Parental Care (NJ Royle et al., eds), pp. 1–17. Oxford University Press, Oxford.

Smiseth PT, Lennox L, Moore AJ (2007a) Interaction between parental care and sibling competition: parents enhance offspring growth and exacerbate sibling competition. Evolution 61:2331–2339.

Smiseth PT, Lorentsen SH (2001) Begging and parent-offspring conflict in grey seals. Animal Behaviour 62:273–279.

Smiseth PT, Moore AJ (2002) Does resource availability affect offspring begging and parental provisioning in a partially begging species? Animal Behaviour 63:577–585.

Smiseth PT, Moore AJ (2004a) Behavioral dynamics between caring males and females in a beetle with facultative biparental care. Behavioral

Ecology 15:621–628.

Smiseth PT, Moore AJ (2004b) Signalling of hunger when offspring forage by both begging and self-feeding. Animal Behaviour 67:1083–1088.

Smiseth PT, Parker HJ (2008) Is there a cost to larval begging in the burying beetle *Nicrophorus vespilloides*? Behavioral Ecology 19:1111–1115.

Smiseth PT, Ward RJS, Moore AJ (2006) Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. Functional Ecology 20:151–156.

Smiseth PT, Ward RJ, Moore AJ (2007b) Parents influence asymmetric sibling competition: experimental evidence with partially dependent young. Ecology 88:3174–3182.

Smiseth PT, Wright J, Kölliker M (2008) Parentâoffspring conflict and co-adaptation: behavioural ecology meets quantitative genetics. Proceedings of the Royal Society of London B: Biological Sciences 275:1823–1830.

Snell-Rood EC (2013) An overview of the evolutionary causes and consequences of behavioural plasticity. Animal Behaviour 8:1004–1011.

Sol D, Timmermans S, Lefebvre L (2002) Behavioral flexibility and invasion success in birds. Animal Behaviour 63:495–502.

Steiger S (2013) Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. Proceedings of the Royal Society of London B: Biological Sciences 280:20131225.

Steiger S, Gershman SN, Pettinger AM, Eggert A, Sakaluk SK (2011) Sex differences in immunity and rapid upregulation of immune defence during parental care in the burying beetle, *Nicrophorus orbicollis*. Functional Ecology 25:1368–1378.

Steiger S, Haberer W, Müller JK (2011) Social environment determines degree of chemical signalling. Biology Letters 7:822–824.

Steiger S, Peschke K, Francke W, Müller JK (2007) The smell of parents: Breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides*. Proceedings of the Royal Society of

London B: Biological Sciences 274:2211–2220.

Steiger S, Stökl J (2018) Pheromones regulating reproduction in subsocial beetles: insights with references to eusocial insects. Journal of chemical ecology 44:785–795.

Stein LR, Bell AM (2019) The role of variation and plasticity in parental care during the adaptive radiation of three-spine sticklebacks. Evolution 73:1037–1044.

Stillwell RC, Blanckenhorn WU, Teder T, Davidowitz G, Fox CW (2010) Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. Annual Review of Entomology 55:227–245.

Stockmaier S, Bolnick DI, Page RA, Carter GG (2020) Sickness effects on social interactions depend on the type of behaviour and relationship. Journal of Animal Ecology 89:1387–1394.

Stoehr AM, Hill GE (2000) Testosterone and the allocation of reproductive effort in male house finches (*Carpodacus mexicanus*). Behavioral Ecology and Sociobiology 48:407–411.

Stroeymeyt N, Grasse AV, Crespi A, Mersch DP, Cremer S, Keller L (2018) Social network plasticity decreases disease transmission in a eusocial insect. Science 362:941–945.

Suzuki S (2015) Recognition of the sex of the parents by young: Effects of hunger and biparental care on the begging behavior of *Nicrophorus quadripuncatus* larvae. Journal Insect Behaviour 28:38–344.

Suzuki S (2016) When the male determines his provisioning effort: does the timing of handicapping affect the negotiation between parents in *Nicrophorus quadripunctatus*? Behaviour 153:1435–1443.

Suzuki S, Nagano M (2009) To compensate or not? Caring parents respond differentially to mate removal and mate handicapping in the burying beetle, *Nicrophorus quadripunctatus*. Ethology 115:1–6.

Székely T, Cuthill IC, Kis J (1999) Brood desertion in Kentish plover: sex differences in remating opportunities. Behavioral Ecology 10:185–190.

197

Székely T, Williams TD (1995) Costs and benefits of brood desertion in female Kentish plovers, *Charadrius alexandrinus*. Behavioral Ecology and Sociobiology 37:155–161.

Szulkin M, Stopher KV, Pemberton JM, Reid JM (2013) Inbreeding avoidance, tolerance, or preference in animals?. Trends in ecology evolution, 28:205–211.

Taborsky B, Oliveira RF (2012) Social competence: an evolutionary approach. Trends in Ecology and Evolution 27:679–688.

Takata M, Mitaka Y, Steiger S, Mori N (2019) A parental volatile pheromone triggers offspring begging in a burying beetle. iScience 19:1260–1278.

Tallamy DW, Wood TK (1986) Convergence patterns in subsocial insects. Annual Review of Entomology 31:369–390.

Therneau T (2015) A Package for Survival Analysis in S. version 2.38.

Thomson CE, Bayer F, Crouch N, Farrell S, Heap E, Mittell E, Zurita-Cassinello M, Hadfield JD (2017) Selection on parental performance opposes selection for larger body mass in a wild population of blue tits. Evolution 71:716–732.

Tretzel E (1961) Biologie, ökologie und brutpflege von Coelotes terrestris (wider) (Araneae, Agelenidae). Zeitschrift für Morphologie und Ökologie der Tiere 50:375–542.

Trivers RL (1972) Parental investment and sexual selection. In: Sexual selection and the descent of man (B Campbell eds), PP. 136–79. Heinemann, London.

Trivers RL (1974) Parent-offspring conflict. Integrative and Comparative Biology 14:249–264.

Trumbo ST (1991) Reproductive benefits and the duration of paternal care in a biparental burying beetle, *Necrophorus orbicollis*. Behaviour 117:82–105.

Trumbo ST (1992) Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*). Ecological Entomology 17:289–298.

Trumbo ST (2007) Defending young biparentally: female risk-taking with and without a male in the burying beetle, *Nicrophorus pustulatus*. Behavioral Ecology and Sociobiology 61:1717–1723.

Trumbo ST (2012) Patterns of parental care in invertebrates. In: The evolution of parental care (NJ Royle et al., eds), pp. 81–100. Oxford University Press, Oxford.

Trumbo ST, Robinson GE (2008) Social and nonsocial stimuli and juvenile hormone titer in a male burying beetle, *Nicrophorus orbicollis*. Journal of Insect Physiology 54:630–635.

Trumbo ST, Valletta RC (2007) The costs of confronting infanticidal intruders in a burying beetle. Ethology 113:386–393.

Tveera T, Saether BE, Aanes R, Erikstad KE (1998) Regulation of food provisioning in the Antarctic petrel; the importance of parental body condition and chick body mass. Journal of Animal Ecology 67:699–704

Vale PF, Siva-Jothy JA, Morrill A, Forbes MR (2018) The influence of parasites. In: Insect Behavior: from mechanisms to ecological and evolutionary consequences (A Córdoba-Aguilar et al., eds), pp. 273–291. Oxford University Press, Oxford.

Venesky MD, Parris MJ, Storfer A (2009) Impacts of *Batrachochytrium dendrobatidis* infection on tadpole foraging performance. EcoHealth 6:565–575.

Vilcinskas A, Stoecker K, Schmidtberg H, Röhrich CR, Vogel H (2013a) Invasive harlequin ladybird carries biological weapons against native competitors. Science 340:862–863.

Vilcinskas A, Mukherjee K, Vogel H (2013b) Expansion of the antimicrobial peptide repertoire in the invasive ladybird H*armonia axyridis*. Proceedings of the Royal Society B: Biological Sciences 280:20122113.

Vincze O, Székely T, Küpper C, AlRashidi M, Amat JA, Ticó AA, Burgas D, Burke T, Cavitt J, Figuerola J, Shobrak M (2013) Local environment but not genetic differentiation influences biparental care in ten plover populations. PLoS One 8:e60998.

Waller DM, Dole J, Bersch AJ (2008) Effects of stress and phenotypic variation on inbreeding depression in *Brassica rapa*. Evolution 62:917–931.

Walling CA, Stamper CE, Smiseth PT, Moore AJ (2008) The quantitative genetics of sex differences in parenting. Proceedings of the National Academy of Sciences 105:18430–18435.

Wang Y, Rozen DE (2018) Gut microbiota in the burying beetle, *Nicrophorus vespilloides*, provide colonization resistance against larval bacterial pathogens. Ecology and evolution 8:1646–1654.

Ward RJ, Cotter SC, Kilner RM (2009) Current brood size and residual reproductive value predict offspring desertion in the burying beetle N*icrophorus vespilloides*. Behavioral Ecology 20:1274–1281.

West HE, Capellini I (2016) Male care and life history traits in mammals. Nature Communications 7:11854.

West-Eberhard MJ (1989) Phenotypic plasticity and the origins of diversity. Annual review of Ecology and Systematics 20:249–278.

Westneat DF, Hatch MI, Wetzel DP, Ensminger AL (2011) Individual variation in parental care reaction norms: integration of personality and plasticity. American Naturalist 178:652–667.

Westneat DF, Sargent RC (1996) Sex and parenting: the effects of sexual conflict and parentage on parental strategies. Trends in Ecology and Evolution 11:87–91.

Weygoldt P (1980) Complex brood care and reproductive behaviour in captive poison-arrow frogs, *Dendrobates pumilio* O. Schmidt. Behavioral Ecology and Sociobiology 7:329–332.

Whittingham LA (1989) An experimental study of paternal behavior in red-winged blackbirds. Behavioral Ecology and Sociobiology 25:73–80.

Williams GC (1966) Natural selection, the costs of reproduction, and a refinement of Lack's principle. American Naturalist 100:687–690.

Wilson DS, Fudge J (1984) Burying beetles: intraspecific interactions and reproductive success in the field. Ecological Entomology 9:195ââ203.

Wolf JB, Brodie ED III, Cheverud JM, Moore AJ, Wade MJ (1998) Evolutionary consequences of indirect genetic effects. Trends in Ecology and Evolution 13:64–69.

Wong JW, Kölliker M (2012) The effect of female condition on maternal care in the European earwig. Ethology 118:450–459.

Wright J, Cuthill I (1989) Manipulation of sex differences in parental care. Behavioral Ecology Sociobiology 25:171–181.

Xu H, Suzuki N (2001) Effects of carcass size and parental feeding on reproductive success of the burying beetle *Nicrophorus quadripunctatus* (Coleoptera: Silphidae). Entomological Science 4:217–222.

Yoshioka M, Meeks C, Summers K (2016) Evidence for begging as an honest signal of offspring need in the biparental mimic poison frog. Animal Behaviour 113:1–11.

Ziadie MA, Ebot-Ojong F, McKinney EC, Moore AJ (2019) Evolution of personal and social immunity in the context of parental care. American Naturalist 193:296–308.

Appendix: Journal articles arising from this thesis

Original Article

Parental responses to increasing levels of handicapping in a burying beetle

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Parental care is highly variable, reflecting that parents make flexible decisions about how much care to provide in response to variation in the cost and/or benefit of care. Handicapping has traditionally been used as a tool for increasing the energetic cost of care, thereby inducing a reduction in care by handicapped parents. However, recent evidence shows that handicapped parents sometimes provide more care, suggesting that handicapping can trigger terminal investment. Here, we investigate responses to different levels of handicapping in the burying beetle *Nicrophorus vespilloides* by comparing handicapped female parents fitted with a wide range of handicaps, as well as control females without a handicap. We found that handicapped females spent more time provisioning food and less time being absent from the crypt than control females, while there was no effect of the level of handicapping among handicapped females. We found no effect of handicapping on larval begging behavior, larval performance (mean larval mass and brood size at dispersal), or female investment in future reproduction (i.e., weight gain while breeding and life span after breeding). Our findings provide no support for the widely held assumption that handicapping simply increases the cost of care. Instead, our results are consistent with the suggestion that handicapping triggers terminal investment by suppressing the condition of parents below the threshold at which terminal investment is triggered.

Key words: cost of care, *Nicrophorus vespilloides*, parental decision, reproductive trade-off, terminal investment.

INTRODUCTION

Parental care encompasses any parental trait that enhances the survival and/or growth of a parent's offspring, often at a cost to the parent's ability to invest in other current or future offspring (Clutton-Brock 1991; Royle et al. 2012). Parental care is highly variable (Clutton-Brock 1991; Royle et al. 2012), reflecting that parents make flexible decisions about how much care to provide due to variation in the cost of care to themselves and/or the benefit to their offspring (Royle et al. 2014; Ratz and Smiseth 2018). For example, as shown by handicapping experiments on birds and insects, parents are expected to provide less care given an increase in the cost of care (Wright and Cuthill 1989; Harrison et al. 2009; Suzuki and Nagano 2009). Handicapping experiments are used to study negotiation between parents in birds with biparental care (Harrison et al. 2009), and their rationale is to increase the energetic cost of providing care at a given level by attaching a lead weight to the base of the handicapped parent's tail feathers (Wright and Cuthill 1989) or by clipping some of its flight feathers (Slagsvold and Lifjeld 1988, 1990). Most such experiments find that handicapped parents provide less care than control parents (e.g., Wright and

Cuthill 1989; Harrison et al. 2009), confirming that parents plastically reduce the amount of care they provide when the cost of care increases. However, a recent study on the burying beetle *Nicrophorus vespilloides* found that handicapped females provided *more* care than control females (Ratz and Smiseth 2018). This finding contradicts the implicit assumption that handicapping simply increases the cost of care. In light of this, there is now a need to improve our understanding of how parents respond to handicapping given its important role in the study of parental care.

One potential explanation for why handicapped parents sometimes provide more care than control parents is that handicapping can trigger a shift towards greater investment in current reproduction (Ratz and Smiseth 2018), often referred to as terminal investment (Williams 1966; Clutton-Brock 1984). Theory suggests that terminal investment is triggered when an individual's condition deteriorates below a certain threshold value, thereby reducing its future survival prospects (Duffield et al. 2017). Handicapping could trigger terminal investment if it suppresses the parent's condition below this threshold value by, for example, reducing its foraging ability or increasing its energy expenditure. Thus, current evidence suggests that handicapping might influence the parent's behavior either by increasing the energetic cost of care or by triggering terminal investment. We note that these two effects are not mutually

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exclusive, as handicapping could both increase the cost of care and trigger terminal investment. If so, we might expect more complex responses to handicapping that are determined by a combination of whether or not the handicap suppresses the parent's condition below the threshold triggering terminal investment and the extent to which the handicap increases the energetic cost of care. As outlined below, in order to advance our understanding of the effects of handicapping, we now need novel experimental designs that monitor how caring parents respond to different levels of handicapping.

In this study, we investigated how female parents responded to different levels of handicapping in a burying beetle. Burying beetles of the genus *Nicrophorus* are ideal study systems to explore this issue because they show highly elaborate forms of parental care, including provisioning of predigested carrion to the larvae and depositing antimicrobial secretions to preserve the small vertebrate carcass used for breeding as a food source throughout larval development (Scott 1998). Furthermore, these beetles have been subject to handicapping experiments, showing that handicapped parents either provide less care, as reported in studies on *Nicrophorus quadripunctatus* and *N. orbicollis* (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016), or more care, as reported in *N. vespilloides* (Ratz and Smiseth 2018). One potential explanation for why studies have reported contrasting effects of handicapping is that these studies used different levels of handicapping. For example, studies showing that handicapped parents provide less care used larger weights that were about 40–50% of a parent's body mass (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016), whereas the study reporting that handicapped parents provide more care used smaller weights that were about 20–30% of a parent's body mass (Ratz and Smiseth 2018). Although this pattern suggests that parents provide more care in response to a relatively small handicap but less care in response to a relatively large handicap, there is now a need for experimental work monitoring how parents respond to different levels of handicapping within a single species.

Our aim was to investigate how single female parents respond to different levels of handicapping in the burying beetle *N. vespilloides*. We handicapped females by attaching a small weight to their pronotum (Suzuki and Nagano 2009). The weights weighed 0.037–0.242 g, corresponding to 11–103% of a female's body mass. We also included a control treatment, where females were not fitted with a weight but otherwise were handled in the same way as handicapped females. Prior work shows that females respond by providing more care when fitted with a 0.05 g weight (Ratz and Smiseth 2018), suggesting that the threshold triggering terminal investment is below this level of handicapping. We then tested for subsequent effects on the amount of care provided by females (i.e., time spent provisioning food and maintaining the carcass) during the period where females provide direct care for larvae, as well as on offspring performance (i.e., mean larval mass, number of larvae at dispersal, and larval begging behavior) and female investment in future reproduction (i.e., weight change while breeding and life span after breeding).

If handicapping primarily increased the cost of care, we predicted that females should provide progressively less care as the level of handicapping increased (Figure 1a). Furthermore, offspring performance should gradually decline as the level of handicapping increases, and females should pay a progressively higher cost in terms of their investment in future reproduction. Conversely, if handicapping primarily triggered terminal investment, we predicted that the effects of the level of handicapping should be discontinuous with handicapped females providing more care than control
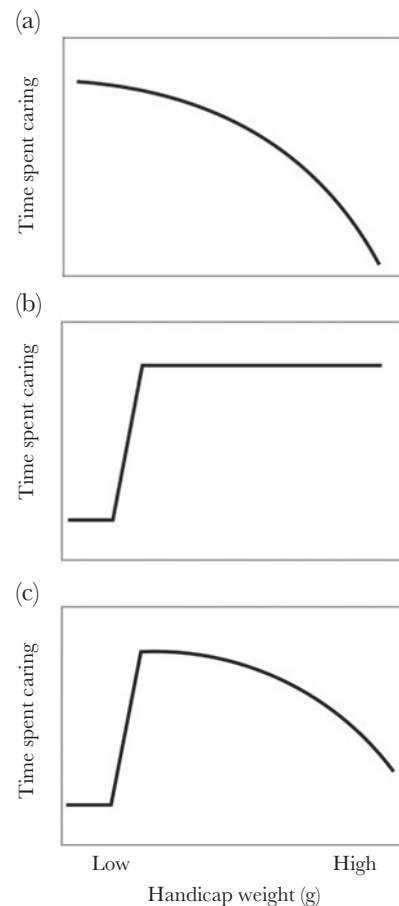

Figure 1
Predicted effects of the level of handicapping on the amount of care provided by parents. If handicapping primarily elevates the cost of care, parents should provide progressively less care as the level of handicapping increases (a). If handicapping primarily triggers terminal investment, the effects of the level of handicapping should be discontinuous with a marked increase in care by handicapped parents at the threshold value (b). Handicapped parents should provide as much care as control parents below this threshold, while they should provide more care than control parents above the threshold. Handicapped parents should provide the same level of care regardless of the level of handicapping above the threshold. If handicapping both elevates the cost of care and triggers terminal investment, the effects of the level of handicapping should also be discontinuous with a marked increase in care by handicapped parents at the threshold value (c). However, in this case, handicapped parents should provide progressively less care as the level of handicapping increases above the threshold.

females provided that the handicap suppressed the parent's condition below the threshold value (Figure 1b). Below this threshold, handicapped parents should provide as much care as control parents. Above the threshold, handicapped parents should provide more care than control parents, but the former should provide the same level of care regardless of the level of handicapping (Figure 1b). Furthermore, offspring performance should be higher, while female investment in future reproduction should be lower, above the threshold than below. Finally, if handicapping both elevates the cost of care and triggers terminal investment, we predicted that the effects of the level of handicapping should be discontinuous with a marked increase in care by handicapped parents at the threshold value (Figure 1c). However, above this threshold, handicapped

parents should provide progressively less care as the level of handicapping increases. Furthermore, offspring performance and female investment in future reproduction should gradually decline with the level of handicapping above the threshold.

MATERIALS AND METHODS

Source and rearing of experimental beetles

The beetles used in this experiment came from a laboratory stock population originating from beetles collected at Corstorphine Hill Local Nature Reserve and Hermitage of Braid and Blackford Hill Local Nature Reserve, Edinburgh, UK. Nonbreeding adult beetles were housed in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil. All beetles were fed organic beef twice a week and maintained under a constant temperature (20 °C) and a 16:8 h light:dark photoperiod.

Experimental design and procedures

We manipulated the level of handicapping by attaching a nontoxic fishing weight (Dinsmores, Aldridge, UK and DGT, Shirley, UK) to the pronotum of caring females (see below for further details). The weights used in our experiment weighed 0.037–0.242 g, corresponding to 11–103% of the initial body mass of females. We used this range to ensure that our handicaps overlapped the range used in prior work on this species (20–30%; Ratz and Smiseth 2018) and on *N. quadripunctatus* and *N. orbicollis* (40–50%; Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016). We also included weights that went beyond this range to ensure that our handicaps were large enough to induce a potential increase in the energetic cost of care. Our design included a control treatment, where females were not fitted with a weight but were otherwise handled and treated in the same way as handicapped females. In this experiment, we focused on the response of a single parent to exclude potential compensatory responses by its partner. We did this given that our aim was to establish whether handicapping increases the cost of care, triggers terminal investment, or both. We specifically focused on single female parents because females provide more parental care than males in this species (Eggert et al. 1998; Rauter and Moore 2004) and because the experimental removal of the male has no effect on offspring fitness under laboratory conditions (Smiseth et al. 2005).

We began the experiment by pairing females and males at random, transferring each pair into a larger plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass (Livefoods Direct, Sheffield, UK) of a standardized size (14.68–19.98 g). One day before the expected date of hatching (i.e., 2 days after the beginning of egg laying), we randomly assigned each female to the handicapping or the control treatment (i.e., no weight; hereafter referred to as 0g). Although the nominal mass of the weights was categorical (0.05 g, 0.10 g, or 0.20 g), there was considerable variation in the mass of weights within each category (range, mean ± SE for 0.05 g, 0.10 g, and 0.20 g weights, respectively: 0.0370–0.0757 g, 0.0544 ± 0.0017 g; 0.0716–0.1241 g, 0.0959 ± 0.0019; 0.1702–0.2423 g, 0.1988 ± 0.0026). We weighed all females before and after subjecting them to the handicapping treatment, using the difference in mass as a measure of the mass of the handicap provided to each female. We attached the weight to the pronotum of each handicapped female using instant-adhesive glue (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016; Ratz and

Smiseth 2018). Before attaching the weight, we gently scraped the surface of the apex of the pronotum using fine sandpaper (P600). We did so to remove impurities, thereby improving adhesion of the weight. We treated females assigned to the control treatment in the same way as handicapped females (i.e., we weighed them before and after handling, handled them, and scraped the surface of, and applied glue to, their pronotum), except that no weight was attached to their pronotum. For further details on the handicapping procedure, we refer to Ratz and Smiseth (2018).

Once handicapped females had been fitted with a weight and control females had been handled, we moved them together with their mouse carcass to a fresh container filled with moist soil. We did this to separate females from their eggs, thereby allowing us to provide them with standardized experimental broods. Once the larvae started hatching, we collected them in a temporary holding container, using them to generate experimental broods comprised of 10 same-aged larvae of mixed maternal origin (Smiseth, Lennox, et al. 2007). For practical reasons, we allocated females broods comprising some larvae that were their own and some that were foreign. It is unlikely that this would influence our results as there is no evidence that females differentiate between their own and foreign larvae in this species. Instead, females have a temporal kin discrimination mechanism whereby they kill any larvae arriving on the carcass before their own eggs would have hatched (Müller and Eggert 1990). Thus, to avoid infanticide, we ensured that we only provided females with an experimental brood once their own eggs had hatched. We used experimental rather than natural broods in this experiment to control for potential confounding effects due to variation in the number of larvae in the brood and the age of the brood, both of which are known to influence the amount of care provided by females in *N. vespilloides* (Smiseth et al. 2003; Smiseth, Lennox, et al. 2007; Smiseth, Ward, et al. 2007). We removed male parents at the same time as we moved females to a fresh container.

We recorded data on the amount of care provided by handicapped and control females 24 h (±15 min) after we placed the larvae on the carcass. This time point corresponds to the peak in time spent providing care towards larvae in this species (Smiseth et al. 2003). We collected behavioral data using instantaneous sampling every 1 min for 30 min under red light, in accordance with established protocols (e.g., Smiseth and Moore 2002, 2004a; Ratz and Smiseth 2018). Although the 30 min sampling period is a relatively small part of the period when females provide direct care for the larvae (larvae become nutritionally independent 72 h after hatching), there are positive correlations between different measures of parental care in *N. vespilloides* (Andrews et al. 2017), and the amount of time spent providing care 24 h after hatching is positively correlated with the time at which the parents desert the brood (Pilakouta, N., Hanlon, B., and Smiseth, P.T., personal communication). Thus, our sampling period is representative of the total amount of care provided by females. At each scan, we recorded whether the female was engaged in the following behaviors: *provisioning food*, defined as when there was mouth-to-mouth contact between the female and at least one larva, *maintaining the carcass*, defined as when the female was excavating the soil around the carcass or coating the carcass with secretions or *absent from the crypt*, defined as when the female was away from the crypt (i.e., the depression surrounding the carcass). We conducted the behavioral observations blind with respect to treatments as far as this was practically possible. The observations were blind for the different levels of handicapping, as it was not possible for the observer to identify the size of the handicap in the dim light conditions of under which

the observations were conducted. However, it was not possible to keep the control treatment (i.e., 0 g) blind, as the observer could tell whether females had been provided with a weight or not.

At the same time as we recorded data on the amount of care provided by females, we also recorded data on larval begging to test for potential effects of handicapping on larval behavior. In burying beetles, larval begging is tactile and begging larvae raise their bodies towards the female and touch the female with their legs (Smiseth and Moore 2002). Larval begging only occurs when the parent is in close contact with the larvae, defined as a distance less than or equal to the width of the female's pronotum (Rauter and Moore 1999; Smiseth and Moore 2002). At each scan, we counted the number of larvae that were begging. We calculated the average proportion of time spent begging per larva in the brood as B=(Σb/n)/p, where Σb is the cumulative number of begging events during the 30-min observation period, n is the brood size at the time of observation, and p is the number of scans during which the female was near the larvae. This metric provides a measure of larval begging that is largely independent of variation in female behavior towards the larvae (Smiseth and Moore 2004b).

At the time of larval dispersal from the carcass, which normally takes place about 5 days after hatching, we recorded the number of surviving larvae in the brood and weighed the brood. We did this to test for potential effects of handicapping on offspring performance. We calculated mean larval mass by dividing the total brood mass by the number of surviving larvae in the brood. In this species, body size is a key determinant of an individual's reproductive success and adult body size is highly correlated with larval mass at dispersal (Otronen 1988; Safryn and Scott 2000). At the time of larval dispersal, we also removed the weights from the female's pronotum by gently twisting the weight or lifting it off using soft forceps. We removed the weights at this time to obtain information on the potential fitness cost of handicapping during the period when females provided care for their larvae. We then recorded the postbreeding body mass of each female, which we used to calculate the female's weight change while breeding as the difference between post- and prebreeding body mass. Finally, we recorded female life span after breeding. To this end, we moved all females into individual containers and we then checked each container twice a week and recorded the date of death for each female.

We set up 137 pairs in total in the course of this experiment. We excluded 3 females that did not lay any eggs, 11 females whose eggs did not hatch, and 3 females for which the weight of the handicap was recorded incorrectly, yielding the following final sample sizes for female parental behavior, larval begging, mean larval mass at dispersal, and female weight change: control females (0 g weight: $N = 30$), and handicapped females (0.037–0.242 g: $N = 90$). We further excluded two females from our analyses on brood size at dispersal because the number of larvae was uncertain, yielding the following final sample sizes for brood size: control females ($N = 29$), and handicapped females ($N = 89$). For female life span, we excluded 35 females for the reasons stated above and because we could not remove their weights, yielding the following final sample sizes for this trait: control females ($N = 28$), and handicapped females ($N = 67$).

Statistical analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team 2019). Behavioral traits were recorded as the total number of scans out of a maximum of 30 scans and

were therefore analyzed assuming a binomial error structure. Given that our data on time spent provisioning food, maintaining the carcass and absent from the crypt by females showed over-dispersion and minor zero-inflation, we analyzed these data using a Bayesian approach with the $MCMC_{GLMM}$ R package (Hadfield 2010), fitting the models with a binomial error structure using "multinomial2" and a flat improper prior. We analyzed data on offspring performance and female investment in current and future reproduction using general linear models with a Gaussian error structure for normally distributed traits (mean larval mass at dispersal and female weight change), and using generalized linear models with a binomial error structure for larval begging and a Poisson error structure for other traits representing count data (female life span and brood size at dispersal).

Given that our main aim was to test for an overall effect of the level of handicapping on our traits of interest and given the considerable variation in mass of fishing weights (see above for further details), we treated handicapping as a continuous linear predictor, including a quadratic term to test for possible nonlinear effects of handicapping. Whenever handicapping had significant linear and quadratic effects, we presented the data with a polynomial regression ± 95% CIs (see Results section below). We included the initial weight of the female at the time of treatment as a predictor in the models to account for potential variation among different-sized females in their response to the level of handicapping. We also included brood size at the time of observation as a covariate in the model on female parental behavior, and we included brood size at dispersal in the model on female weight change because brood size influences food provisioning in this species (e.g., Smiseth, Lennox, et al. 2007; Ratz and Smiseth 2018). Finally, we included female weight change as a covariate in the model on female life-span given that prior work shows that life span is positively correlated with weight change (Gray et al. 2018). Parameter estimates for the Bayesian model are given as posterior means ± 95% CIs of 1499 samples ran for 1.5×10^6 iterations with a thinning interval of 1.0×10^3 and a burn-in of 1.0×10^3.

RESULTS

Female parental behavior

Handicapping had a positive linear effect on the amount of time females spent provisioning food to the brood, while there was a negative effect of the quadratic term of handicapping (Figure 2a; Table 1). Visual inspection of confidence intervals suggests that handicapped females spent more time provisioning food than control females, but that there was no effect of the level of handicapping among handicapped females (Figure 2a). This interpretation is supported by posthoc tests, showing that handicapped females spent more time provisioning food than control females (estimate = 1.129, lower 95% = 0.416, upper 95% = 1.940, $P_{MCMC} = 0.001$) and that there was no effect of the level of handicapping when restricting the analysis to handicapped females (estimate = 18.4, lower 95% = −15.07, upper 95% = 50.9, $P_{MCMC} = 0.278$). Handicapping had a negative linear effect on the amount time females were absent from the crypt, and there was a positive effect of the quadratic term of handicapping (Figure 2b, Table 1). Visual inspection suggests that control females were more likely to abandon the brood temporarily than handicapped females, while there was no effect of the level of handicapping among handicapped females (Figure 2b).

This interpretation is supported by posthoc tests, showing that handicapped females spent less time being absent than control females (estimate = −6.510, lower 95% = −10.6, upper 95% = −2.000, P_{MCMC} = 0.001) and that there was no effect of the level of handicapping when restricting the analysis to handicapped females (estimate = −184.7, lower 95% = −451.1, upper
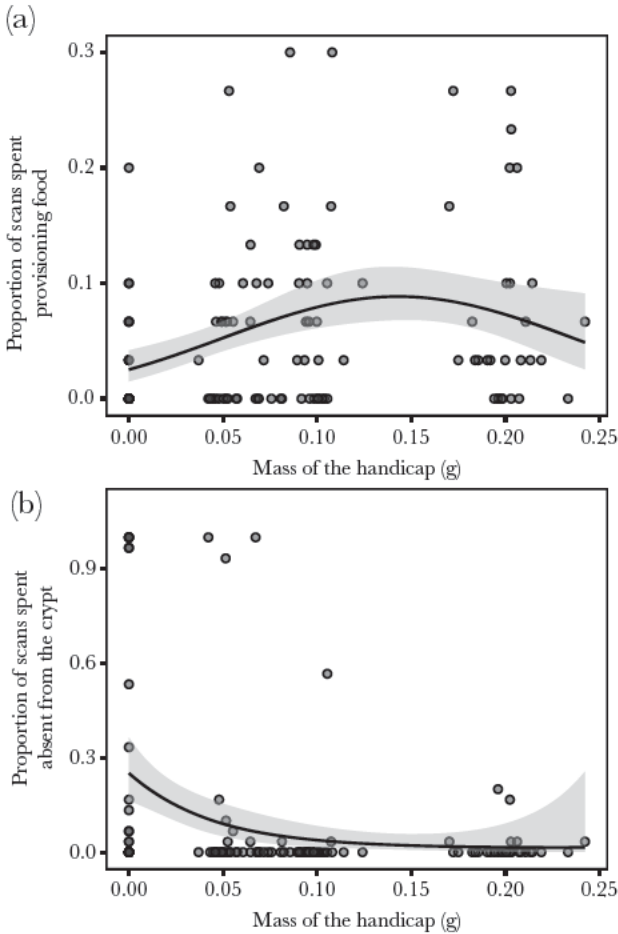

(a)

(b)

Figure 2
Effects of the level of handicapping on the proportion of time spent provisioning by the female (a) and time absent from the crypt (b). Proportions represent the total time spent provisioning or absent from the crypt during the 30-min observation period, divided by 30. The black lines represent polynomial regression lines (± 95% confidence intervals) from GLMs assuming a binomial error structure.

95% = 65.1929, P_{MCMC} = 0.108). There was no linear effect of handicapping and no effect of the quadratic term on time spent maintaining the carcass (Table 1).

There was no effect of brood size at the time of observation on time spent provisioning food (estimate = 0.136, lower 95% = −0.026, upper 95% = 0.288, P_{MCMC} = 0.092), time spent absent from the crypt (estimate = 0.036, lower 95% = −0.882, upper 95% = 0.973, P_{MCMC} = 0.925), or time spent maintaining the carcass (estimate = 0.108, lower 95% = −0.070, upper 95% = 0.282, P_{MCMC} = 0.235). Likewise, there was no effect of the initial weight of females on time spent provisioning food (estimate = −4.63, lower 95% = −10.4, upper 95% = 1.84, P_{MCMC} = 0.111), time spent absent from the crypt (estimate = 22.6, lower 95% = −18.8, upper 95% = 65.3, P_{MCMC} = 0.273), or time spent maintaining the carcass (estimate = 4.25, lower 95% = −2.69, upper 95% = 11.0, P_{MCMC} = 0.272).

Offspring performance

There were no effects of either the linear or the quadratic terms of handicapping on larval begging (Table 2). Likewise, there were no effects of the linear or the quadratic terms of handicapping on mean larval mass at dispersal (Table 2) or brood size at dispersal (Table 2). Thus, there was no evidence that larvae spent less time begging in response to handicapping of their female parent even though handicapped females spent more time provisioning food, and there was no evidence that handicapping of the female affected offspring performance. There was no effect of the initial weight of females on larval begging (estimate = −4.40, SE = 7.49, z = −0.588, P = 0.557), mean larval mass (estimate = −0.070, SE = 0.051, t = −1.38, P = 0.171), or brood size (estimate = −0.340, SE = 2.28, t = −0.149, P = 0.882).

Female investment in current and future reproduction

There were no effects of the linear or quadratic terms of handicapping on female weight change while breeding (Table 2) or female life span after breeding (Table 2). Likewise, brood size at dispersal had no effect on female relative weight change (estimate = −0.412, SE = 0.519, t = −0.795, P = 0.429). The initial weight of females had no effect on female relative weight change (estimate = 25.4, SE = 28.7, t = 0.886, P = 0.378), but it had a significant positive effect on female life span with heavier females living for longer (estimate = 0.823, SE = 0.240, z = 3.43, P = 0.001).

Table 1
Effects of handicapping (linear and quadratic terms) on time spent provisioning food, maintaining the carcass and being absent from crypt by females

	Handicapping				Handicapping²			
	Estimate	Lower 95%	Upper 95%	P_{MCMC}	Estimate	Lower 95%	Upper 95%	P_{MCMC}
Provisioning food	19.4	4.83	33.2	**0.004**	−66.1	−125.3	1.42	**0.033**
Absent from the crypt	−134.1	−238.6	−49.6	**<0.0001**	491.0	83.5	919.7	**0.008**
Maintenance of carcass	15.2	−1.18	30.7	0.056	−47.5	−124.4	18.8	0.192

Values were obtained from Bayesian Generalized Linear Models using *MCMCGLMM*. The sample sizes were 30 for control females (i.e., 0 g weight) and 90 handicapped females (i.e., 0.037–0.242 g weight), respectively.

Table 2

Effects of handicapping (linear and quadratic terms) on larval begging behavior, larval performance (mean larval mass and brood size), and female investment in current and future reproduction (female weight change and female life span)

	Handicapping				Handicapping[2]			
	Estimate	SE	t/z-value	*P*-value	Estimate	SE	t/z-value	*P*-value
Larval begging	2.17	18.1	0.120	0.904	−3.70	79.5	−0.047	0.963
Mean larval mass	0.051	0.116	0.444	0.658	−0.235	0.519	−0.454	0.651
Brood size	8.94	4.89	1.827	0.070	−35.4	22.3	−1.59	0.115
Female weight change	11.6	63.5	0.182	0.856	132.9	292.3	0.454	0.651
Female life span	0.334	0.526	0.635	0.526	−2.01	2.42	−0.830	0.406

Values were obtained from General and Generalized Linear Models. The sample sizes for larval begging, mean larval mass and female weight change were 30 for control females (i.e., 0 g weight) and 90 for handicapped females (i.e., 0.037–0.242 g weight), respectively. The sample sizes for brood size were 29 for control and 118 for handicapped females, and the sample sizes for female life span were 28 for control and 67 for handicapped females.

Finally, female weight change had no effect on female life span (estimate = −0.0003, SE = 0.0009, z = −0.300, *P* = 0.764).

DISCUSSION

Here, we tested for effects of different levels of handicapping on the amount of care provided by female parents, the performance of their offspring and female investment towards current reproduction in the burying beetle *N. vespilloides*. At the time point in larval development corresponding to the peak in parental care, handicapped females spent more time provisioning food to the brood and less time being away from the crypt than control females. This finding confirms evidence from a recent study on *N. vespilloides* reporting that handicapped females provide more care than control females (Ratz and Smiseth 2018). We found no evidence of females providing less care as the level of handicapping increased. Furthermore, there was no evidence that handicapping influenced time spent maintaining the carcass by females, larval begging behavior, larval performance (i.e., mean larvae size at dispersal and larval survival until dispersal), or female investment in current and future reproduction (i.e., weight change over the reproductive attempt or female life span after breeding). Below, we provide a more detailed discussion of our results and their implications for our understanding of how handicapping affects parental care decisions.

Our main finding was that handicapped females spent more time provisioning food than control females, but that there was no effect of the level of handicapping among handicapped females. The first finding is consistent with prior work on this species showing that handicapped females spend more time provisioning food (Ratz and Smiseth 2018). Handicapped females are predicted to provide more care than control females if handicapping suppresses the female's condition below the threshold value triggering terminal investment (Duffield et al. 2017). Thus, our results provide further evidence that handicapping can trigger terminal investment and suggest that even the smaller handicaps used in our experiment were sufficient to suppress the female's condition below the threshold value. The second finding (i.e., that there was no effect of the level of handicapping among handicapped females) is consistent with what we predicted if handicapping primarily induced a shift towards greater investment in current reproduction (Figure 1b). In contrast, if handicapping both induced such a shift and increased the energetic cost of care, we predicted that handicapped females should provide progressively less care as the level of handicapping increased (Figure 1c). One potential explanation for why we found no evidence that handicapped females provided less care as the level

of handicapping increased is that our handicaps were too small to increase the energetic cost of care. This explanation seems unlikely given that our experiment included handicaps that were substantially larger than those used in prior studies on burying beetles reporting that handicapped females provided less care than control females (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016), Thus, our results have important implications for our understanding of handicapping by confirming that its effects on parental behavior cannot be explained simply as a consequence of an increase in the energetic cost of providing a given level care, as implicitly assumed in prior handicapping experiments (Ratz and Smiseth 2018).

An alternative explanation for why handicapped females provide more care than control females is that handicapping might have a differential effect on activities associated with different modes of locomotion. For example, in burying beetles, females walk while caring for their current brood, while they fly while searching for carcasses for use in future reproductive attempts (Scott 1998). Increasing the level of handicapping might trigger a shift towards greater investment in current reproduction if handicapping has a greater impact on the energetic cost of flight than on the energetic cost of walking. There is some support for this suggestion from prior work on the burying beetle *N. quadripunctatus* indicating that handicapped females cease flying but continue walking (Suzuki and Nagano 2009). Handicapping may have limited impact on walking in these beetles given that females have been reported to move vertebrate carcasses weighing up to 30 g (i.e., objects weighing over 100 times more than the largest handicaps used in our experiment) for several meters (Scott 1998). Thus, our results may reflect that handicapping in burying beetles may have a greater impact on the cost of locating a new carcass required for initiating a future reproductive attempt than on the cost of providing care in the current reproductive attempt.

Our finding that handicapped females provided more care than control females contrasts with prior handicapping experiments on birds (e.g., Wright and Cuthill 1989; Harrison et al. 2009) and other species of burying beetles (*N. quadripunctatus*: Suzuki and Nagano 2009; Suzuki 2016; *N. orbicollis*: Creighton et al. 2015) reporting that handicapped parents provide less care than controls. One potential explanation for why our results differ from those of prior studies is that handicapping primarily increases the cost of care in birds and other species of burying beetles, while it primarily triggers a shift towards greater investment in current reproduction in our study species. For example, in altricial birds, parents fly continuously between the nest and the foraging sites in the surrounding environment to

provision their nestlings with arthropods or other sources of food. Thus, we might expect handicapping to have greater impact on the energetic cost of care in birds than in our study species. Although this suggestion might explain why our results differ from prior studies on birds, it seems unlikely that it accounts for the difference between our study species and other species of burying beetles. The reason for this is that all burying beetles breed on carcasses of small vertebrates and that, in all species, parents walk rather than fly while caring for their larvae. Instead, the different results from studies on different species of burying beetles might reflect differences in their life histories. For example, a recent study shows that larval survival is more dependent on parental care in *N. orbicollis* than in *N. vespilloides* (Capodeanu-Nägler et al. 2016). Thus, there may be differences between species of burying beetles with respect to the returns on investment in current reproduction. Alternatively, there might be differences in the availability of resources for investment in future reproduction between different species. If so, this might lead to interspecific variation in the trade-off between current and future reproduction. Currently, relatively little is known about differences between species of burying beetles with respect to availability of resources and the trade-off between current and future reproduction. Thus, obtaining such information should now be a priority to help explaining why studies on different species of burying beetles sometimes find somewhat different results.

One potential explanation for why our results differ from those of prior studies on burying beetles is that females may respond differentially to handicapping depending on whether they are assisted by a male partner or not. In our study, as well as in the prior study reporting that handicapped females provided more care than controls (Ratz and Smiseth 2018), handicapped and control females reared their brood on their own without assistance from a male partner. In contrast, handicapped and control females reared their brood with the assistance from a male partner in studies reporting that handicapped females provided less care than controls (Suzuki and Nagano 2009; Creighton et al. 2015; Suzuki 2016). Thus, handicapped females might provide less care when assisted by a male partner, while they provide more care when rearing the brood on their own. Such a differential response to handicapping might be expected if the presence of a male partner buffers against any negative effects on offspring should females provide less care. If so, handicapped females could reduce their contribution towards care without harming their offspring's fitness when assisted by a male partner, while this would not be the case when rearing the brood on their own. Thus, there is now a need for studies that investigate whether female burying beetles respond differentially to handicapping depending on whether they are assisted by a male partner or not.

We found that handicapped females spent less time being absent from the crypt than control females. Currently, little is known about why breeding females temporarily leave the crypt in this species, but potential explanations are that females do so to explore the surrounding area for signs of conspecific intruders and/or predators. Thus, our results suggest that handicapped females are less inclined to explore the surrounding area than control females. An alternative explanation is that handicapped females remained within the crypt simply as a consequence of reduced mobility. However, if this was the case, we should also expect handicapped females to spend less time provisioning food than control females given that this behavior also requires mobility. Thus, given that we found that handicapped females spent more time provisioning food, this explanation seems unlikely (Figure 2). Our study highlights that there is a need to investigate why breeding females temporarily leave the crypt.

We found no evidence that handicapping affected larval begging behavior, larval performance (i.e., mean larval mass or larval survival until dispersal), or female investment in current and future reproduction (i.e., weight change over reproduction and life span after reproduction). These findings are surprising given that handicapped females spent more time provisioning food towards larvae than control females. Prior work shows that larval begging in *N. vespilloides* reflects larval hunger state (Smiseth and Moore 2004a) and that larvae grow to a larger size when receiving more care from female parents (Andrews et al. 2017). Thus, we might expect larvae reared by handicapped females to be less hungry, therefore spending less time begging, and to grow to be a larger size than larvae reared by control females. One potential explanation for why we found no such effects is that the quality of care (e.g., nutritional quality of predigested carrion transferred to larvae via mouth-to-mouth contact) was lower in handicapped females than in control females. If so, larvae might receive a similar amount of care regardless of whether they are reared by handicapped or control females. An alternative explanation is that handicapping had a differential effect at different times of the larvae's development. Our results show that handicapped females spent more time providing care at the time point in larval development corresponding to the peak in parental care (i.e., 24 h after hatching) than control females. Given that we recorded effects on female parental behavior at a single time point, we cannot rule out the possibility that handicapped females provided less care either earlier or later in development. Finally, we found that handicapping had no effect on female weight change during breeding or female life span. These results contrast with those of most studies on birds, showing that handicapped females lose more weight than control females (e.g., Slagsvold and Lifjeld 1990; Markman et al. 1995; Sanz et al. 2000). As discussed above, the energetic cost of care might be relatively high in birds, in which case we might expect handicapped females to lose more weight than controls. In contrast, the energetic cost of care might be relatively low in burying beetles. There is also evidence that parents forage from the carcass while breeding (Pilakouta et al. 2016), which may allow handicapped females to compensate for the energetic cost of handicapping by consuming more food from the carcass (Ratz and Smiseth 2018).

Our study adds to our understanding of the terminal investment hypothesis, that is, the suggestion that parents should increase their investment in reproduction during their final reproductive attempt (Williams 1966; Hirshfield and Tinkle 1975; Clutton-Brock 1984). Traditionally, the terminal investment hypothesis has focused on increases in investment in reproduction with age (Clutton-Brock 1984), but its rationale applies to any factor that suppresses the condition of parents below a certain threshold that reduces their prospects for future reproduction. Indeed, there is mounting evidence that terminal investment is triggered by a range of factors other than age, including immune challenges (e.g., Podmokła et al. 2014), intraspecific competition (e.g., Rebar and Greenfield 2017) and predation risk (e.g., Knight and Temple 1986). Thus, our results suggest that handicapping can be added to the list of factors that can induce terminal investment by suppressing the parent's condition. We suggest that handicapping would provide a useful tool for studying terminal investment as it provides a simple experimental tool for suppressing an individual's condition. Given that handicaps can be removed, such experiments could be used to establish whether individuals reverse their decisions to invest more in current reproduction should their condition improve at a later stage.

In conclusion, we found that handicapped females spent more time providing care than control females, possibly reflecting that handicapping suppresses the condition of females below the threshold triggering terminal investment (Duffield et al. 2017). Our results have important implications for our understanding of the effects of handicapping, which is a key experimental tool used by behavioral ecologists to study negotiation between parents in species with biparental care (Harrison et al. 2009). Such studies are based on the assumption that handicapping primarily increases the energetic cost of care, and our results show that this is not necessarily the case. This conclusion emphasizes that handicapping experiments can lead to different outcomes in different species, presumably reflecting differences in the modes of locomotion of caring parents, differences in life histories, and/or differential responses depending on the presence or absence of a partner. Thus, we encourage further handicapping experiments across a variety of different taxa and social contexts.

Data accessibility: Analyses reported in this article can be reproduced using the data provided by Ratz et al. (2019).

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REFERENCES

Andrews CP, Kruuk LE, Smiseth PT. 2017. Evolution of elaborate parental care: phenotypic and genetic correlations between parent and offspring traits. Behav Ecol. 28:39–48.

Capodeanu-Nägler A, Keppner EM, Vogel H, Ayasse M, Eggert AK, Sakaluk SK, Steiger S. 2016. From facultative to obligatory parental care: interspecific variation in offspring dependency on post-hatching care in burying beetles. Sci Rep. 6:29323.

Clutton-Brock TH. 1984. Reproductive effort and terminal investment in iteroparous animals. Am Nat. 123:212–219.

Clutton-Brock TH. 1991. The evolution of parental care. Princeton (NJ): Princeton University Press.

Creighton CJ, Smith AN, Komendat A, Belk MC. 2015. Dynamics of biparental care in a burying beetle: experimental handicapping results in partner compensation. Behav Ecol Sociobiol. 69:265–271.

Duffield KR, Bowers EK, Sakaluk SK, Sadd BM. 2017. A dynamic threshold model for terminal investment. Behav Ecol Sociobiol. 71:185.

Eggert Ak, Reinking M, Muller JK. 1998. Parental care improves offspring survival and growth in burying beetles. Anim Behav. 55:97–107.

Gray F, Richardson J, Ratz T, Smiseth PT. 2018. No evidence for parent-offspring competition in the burying beetle *Nicrophorus vespilloides*. Behav Ecol. 29:1142–1149.

Hadfield JD. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. J Stat Softw. 33:1–22.

Harrison F, Barta Z, Cuthill I, Székely T. 2009. How is sexual conflict over parental care resolved? A meta-analysis. J Evol Biol. 22:1800–1812.

Hirshfield MF, Tinkle DW. 1975. Natural selection and the evolution of reproductive effort. Proc Natl Acad Sci USA 72:2227–2231.

Knight RL, Temple SA. 1986. Nest defence in the American goldfinch. Anim Behav. 34:887–897.

Markman S, Yom-Tov Y, Wright J. 1995. Male parental care in the orange-tufted sunbird: behavioural adjustments in provisioning and nest guarding effort. Anim Behav. 50:655–669.

Müller JK, Eggert A-K. 1990. Time-dependent shifts between infanticidal and parental behavior in female burying beetles: a mechanism of indirect mother-offspring recognition. Behav Ecol Sociobiol. 27:11–16.

Otronen M. 1988. The effect of body size on the outcome of fights in burying beetles. Ann Zool Fenn. 25:191–201.

Pilakouta N, Richardson J, Smiseth PT. 2016. If you eat, I eat: resolution of sexual conflict over consumption from a shared resource. Anim Behav. 111:175–180.

Podmokła E, Dubiec A, Drobniak SM, Arct A, Gustafsson CM. 2014. Avian malaria is associated with increased reproductive investment in the blue tit. J Avian Biol. 45:219–224.

Ratz T, Nichol TW, Smiseth PT. 2019. Data from: parental responses to increasing levels of handicapping in a burying beetle. Dryad Digital Repository. http://dx.doi.org/10.5061/dryad.3q4g02f

Ratz T, Smiseth PT. 2018. Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*. J Evol Biol. 31:646–656.

Rauter CM, Moore AJ. 1999. Do honest signalling models of offspring solicitation apply to insects? Proc Biol Sci. 266:1691–1696.

Rauter CM, Moore AJ. 2004. Time constraints and trade-offs among parental care behaviours: effects of brood size, sex and loss of mate. Anim Behav. 68:695–702.

R Development Core Team 2019. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Rebar D, Greenfield MD. 2017. When do acoustic cues matter? Perceived competition and reproductive plasticity over lifespan in a bushcricket. Anim Behav. 128:41–49.

Royle NJ, Russell AF, Wilson AJ. 2014. The evolution of flexible parenting. Science. 345:776–781.

Royle NJ, Smiseth PT, Kölliker M. 2012. The evolution of parental care. Oxford (UK): Oxford University Press.

Safryn SA, Scott MP. 2000. Sizing up the competition: do burying beetles weigh or measure their opponents? J Insect Behav. 13:291–297.

Sanz JJ, Kranenbarg S, Tinbergen JM. 2000. Differential response by males and females to manipulation of partner contribution in the great tit (*Parus major*). J Anim Ecol. 69:74–84.

Scott MP. 1998. The ecology and behavior of burying beetles. Annu Rev Entomol. 43:595–618.

Slagsvold T, Lifjeld JT. 1988. Ultimate adjustment of clutch size to parental feeding capacity in a passerine bird. Ecology. 69:1918–1922.

Slagsvold T, Lifjeld JT. 1990. Influence of male and female quality on clutch size in tits (Parus spp.). Ecology. 71:1258–1266.

Smiseth PT, Darwell CT, Moore AJ. 2003. Partial begging: an empirical model for the early evolution of offspring signalling. Proc Biol Sci. 270:1773–1777.

Smiseth PT, Dawson C, Varley E, Moore AJ. 2005. How do caring parents respond to mate loss? Differential response by males and females. Anim Behav. 69:551–559.

Smiseth PT, Lennox L, Moore AJ. 2007. Interaction between parental care and sibling competition: parents enhance offspring growth and exacerbate sibling competition. Evolution. 61:2331–2339.

Smiseth PT, Moore AJ. 2002. Does resource availability affect offspring begging and parental provisioning in a partially begging species? Anim Behav. 63:577–585.

Smiseth PT, Moore AJ. 2004a. Signalling of hunger when offspring forage by both begging and self-feeding. Anim Behav. 67:1083–1088.

Smiseth PT, Moore AJ. 2004b. Behavioral dynamics between caring males and females in a beetle with facultative biparental care. Behav Ecol. 15:621–628.

Smiseth PT, Ward RJ, Moore AJ. 2007. Parents influence asymmetric sibling competition: experimental evidence with partially dependent young. Ecology. 88:3174–3182.

Suzuki S. 2016. When the male determines his provisioning effort: does the timing of handicapping affect the negotiation between parents in *Nicrophorus quadripunctatus*? Behaviour. 153:1435–1443.

Suzuki S., Nagano M. 2009. To compensate or not? Caring parents respond differentially to mate removal and mate handicapping in the burying beetle, *Nicrophorus quadripunctatus*. Ethology. 115:1–6.

Williams GC. 1966. Natural selection, the costs of reproduction, and a refinement of Lack's principle. Am Nat. 100: 687–690.

Wright J., Cuthill I. 1989. Manipulation of sex differences in parental care. Behav Ecol Sociobiol. 25:171–181.

Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*

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Abstract

Parental care is highly variable, reflecting that parents make flexible deci
sions in response to variation in the cost of care to themselves and the bene
fit to their offspring. Much of the evidence that parents respond to such
variation derives from handicapping and brood size manipulations, the sepa
rate effects of which are well understood. However, little is known about
their joint effects. Here, we fill this gap by conducting a joint handicapping
and brood size manipulation in the burying beetle *Nicrophorus vespilloides*.
We handicapped half of the females by attaching a lead weight to their
pronotum, leaving the remaining females as controls. We also manipulated
brood size by providing each female with 5, 20 or 40 larvae. In contrast to
what we predicted, handicapped females spent *more* time provisioning food
than controls. We also found that handicapped females spent more time
consuming carrion. Furthermore, handicapped females spent a similar
amount of time consuming carrion regardless of brood size, whereas con
trols spent more time consuming carrion as brood increased. Females spent
more time provisioning food towards larger broods, and females were more
likely to engage in carrion consumption when caring for larger broods. We
conclude that females respond to both handicapping and brood size manipu
lations, but these responses are largely independent of each other. Overall,
our results suggest that handicapping might lead to a higher investment into
current reproduction and that it might be associated with compensatory
responses that negate the detrimental impact of higher cost of care in handi
capped parents.

Introduction

Parental care is defined as any parental trait that has
evolved to enhance the survival and/or growth of the
parent's offspring, often at cost to the parent's own fit
ness (Royle *et al.*, 2012). Typical forms of care include
protection against predators and other environmental
hazards, and provisioning of food or other resources
after hatching or birth (Smiseth *et al.*, 2012). In many
species, parental care is highly variable, reflecting that
parents make flexible decisions about how much care
to provide in response to variation in the cost of care to

themselves and the benefit of care to their offspring
(Alonso alvarez & Velando, 2012; Royle *et al.*, 2014). In
general, parents are expected to provide less care when
the cost of care is higher and provide more care when
the benefit of care is higher (Grodzinski & Johnstone,
2012). Much of the experimental evidence for these
two predictions derives from handicapping and brood
size manipulations, respectively. For example, handi
capping experiments in birds and insects (based on
attachments of weights or feather clipping) show that
handicapped parents decrease their care, presumably
because handicapping elevates the cost of care to par
ents (Wright & Cuthill, 1989; Harrison *et al.*, 2009;
Suzuki & Nagano, 2009). Likewise, brood size manipu
lations in birds, fishes and insects show that parents
usually provide more care towards enlarged broods,
presumably because the benefit of care is higher, while
parents provide less care towards reduced broods,

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presumably because the benefit of care is lower (e.g. Ridgway, 1989; Sanz, 1997; Rauter & Moore, 2004; Smiseth *et al.*, 2007). Thus, handicapping and brood size manipulations have been instrumental in providing experimental evidence showing that variation in the cost and benefit of care are key determinants of how parents make flexible decisions regarding how much care to provide for their offspring.

Although we have a good understanding of the sepa rate effects of handicapping and brood size manipula tions on the amount of care provided by parents, little is known about their joint effects. Despite the lack of formal theory, we can derive predictions from simple graphical models based on assumptions about how handicapping and brood size manipulations influence the cost and benefit functions of care (Fig. 1). These functions describe the effect that specific levels of par ental care have on parental and offspring fitness, respectively (Smiseth, 2017). The cost function is assumed either to increase at an accelerating rate or to be linear. In either case, if handicapping increases the cost of care, handicapped parents are predicted to reduce their level of care (Fig. 1), as reported for birds (Wright & Cuthill, 1989; Harrison *et al.*, 2009) and insects (Suzuki & Nagano, 2009). Meanwhile, the bene fit function is assumed to increase at a decelerating rate to reach an asymptote above which any further increase in care has no effect on offspring fitness (Tri vers, 1974; Royle *et al.*, 2012). The benefit function describes the fitness effect on an individual offspring. Thus, in order to derive the indirect benefit function to the parent, we need to account for both the coefficient of relatedness between the parent and its offspring and the number of offspring in the brood (Fig. 1). If brood size enlargement increases the benefit of care, parents should increase their care towards enlarged broods (Fig. 1), as reported for fishes (e.g. Ridgway, 1989), birds (Sanz, 1997) and insects (e.g. Rauter & Moore, 2004; Smiseth *et al.*, 2007). Furthermore, this model predicts no interaction effect (or one that is too small to be detected) if handicapping leads to only minor diver gence in the cost function at higher levels of care (Fig. 1a,b). On the other hand, it predicts an interaction effect if handicapping leads to a greater divergence in the cost function at higher levels of care (Fig. 1c,d). These predictions have never before been tested empiri cally, and here, we address this gap by conducting a joint handicapping and brood size manipulation experi ment in the burying beetle *Nicrophorus vespilloides*.

Burying beetles of the genus *Nicrophorus* are ideal for studying the joint effects of handicapping and brood size as prior studies show that parents respond to both treatments (handicapping: Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Suzuki, 2016; brood size manip ulations: Rauter & Moore, 2004; Smiseth *et al.*, 2007). These beetles breed on carcasses of small vertebrates that serve as the sole food source for the brood during

larval development (Eggert *et al.*, 1998; Scott, 1998). Larvae can obtain resources by either feeding directly from the carcass or begging for predigested carrion from the parents (Smiseth & Moore, 2002; Smiseth *et al.*, 2003). In *N. vespilloides*, begging reflects the offspring's nutritional need (Smiseth & Moore, 2004b) and is costly to the offspring in terms of increased risk of filial cannibalism (Andrews & Smiseth, 2013). Prior work on *N. vespilloides* and *Nicrophorus orbicollis* shows that par ents respond to brood size manipulations by increasing their food provisioning rate towards larger broods (Rau ter & Moore, 2004; Smiseth *et al.*, 2007). Moreover, prior work on *Nicrophorus quadripunctatus* and *N. orbicol lis* shows that handicapped parents provide less care than control parents (Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Suzuki, 2016). Although the reduction in parental care by handicapped parents is generally attributed to an increase in the cost of care, this response may also be caused by deteriorating con dition of handicapped parents (Pilakouta *et al.*, 2015) or by stress induced by handicapping. Regardless of how handicapping leads to a reduction in parental care, there is no information on the joint effects of handicap ping and brood size manipulations on the amount of care provided by parents.

Our main aim was to examine joint effects of handi capping and brood size on the overall level of care pro vided by females and on female weight change during breeding. The latter is used as a proxy for how much females consume from the carcass to invest into their future reproduction (Creighton *et al.*, 2009; Billman *et al.*, 2014). We expect an effect of the interaction between handicapping and brood size only if handicap ping leads to a greater divergence in the cost function at higher levels of care (Fig. 1d). We predict main effects of handicapping and brood size, reflecting that weighted females provide less care to the brood than control females and that females provide more care to larger broods than to smaller ones. We predict an effect of the interaction between handicapping and brood size and main effects of handicapping and brood size on the amount of time spent provisioning food by parents. The reason for this is that this form of parental care is direc ted towards individual offspring within the brood (un like other forms of care, such as carcass maintenance). We also predict that handicapping and an increase in brood size would be associated with a greater loss in weight of females, reflecting that weighted females pay a greater cost from their investment into the current brood and that larger broods require more care. Our second main aim was to test for subsequent conse quences of handicapping and brood size on offspring begging and offspring performance. We predict that handicapping of females would lead to an increase in larval begging and have a detrimental impact on larval fitness given that weighted females would spend less time provisioning food to the brood. Similarly, we
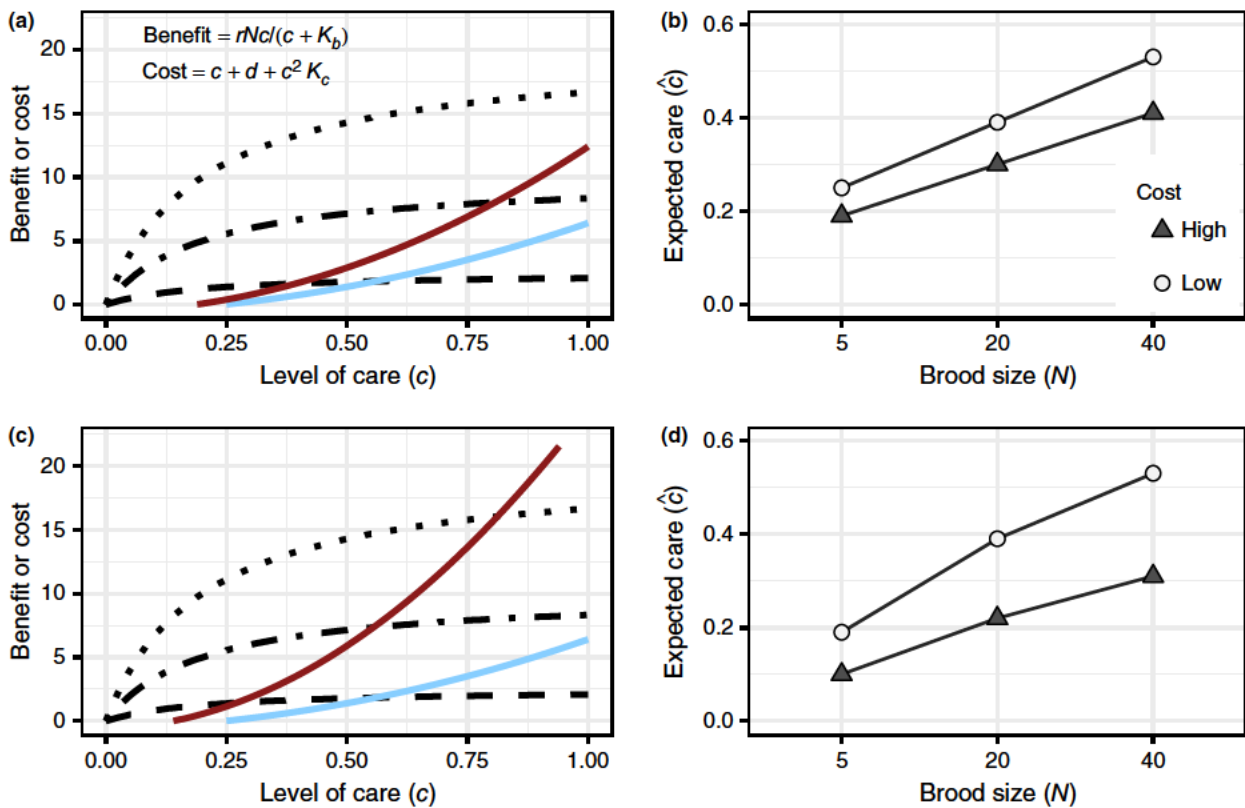
Fig. 1 Direct cost and indirect benefit functions of parental care in relation to the level of care (a, c). The cost functions (blue and red lines) increase at an accelerating rate, and the benefit functions (black lines) increase at a decelerating rate when a parent increases its level of parental care c. The specific cost and benefit returns to a parent depend on the coefficient of relatedness between the parent and its offspring (here, r 0.5), the brood size N, the intercept b of the cost function, and the shapes k_c and k_b of the cost and benefit functions, respectively (adapted from Kölliker et al., 2010). The indirect benefit of care to the parent increases with the number of offspring in the brood, which in this case varies between 5 (dashed line), 20 (dashed dotted line) or 40 offspring (dotted line). The direct cost of care to the parent may be relatively low (blue line, $k_{c\ Low}$ 6) or high (red line), depending on whether females are handicapped or not. Handicapping may affect the slope of the cost function, shown here as the divergence in functions (red lines) at higher levels of care, here with $k_{c\ High}$ 10 (a) and $k_{c\ High}$ 24 (c). This model predicts that parents should provide less care when the cost of care is higher and the benefit of care is lower. The model also predicts that there should be an effect of the interaction between the cost and benefit of care if handicapping leads to a greater divergence in the cost function at higher levels of care (d). On the other hand, there may be no such an effect (or it may not be detectable) if handicapping leads to a minor divergence in the cost function at higher levels of care (b).

predict that an increase in brood size would lead to an increase in larval begging and have a detrimental impact on larval performance given that larger broods should be associated with more intense sibling competition (Smiseth et al., 2007).

Materials and methods

Beetle husbandry

We used the second to the fifth generation of beetles from a laboratory population of outbred beetles descending from a population collected in Corstorphine Hill, Edinburgh, UK. Adult beetles were kept individually in transparent plastic containers (12 × 8 × 2 cm)

filled with moist soil. The laboratory conditions were kept constant throughout the experiment; that is, the beetles were kept at 20 °C and under a 16 : 8 h light: dark photoperiod. Nonbreeding beetles were fed small pieces of beef twice a week.

Experimental design

We used a 2 × 3 factorial design to examine effects of handicapping of the female parent (weighted or control females) as one factor and brood size (5, 20 or 40 larvae) as the other factor. Previous work has found that weighted (i.e. handicapped) parents reduce their amount of parental care in the closely related N. quadripunctatus (Suzuki & Nagano, 2009) and

N. orbicollis (Creighton *et al.*, 2015). Meanwhile, brood size manipulations on *N. vespilloides* and *N. orbicollis* show that parents provide more care towards larger broods (Rauter & Moore, 2004; Smiseth *et al.*, 2007). In this experiment, we chose brood sizes of 5, 20 and 40 larvae as treatment levels reflecting that broods range in size from 2 to 47 larvae under laboratory conditions with a mean brood size of 21 larvae (Smiseth & Moore, 2002).

We selected an initial number of 231 virgin females for use in the experiment. At the start of the experiment, each female was paired with an unrelated virgin male. The pair was placed in a larger plastic container ($17 \times 12 \times 6$ cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass of a standardized size (22.31 ± 0.002 g; range: 20.45 23.51 g; Livefoods Direct, Sheffield, UK). Containers were checked for the presence of eggs the following days, and egg laying date was recorded as the day where the first eggs were laid. Females were randomly assigned to a handicapping treatment (weighted or control) 1 day before the expected hatching date. At this stage, we moved females and their mouse carcasses into new boxes filled with fresh soil. We did this to separate females from their eggs, such that the larvae hatching from the eggs could be used to generate experimental broods of different sizes (Smiseth *et al.*, 2007). At this time, we also removed males because males often desert the brood before hatching and the presence or absence of males in *N. vespilloides* has no detectable impact on offspring fitness under laboratory conditions (Smiseth *et al.*, 2005). As soon as the eggs hatched, we randomly allocated each female a brood of newly hatched unrelated offspring made up of either 5, 20 or 40 larvae. We only allocated a female with an experimental brood once her own eggs had hatched given that parents will kill any larvae that emerge on the carcass before their own eggs have hatched (Müller & Eggert, 1990).

In parallel with the experimental females used in the experiments, we set up a total of 485 pairs of nonexperimental parents. These parents produced foster larvae that were used to generate the experimental foster broods. The foster broods were always of mixed maternity, which allowed us to eliminate any potential prenatal maternal effects associated with our handicapping treatments that can have had confounding effects on offspring and parental behaviours (Paquet *et al.*, 2015).

Handicapping procedure

To test the effects of handicapping on parental care, we weighted breeding females in the gap between the end of egg laying and the beginning of hatching. In this species, this gap occurs during the 2 days following the beginning of egg laying (Müller & Eggert, 1990). For weighted (handicapped) females, we attached a small lead weight to the pronotum of the female using instant adhesive glue, as described in previous studies on the closely related *N. quadripunctatus* (Suzuki & Nagano, 2009; Suzuki, 2016) and *N. orbicollis* (Creighton *et al.*, 2015). In both species, handicapping reduced mobility of adult beetles and affected parental care behaviours by reducing the frequency of direct and indirect care (Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Suzuki, 2016). In our study, the mass of the weight together with the glue (0.06 ± 0.0008 g) represented approximately 20% of the initial female body mass (n 116, 0.30 ± 0.004 g) measured shortly before handicapping. During the course of the experiment, we noticed that sizeable amounts of dirt were accumulating around the weight due to the digging behaviour of the burying beetles. This formed a lump on the pronotum and induced handicapped females to carry a total mass (i.e. lead weight + dirt) of approximately 30% their initial body mass (mean $\pm$ SE mass of dirt: 0.014 ± 0.0013 g). We had a control treatment of females that were of a similar body mass to the experimental females (n 101, 0.30 ± 0.005 g). The control females were treated in the same way as the experimental females (i.e. these beetles were handled and disturbed), except that they had no weight attached to them.

Among the initial 231 experimental broods, 41 were excluded from the analysis for the following reasons: females lost their weights (n 12) or died (n 3) before the behavioural observations, females could not be allocated a foster brood (n 4), females failed to produce eggs (n 6), no eggs hatched from the clutch (n 9), or eggs hatched before females were handicapped (n 7). In addition to this, 11 broods were included in the behavioural analysis but excluded from analyses on fitness related traits because the females had lost their weights or died between the time of observation and the time of larval dispersal. The final sample sizes for the different treatment groups were as follows for the behavioural traits measured 1 day after hatching (n_{d1}) and the fitness traits measured at larval dispersal (n_{disp}): control females with brood size of five larvae: n_{d1} n_{disp} 29; control females with a brood size of 20 larvae: n_{d1} n_{disp} 29; control females with a brood size of 40 larvae: n_{d1} n_{disp} 34; weighted females with a brood size of five larvae: n_{d1} 33 and n_{disp} 29; weighted females with a brood size of 20 larvae: n_{d1} 35 and n_{disp} 31; and weighted females with a brood size of 40 larvae: n_{d1} 30 and n_{disp} 27.

Female and offspring behaviours

We recorded parental and larval behaviours 24 h (± 15 min) after the larvae were placed on the carcass, as this stage corresponds to the period when there is a peak in female food provisioning (Smiseth *et al.*, 2003, 2007). Behavioural observations were performed under

red light using instantaneous sampling every 1 min for 30 min. Both parental and larval behaviours were simultaneously observed and scored following methods described in previous studies (e.g. Smiseth & Moore, 2002, 2004a, b). To summarize briefly, we recorded the occurrence of parental food provisioning as the number of scans where there was mouth to mouth contacts with larvae, carcass maintenance as the number of scans where the female was spreading secretions on the surface of the carcass or excavating the crypt (i.e. the depression in the soil surrounding the carcass), and carrion consumption as the number of scans where female was feeding within the crater (i.e. the opening on the top of the carcass).

At each scan, we also recorded the number of larvae that were begging. We considered a larva to be begging when it raised its head towards the female while waving its legs or when it touched the female with its legs (Smiseth & Moore, 2002). We then calculated the average proportion time spent begging per larva in the brood as B $(\Sigma b/n)/l$, where Σb is the total number of begging events during an observation session, n is the number of larvae in the brood at the time of observation, and l is the number of scans for which the female was near the larvae (Smiseth et al., 2003). We included the latter because larvae only beg when the parent is in close vicinity (i.e. less than or equal to the female's pronotum width; Rauter & Moore, 1999; Smiseth & Moore, 2002; Smiseth et al., 2007). Thus, this measure of begging is largely independent of the female's behaviour towards the larvae (Smiseth & Moore, 2004a).

Female weight change and offspring performance

To assess the consequences of handicapping and brood size on how much females consume from the carcass to invest in future reproduction, we measured the relative change in mass of females over the reproductive period. We estimated female weight change as the difference between the female's initial weight on the day preceding the hatching of her eggs and her final weight at the time of larval dispersal. We also tested for effects of handicapping and brood size on two measures of offspring performance: larval survival until dispersal and mean larval mass at dispersal. We measured effects on larval mass at dispersal because it determines adult body size, which in turn is known to be a major determinant of competitive ability and breeding success as adult in Nicrophorus species (Otronen, 1988; Safryn & Scott, 2000).

Statistical analyses

All statistical analyses were conducted using R v 3.3.3 (R Development Core Team, 2011) loaded with the packages car (Fox & Weisberg, 2017), MASS (Ripley et al., 2017), aod (Lesnoff & Lancelot, 2012) and MCMCglmm (Hadfield, 2010). Given that the behavioural traits in our experiment were count data bounded between 0 and 30 scans, we analysed the data using a binomial error distribution. We used general linear models for traits with a Gaussian distribution (female relative mass change and larval body mass at dispersal) and generalized linear models with a quasi binomial distribution for traits that represent binary or count data with an upper limit (larval survival rate and larval begging). We used Bayesian generalized linear models fitted with a binomial error distribution to analyse food provisioning to the brood and carcass maintenance, whereas we used a Bayesian zero inflated binomial model for carrion consumption to control for overdispersion and zero inflation. All Bayesian models were run using flat improper priors. We present parameter estimates for the Bayesian models as posterior means with 95% credible intervals of 2600 samples ran for 5.2×10^5 iterations with a thinning interval of 200 and a burn in of 6×10^4. Outputs from the Bayesian zero inflated binomial model allow us to test both the probability that females engaged into carrion consumption and, when consuming carrion at least once, how much time (i.e. number of scans) females spent consuming carrion during the observation period. All models included female handicapping treatment (control or weighted) and brood size (5, 20 or 40 larvae) and the interaction between them as fixed effects. Brood size was treated as a categorical predictor in the general linear and generalized linear models, whereas it had to be treated as a continuous predictor in the Bayesian models. In the general linear and generalized linear models, we used post hoc contrasts whenever handicapping and/or brood size had a significant effect on the variable of interest to test for differences between each treatment group or brood size category. In these tests, we used the Bonferroni correction for multiple testing.

Results

Female parental behaviour and weight change

There was no evidence of an effect of the interaction between handicapping and brood size on any of the two female parental behaviours (i.e. food provisioning and carcass maintenance) (Table 1; Fig. 2a,b) or on female weight change during the breeding attempt (Table 2; Fig. 2d). However, there was an effect of this interaction on the amount of time spent consuming carrion by females (Count model; Table 1). This interaction effect reflected that control females spent more time consuming carrion as brood increased, whereas weighted females spent a similar amount of time at this behaviour regardless of brood size (Fig. 2c).

Handicapping had a significant effect on the amount of time spent provisioning food to the brood and consuming carrion (Table 1). Contrary to what we

Table 1 Effects of the interaction between handicapping (weighted or control females) and brood size (5, 20 or 40 larvae) and the main effects of handicapping and brood size on female parental behaviours, that is food provisioning, carcass maintenance and female carrion consumption. Values are obtained from Bayesian GLMs using *MCMCglmm*.

	Interaction				Handicapping (weighted vs. control)				Brood size (continuous)			
	Mean	l 95%	u 95%	P_{MCMC}	Mean	l 95%	u 95%	P_{MCMC}	Mean	l 95%	u 95%	P_{MCMC}
Food provisioning	0.011	0.035	0.014	0.368	1.00	0.340	1.74	**0.002**	0.062	0.043	0.080	**< 0.0005**
Carcass maintenance	0.005	0.021	0.028	0.699	0.296	0.949	0.330	0.380	0.015	0.003	0.031	0.098
Carrion consumption												
Binary model	0.056	0.050	0.174	0.239	1.94	3.66	0.309	**0.023**	0.094	0.181	0.028	**< 0.0005**
Count model	0.023	0.046	0.001	**0.039**	0.856	0.249	1.45	**0.007**	0.008	0.009	0.024	0.377

Statistically significant *P* values (< 0.05) are shown in boldface.
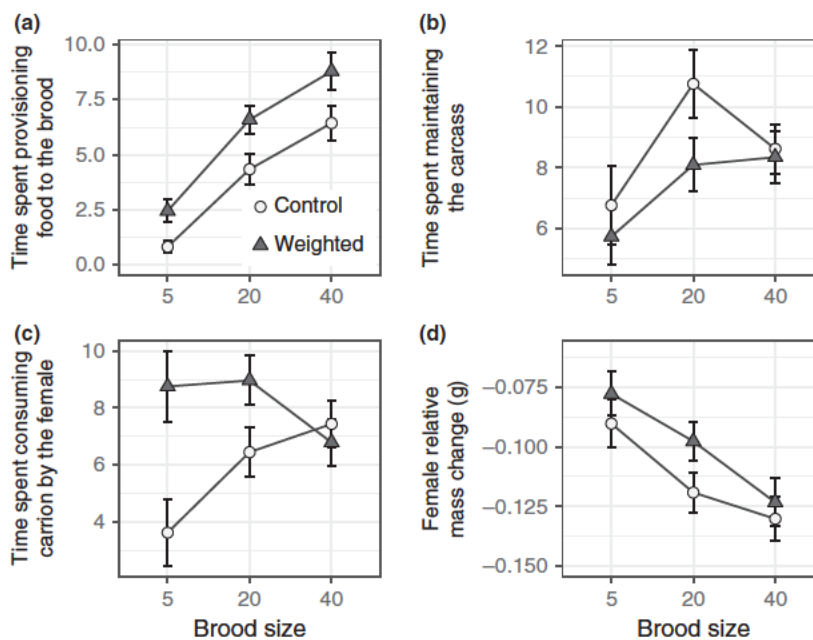
Fig. 2 Effects of handicapping (weighted or control females) and brood size manipulation (5, 10 or 20 larvae) on the time spent (number of scans) by the female provisioning food to the brood (a), maintaining the carcass (b), consuming carrion (c) and on female weight change over the reproductive attempt (d). Mean ± SE.

Table 2 Effects of the interaction between handicapping (weighted or control females) and brood size (5, 20 or 40 larvae) and the main effects of handicapping and brood size on female mass change, larval begging and offspring performance (larval survival and mean larval mass). Values obtained from general linear models (female mass change and mean larval mass) and generalized linear models (larval begging and larval survival).

	Interaction			Handicapping			Brood size		
	LR χ^2	d.f.	P	LR χ^2	d.f.	P	LR χ^2	d.f.	P
Female mass change	0.645	2	0.724	3.34	1	0.067	21.2	2	**< 0.0001**
Larval begging	1.15	2	0.535	0.006	1	0.938	21.3	2	**< 0.0001**
Larval survival	2.34	2	0.310	0.016	1	0.899	12.6	2	**0.002**
Mean larval mass	5.17	2	0.075	0.029	1	0.864	167	2	**< 0.0001**

LR, likelihood ratio.
Statistically significant *P* values (< 0.05) are shown in boldface.

predicted, weighted females spent *more* time provisioning food to the brood than control females (Table 1; Fig. 2a). Weighted females were also more likely to engage in carrion consumption and spent more time consuming carrion overall (Table 1; Fig. 2c). There was no evidence that handicapping had an effect on carcass

maintenance or female weight change (Tables 1 and 2; Fig. 2b,d).

Brood size had a significant effect on the amount of time spent provisioning food to the brood, the probability that females engaged in carrion consumption, as well as on female weight change (Tables 1 and 2; Fig. 2). Females spent more time provisioning food towards larger broods (Table 1, Fig. 2a). Likewise, females were more likely to engage in carrion consumption when caring for larger brood (Binary model; Table 1). Finally, females lost more weight when caring for broods of 20 than for broods of five larvae (Contrast 20 vs. 5 larvae: Estimate 0.024, SE 0.009, z 2.67, P 0.02), but lost a similar amount of weight when caring for broods of 20 and 40 larvae (Contrast 40 vs. 20 larvae: Estimate 0.018, SE 0.009, z 1.98, P 0.14). There was no effect of brood size on the amount of time spent maintaining the carcass (Table 1; Fig. 2b).

Offspring begging and performance

There was no effect of the interaction between handicapping and brood size on the average amount of time spent begging by the larvae, larval survival or mean larval mass at the time of dispersal (Table 2; Fig. 3). Likewise, there were no effects of handicapping on larval begging, larval survival or mean larval mass (Table 2). However, there was an effect of brood size on larval begging, larval survival and mean larval mass (Table 2; Fig. 3a c). Larvae spent more time begging in broods of 20 or 40 larvae than in broods of five larvae (Contrast 20 vs. 5 larvae: Estimate 0.778, SE 0.183, z 4.25, $P < 0.0001$; Contrast 40 vs. 5 larvae: Estimate 0.471, SE 0.191, z 2.47, P 0.041). Likewise, larval survival and mean larval mass were higher in broods of 20 compared to broods of five larvae (Contrast 20 vs. 5 larvae: Estimate 0.579, SE 0.232, z 2.50, P 0.038 and Estimate 0.012, SE 0.004, z 2.70, P 0.02, respectively) or 40 larvae (Contrast 40 vs. 20 larvae: Estimate 0.757, SE 0.224, z 3.38, P 0.002 and Estimate 0.052, SE 0.004, z 12.22, $P < 0.0001$, respectively).

Discussion

The main aim of our study was to investigate effects of the interaction between handicapping and brood size on parental care and offspring performance in the burying beetle *N. vespilloides*. Assuming that handicapping increases the cost of care whereas brood size enlargement increases the benefit of care, we expected such interaction effects if handicapping leads to a greater divergence in the cost function at higher levels of care (Fig. 1d). We found no evidence for the presence of such an interaction effect on female parental behaviours (food provisioning and carcass maintenance),
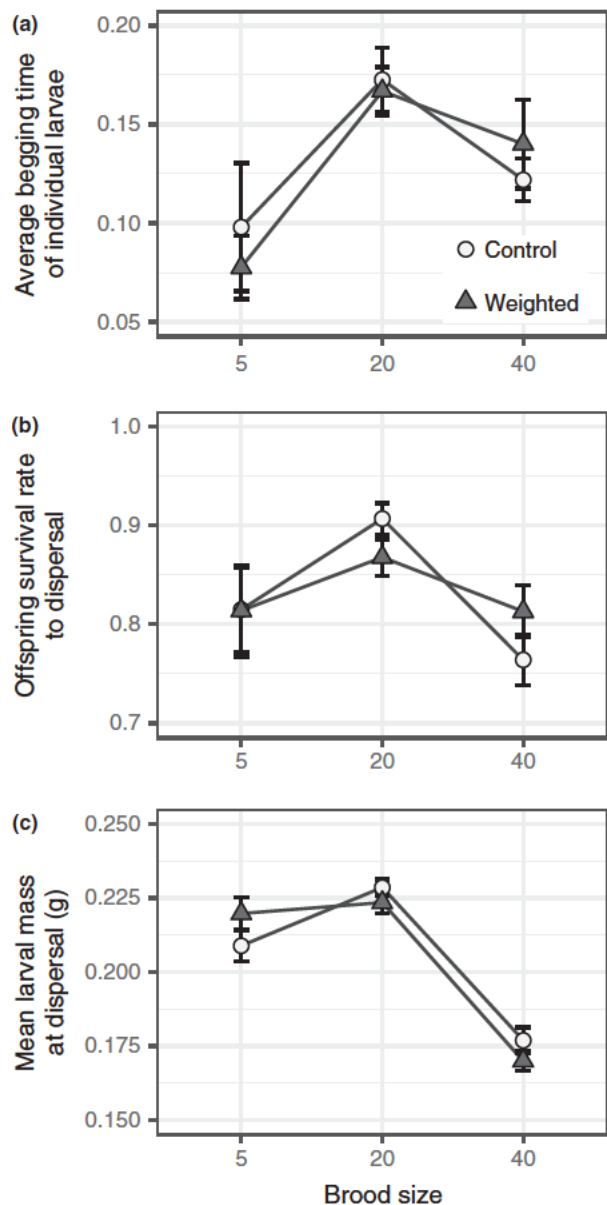

Fig. 3 Effects of handicapping (weighted or control females) and brood size manipulation (5, 10 or 20 larvae) on the average time spent begging by individual larvae in the brood (a), larval survival to dispersal (b) and mean larval mass at dispersal (c). Mean ± SE.

suggesting that these assumptions were not met in our study. Currently, we have little empirical information on the shape of the cost and benefit functions, and obtaining empirical estimates of these functions should now be a priority to guide future theoretical and empirical work in this field (Smiseth, 2017). However, there was an effect of this interaction on female carrion consumption, reflecting that control females consumed more carrion as brood size increased, whereas weighted

females consumed a similar amount of carrion regard less of brood size. This finding suggests that weighted females may compensate for the negative effects of handicapping by consuming more food. Moreover, brood size had an effect on most traits; that is, increas ing female food provisioning and female probability to engage in carrion consumption, reducing female weight change during breeding, increasing larval begging and decreasing larval performance (larval survival and mean larval mass). In contrast, we found that handi capping had an effect on two female parental beha viours only; that is, increased carrion consumption and, contrary to what we predicted, *increased* time provision ing food to the brood. These results imply that handi capping can lead to an increase in parental care, suggesting that the effects of handicapping on parental care may be more complex than has been assumed in prior work using such experimental designs. Below, we provide a more in depth discussion of our results and their implications for our understanding of flexible par ental care.

A surprising finding of our study was that weighted females spent more time provisioning food than control females. This finding contradicts the widely held assumption that handicapping causes a reduction in parental care by increasing the cost of care. Handicap ping experiments are traditionally used to study negoti ation between parents in birds with biparental care, and their rationale is to increase the flight cost of care to the handicapped parent, thereby forcing it to reduce its contribution towards care (Harrison *et al.*, 2009). Such experiments are based on several types of handi capping treatments, including attachment of lead weights (e.g. Wright & Cuthill, 1989), clipping of flight feathers (Slagsvold & Lifjeld, 1988) and hormone manipulation (Hegner & Wingfield, 1987b). There is good evidence that handicapped parents provide less care than control parents regardless of which handicap ping treatment is used (Harrison *et al.*, 2009). More recently, handicapping based on attachment of lead weights has been used to study negotiation between parents in two species of burying beetle, *N. quadripunc tatus* and *N. orbicollis*, and these studies show that, as in birds, weighted females provide less care than control females (Suzuki & Nagano, 2009; Creighton *et al.*, 2015; Suzuki, 2016). The opposite effects of handicap ping on parental care reported in studies on *Nicrophorus* species might reflect differences in the level of handi capping as our weights were of 20 30% relative to body mass of the beetles, whereas studies in *N. quadripunctatus* (Suzuki & Nagano, 2009; Suzuki, 2016) and *N. orbicollis* (Creighton *et al.*, 2015) used weights of 40% and about 50%, respectively. As we discuss in greater detail below, handicapping may not only increase the cost of care, but also impact upon parental decisions through its effect on the parent's state (Pilakouta *et al.*, 2015). For example, the relatively

minor handicaps used in our study might have a greater impact on the parent's state than its costs of care, whereas the relatively major handicaps used in previous work might have greater impact on the cost of care. An alternative explanation is that these differ ences reflect species specific response to handicapping due to divergent life histories.

As hinted at above, handicapping may alter parental decisions about how much care to provide if it causes a decline in the parent's state (i.e. its condition, energy reserves or stress level; Pilakouta *et al.*, 2015). This in turn may lead to a reduction in parental care by weighted parents given that a decline in the parent's state should be associated with lower resources for investment in parental care and other priorities. Why then did we find that weighted females provided *more* care? One potential explanation for this finding is that weighted females responded to a decline in their state by shifting their investment towards their current brood at the expense of future reproduction. The terminal investment hypothesis predicts that parents should increase their investment into current reproduction when their prospects of future reproduction are lower (Clutton Brock, 1984).

We would expect an increase in care by weighted females if this shift towards current investment more than outweighs the impact of the higher cost of care. There is some evidence for terminal investment from prior studies on species within the genus *Nicrophorus*. For example, in *N. vespilloides*, immune challenged par ents, which face higher risks of death from pathogens, increase their investment into current reproduction (Cotter *et al.*, 2010; Reavey *et al.*, 2015). Likewise, inbred males, which have a shortened lifespan, invest more into current reproduction and are more likely to risk injury in fights with conspecific competitors (Richardson & Smiseth, 2017). Finally, there is evi dence that investment into current reproduction increases with the age of the female parent in *N. or bicollis* as predicted by the terminal investment hypoth esis (Creighton *et al.*, 2009). Thus, if handicapping leads to terminal investment, we might have expected weighted females to gain less weight during breeding, as this trait is used as a proxy for investment in future reproduction (Creighton *et al.*, 2009; Billman *et al.*, 2014). We found no evidence that weighted females lost more weight during the breeding period than con trol females, suggesting that our results provide no overall support for terminal investment triggered by handicapping. However, as argued below, the lack of evidence for terminal investment based on data on female weight gain might reflect that handicapping also causes an increase in female food consumption.

We found that weighted females consumed a similar amount of carrion regardless of brood size, whereas control females consumed more carrion as brood size increased. In *N. vespilloides*, parents consume carrion

partly to provision food in the form of predigested carrion to their larvae and partly to replenish their own energy reserves (Mattey & Smiseth, 2015). Thus, our results suggest that control females increased their carrion consumption with brood size (Fig. 2c) to match the increase in food provisioning towards larger broods (Fig. 2a). In contrast, weighted females consumed a similar amount of carrion regardless of the brood size (Fig. 2c), presumably reflecting that these females adjusted their carrion consumption based on their own state rather than the brood size. Thus, control females consumed more carrion when they spent more time provisioning food to the brood, while there was no association between carrion consumption and food provisioning for weighted females. This finding also indicates that handicapping might trigger a compensatory response, whereby weighted females attempt to counteract the detrimental effects of handicapping due to an increase in the cost of care by increasing their energy reserves. For example, if handicapping increases the energetic cost of care, females might reduce this cost by building greater energy reserves. In *N. vespilloides*, it is relatively straightforward for females to increase their energy reserves as they can simply consume more from the carcass that is used for breeding (Boncoraglio & Kilner, 2012; Pilakouta *et al.*, 2016). If females increase their energy reserves to reduce the energetic cost of care, this may mask the expected effect of terminal investment on female mass gain.

As predicted, females provided more care and lost more weight when caring for larger broods. Meanwhile, we found that larvae in medium sized broods spent more time begging, gained more weight and had higher survival than larvae in either small or large broods. These results are consistent with findings from previous work showing that parents tend to provide more care as brood size increases in insects, including *N. vespilloides* (e.g. Rauter & Moore, 2004; Smiseth *et al.*, 2007), fishes (e.g. Ridgway, 1989) and birds (e.g. Hegner & Wingfield, 1987a; Sanz, 1997). Thus, our results are in line with the prediction that females provide more care when the indirect benefit of care is higher due to an increase in the number of offspring in the brood (Fig. 1). The finding that females lost more weight when caring for larger broods is likely to reflect that larger broods require more care from females and that it is more costly for parents to care for such broods. Finally, the fact that larvae performed best in broods of intermediate size suggests that larval growth and survival are higher in broods closer to the average size in this species (i.e. 21 larvae; Smiseth & Moore, 2002). This finding may reflect a balance between sibling competition and sibling cooperation (Forbes, 2007; Falk *et al.*, 2014; Schrader *et al.*, 2015), whereby individual offspring in small broods benefit from the presence of other siblings through cooperative begging whereas individual offspring in large broods pay a cost in terms of increased competition (Johnstone, 2004). To sum up, our results confirm that variation in the benefit of care influences female decisions about how much care to provide to the current brood and how much resources to invest into current vs. future reproduction.

Parental care is a highly variable trait (Royle *et al.*, 2012), and this variation reflects that parents make flexible decisions about how much care to provide in response to variation in the cost and benefit of care. Here, we show that parents respond to both handicapping and brood size and that these responses are largely independent of each other. In our experiment, females appear to respond more strongly to variation in brood size than to handicapping, which might reflect that brood size manipulations have a greater impact on the benefit of care compared to the impact of handicapping on the cost of care. Furthermore, weighted females spent more time provisioning food to the brood and consuming carrion than control females. This finding supports the view that parents may respond to handicapping by increasing their investment into the current brood at the expense of investment in future reproduction and/or by increasing their energy reserves to compensate for the increased energetic cost of care. We suggest that future work on parental care based on handicapping should consider that this treatment may not only affect the cost of care, but that it may also lead to an increase in investment into current reproduction and compensatory responses that counteract the increased cost of care.

Acknowledgments

References

Alonso alvarez, C., Velando, A. 2012. Benefits and costs of parental care. In: *The Evolution of Parental Care* (N.J. Royle *et al.*, eds), pp. 40 61. Oxford University Press, Oxford.

Andrews, C.P. & Smiseth, P.T. 2013. Differentiating among alternative models for the resolution of parent offspring conflict. *Behav. Ecol.* **24**: 1185 1191.

Billman, E.J., Creighton, J.C. & Belk, M.C. 2014. Prior experience affects allocation to current reproduction in a burying beetle. *Behav. Ecol.* **25**: 813 818.

Boncoraglio, G. & Kilner, R.M. 2012. Female burying beetles benefit from male desertion: sexual conflict and counter adaptation over parental investment. *PLoS One* **7**: e31713.

Clutton Brock, T.H. 1984. Reproductive effort and terminal investment in iteroparous animals. *Am. Nat.* **123**: 212 219.

Cotter, S.C., Ward, R.J.S., Kilner, R.M., Cotter, S.C., Ward, R.J.S. & Kilner, R.M. 2010. Age specific reproductive invest ment in female burying beetles: independent effects of state and risk of death. *Funct. Ecol.* **25**: 652 660.

Creighton, J.C., Heflin, N.D. & Belk, M.C. 2009. Cost of repro duction, resource quality, and terminal investment in a burying beetle. *Am. Nat.* **174**: 673 684.

Creighton, C.J., Smith, A.N., Komendat, A. & Belk, M.C. 2015. Dynamics of biparental care in a burying beetle: experimen tal handicapping results in partner compensation. *Behav. Ecol. Sociobiol.* **69**: 265 271.

Eggert, A., Reinking, M. & Mu, J.K. 1998. Parental care improves offspring survival and growth in burying beetles. *Anim. Behav.* **55**: 97 107.

Falk, J., Wong, J.W.Y., Kolliker, M. & Meunier, J. 2014. Sib ling cooperation in earwig families provides insights into the early evolution of social life. *Am. Nat.* **183**: 547 557.

Forbes, S. 2007. Sibling symbiosis in nestling birds. *Auk* **124**: 1 10.

Fox, J. & Weisberg, S. 2017. car: An R companion to applied regression. R package version 2.1 6. https://CRAN.R project. org/package car

Grodzinski, U. & Johnstone, R.A. 2012. Parents and offspring in an evolutionary game: the effect of supply on demand when costs of care vary. *Proc. Biol. Sci.* **279**: 109 115.

Hadfield, J.D. 2010. MCMC methods for multi response gener alized linear mixed models: the MCMCglmm R package. *J. Stat. Softw.* **33**: 1 22.

Harrison, F., Barta, Z., Cuthill, I.C. & Székely, T. 2009. How is sexual conflict over parental care resolved? A meta analysis. *J. Evol. Biol.* **22**: 1800 1812.

Hegner, R.E. & Wingfield, J.C. 1987a. Effects of brood size manipulations on parental investment, breeding success, and reproductive endocrinology of house sparrows. *Auk* **104**: 470 480.

Hegner, R.E. & Wingfield, J.C. 1987b. Effects of experimental manipulation of testosterone levels on parental investment and breeding success in male house sparrows. *Auk* **104**: 462 469.

Johnstone, R.A. 2004. Begging and sibling competition: how should offspring respond to their rivals? *Am. Nat.* **163**: 388 406.

Kolliker, M., Ridenhour, B.J. & Gaba, S. 2010. Antagonistic parent offspring co adaptation. *PLoS One* **5**: e8606.

Lesnoff, M. & Lancelot, R. 2012. aod: Analysis of overdispersed data. R package, version 1.3. https://CRAN.R project.org/pac kage aod

Mattey, S.N. & Smiseth, P.T. 2015. Complex effects of inbreed ing on biparental cooperation. *Am. Nat.* **185**: 1 12.

Muller, J.K. & Eggert, A. K. 1990. Time dependent shifts between infanticidal and parental behavior in female bury ing beetles a mechanism of indirect mother offspring recog nition. *Behav. Ecol. Sociobiol.* **27**: 11 16.

Otronen, M. 1988. The effect of body size on the outcome of fights in burying beetles. *Ann. Zool. Fenn.* **25**: 191 201.

Paquet, M., Covas, R. & Doutrelant, C. 2015. A cross fostering experiment reveals that prenatal environment affects beg ging behaviour in a cooperative breeder. *Anim. Behav.* **102**: 251 258.

Pilakouta, N., Richardson, J. & Smiseth, P.T. 2015. State dependent cooperation in burying beetles: parents adjust their contribution towards care based on both their own and their partner's size. *J. Evol. Biol.* **28**: 1965 1974.

Pilakouta, N., Richardson, J. & Smiseth, P.T. 2016. If you eat, I eat: resolution of sexual conflict over consumption from a shared resource. *Anim. Behav.* **111**: 175 180.

R Development Core Team 2011. *R: A Language and Environ ment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Rauter, C.M. & Moore, A.J. 1999. Do honest signalling models of offspring solicitation apply to insects? *Proc. Biol. Sci.* **266**: 1691.

Rauter, C.M. & Moore, A.J. 2004. Time constraints and trade offs among parental care behaviours: effects of brood size, sex and loss of mate. *Anim. Behav.* **68**: 695 702.

Reavey, C.E., Warnock, N.D., Garbett, A.P. & Cotter, S.C. 2015. Aging in personal and social immunity: do immune traits senesce at the same rate? *Ecol. Evol.* **5**: 4365 4375.

Richardson, J. & Smiseth, P.T. 2017. Intraspecific competition and inbreeding depression: increased competitive effort by inbred males is costly to outbred opponents. *Am. Nat.* **189**: 539 548.

Ridgway, M.S. 1989. The parental response to brood size manipulation in smallmouth bass (*Micropterus dolomieui*). *Ethology* **80**: 47 54.

Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A. & Firth, D. 2017. MASS: Modern applied statistics with S. R package, version 7.3 48. https://CRAN.R project.org/packa ge MASS

Royle, N.J., Smiseth, P.T. & Kolliker, M. (eds) 2012. *The Evolu tion of Parental Care*. Oxford University Press, Oxford.

Royle, N.J., Russell, A.F. & Wilson, A.J. 2014. The evolution of flexible parenting. *Science* **345**: 776 781.

Safryn, S.A. & Scott, M.P. 2000. Sizing up the competition: do burying beetles weigh or measure their opponents? *J. Insect Behav.* **13**: 291 297.

Sanz, J.J. 1997. Clutch size manipulation in the Pied Fly catcher: effects on nestling growth, parental care and moult. *J. Avian Biol.* **28**: 157 162.

Schrader, M., Jarrett, B.J.M. & Kilner, R.M. 2015. Parental care masks a density dependent shift from cooperation to competition among burying beetle larvae. *Evolution* **69**: 1077 1084.

Scott, M.P. 1998. The ecology and behavior of burying beetles. *Annu. Rev. Entomol.* **43**: 595 618.

Slagsvold, T. & Lifjeld, J.T. 1988. Ultimate adjustment of clutch size to parental feeding capacity in a passerine bird. *Ecology* **69**: 1918 1922.

Smiseth, P.T. 2017. Parental care. In: *Reference Module in Life Sciences* (B.D. Roitberg & J.C. Perry, eds). Elsevier, Amster dam.

Smiseth, P.T. & Moore, A.J. 2002. Does resource availability affect offspring begging and parental provisioning in a par tially begging species? *Anim. Behav.* **63**: 577 585.

Smiseth, P.T. & Moore, A.J. 2004a. Behavioral dynamics between caring males and females in a beetle with faculta tive biparental care. *Behav. Ecol.* **15**: 621 628.

Smiseth, P.T. & Moore, A.J. 2004b. Signalling of hunger when offspring forage by both begging and self feeding. *Anim. Behav.* **67**: 1083 1088.

Smiseth, P.T., Darwell, C.T. & Moore, A.J. 2003. Partial beg ging: an empirical model for the early evolution of offspring signalling. *Proc. Biol. Sci.* **270**: 1773 1777.

Smiseth, P.T., Dawson, C., Varley, E. & Moore, A.J. 2005. How do caring parents respond to mate loss? Differen tial response by males and females. *Anim. Behav.* **69**: 551 559.

Smiseth, P.T., Lennox, L. & Moore, A.J. 2007. Interaction between parental care and sibling competition: parents enhance offspring growth and exacerbate sibling competi tion. *Evolution* **61**: 2331 2339.

Smiseth, P.T., Kolliker, M., Royle, N.J. 2012. What is parental care? In: *The Evolution of Parental Care* (N.J. Royle *et al.*, eds), pp. 1 17. Oxford University Press, Oxford.

Suzuki, S. 2016. When the male determines his provisioning effort: does the timing of handicapping affect the negotiation between parents in *Nicrophorus quadripunctatus*? *Behaviour* **153**: 1435 1443.

Suzuki, S. & Nagano, M. 2009. To compensate or not? Caring parents respond differentially to mate removal and mate handicapping in the burying beetle, *Nicrophorus quadripuncta tus*. *Ethology* **115**: 1 6.

Trivers, R.L. 1974. Parent offspring conflict. *Am. Zool.* **14**: 249 264.

Wright, J. & Cuthill, I. 1989. Manipulation of sex differences in parental care manipulation. *Behav. Ecol. Sociobiol.* **25**: 171 181.

Original Article

Offspring beg more toward larger females in a burying beetle

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Offspring of many animals beg for food from parents. Begging is often costly, and offspring should seek to reduce such costs to maximize their returns on begging. Whenever multiple adults provide care for a joint brood, as in species where multiple females breed communally, offspring should beg toward the parent that provisions the most food. Here, we investigate whether larvae spend more time begging toward larger females in the burying beetle *Nicrophorus vespilloides*. Prior work on this species shows that larger females provision more food than smaller ones, suggesting that larvae would benefit by preferentially begging toward larger females. To test for such a preference, we provided experimental broods with a simultaneous choice between two dead females: a smaller and a larger one. Larvae spent more time begging toward larger females. We next examined the behavioral mechanism for why larvae begged more toward larger females. Larvae spent more time in close contact with larger females over smaller ones, whereas there was no evidence that larvae begged more *when* in close contact with larger females. Thus, larvae begged more toward the larger female simply as a consequence of spending more time close to larger females. Our findings have important implications for our understanding of parent–offspring communication by showing that offspring can choose between parents based on parental attributes, such as body size, reflecting how much food parents are likely to provision.

Key words: begging, body size, *Nicrophorus vespilloides*, parent–offspring communication, parental care.

INTRODUCTION

Offspring beg for food from their parents across many animal taxa, including birds (e.g., Budden and Wright 2001), mammals (e.g., Smiseth and Lorentsen 2001), amphibians (e.g., Yoshioka et al. 2016), and insects (e.g., Rauter and Moore 1999). Theoretical models propose that costly offspring begging behaviors evolved as a behavioral mechanism for resolving parent–offspring conflict over parental care (Godfray 1991; Parker et al. 2002). The reason for this is that costs of begging ensure that parents benefit by adjusting their food provisioning in response to begging because parents obtain honest information on the nutritional needs of their offspring. There is empirical evidence that begging often incurs costs to offspring in terms of reduced growth (e.g., Kilner 2001; Takata et al. 2019), increased risk of predation (e.g., Redondo and Castro 1992; Haskell 1994), or increased mortality due to filial infanticide (e.g., Andrews and Smiseth 2013). When begging is costly, offspring should be under selection to maximize their returns on begging (Bell 2008; Madden et al. 2009). For instance, in species where both parents provision food for the offspring and where parents of one sex provision more food than the other, offspring may maximize

their returns on begging by begging more toward parents of the sex that provisions the most food, as reported in studies on birds (e.g., Kölliker et al. 1998; Roulin and Bersier 2007; Dickens et al. 2008) and insects (Suzuki 2015; Paquet et al. 2018).

Although there is good evidence that offspring beg more toward parents of the sex that provisions the most food, it is unclear whether begging offspring also respond to other attributes of their parents that might predict the offspring's returns on begging, such as the body size, nutritional state, or age of parents. For example, offspring may beg more toward larger parents if they provision more food than smaller ones. Larger parents produce more milk than smaller parents in mammals (Landete-Castillejos et al. 2003), provision food more often to the nest in insects (Bosch and Vicens 2006), and provision larger loads of food in birds (Tveera et al. 1998). Whenever multiple adults provide care for a joint brood, offspring could potentially maximize their returns on begging by begging more toward the larger adult. Species with communal breeding, where multiple females breed on a shared resource or in a joint nest (Vehrencamp 1978, 2000; Koenig and Dickinson 2004), are particularly useful to test whether offspring beg more toward larger adults given that caring adults differ with respect to body size only. In contrast, caring adults also differ with respect to sex and breeding status

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(i.e., parent vs. helpers) in the contexts of biparental care and cooperative breeding, respectively, in which case there may be confounding effects due to the sex and breeding status of adults.

Here, we conducted an experiment on the burying beetle *Nicrophorus vespilloides* in which we tested whether larvae begged more toward larger females than toward smaller ones. Burying beetles of the genus *Nicrophorus* are ideal study species to explore this issue because multiple females sometimes breed communally on a shared resource: the carcass of a small vertebrate (Eggert and Müller 1992; Trumbo 1992). Communally breeding females provide care indiscriminately to any larva in the joint brood (Komdeur et al. 2013; Richardson and Smiseth 2020). Communally breeding females may differ in body size when breeding on large carcasses (Eggert and Müller 2000), suggesting that larvae sometimes are in a position to choose between different-sized females. Larger females spend more time provisioning food to the brood than smaller females (Steiger 2013) and may process food more efficiently than smaller ones (Pilakouta et al. 2015). Thus, given that begging incurs costs to larvae in terms of increased mortality and reduced growth (Andrews and Smiseth 2013; Takata et al. 2019), larvae might maximize their returns on begging by begging more toward larger females.

Our first aim was to test the hypothesis that larvae maximize their returns on begging by begging more toward larger females. We used a simultaneous choice design, in which larvae could choose between one larger and one smaller female. The hypothesis predicts that larvae would beg more toward the larger female. Our second aim was to test two potential behavioral mechanisms for why larvae begged more toward the larger female. First, larvae may respond directly to female body size by begging more when they are in close contact with the larger female. Second, larvae may respond indirectly by spending more time in close contact with the larger female, thereby having more opportunities to beg toward the larger female. The first mechanism predicts that larvae would beg more when they were in close contact with the larger female as opposed to when they were in close contact with the smaller one but that there would be no difference in the amount of time that larvae spend in close contact with the larger or the smaller females. The second mechanism predicts that larvae would spend more time in close contact with the larger female than with the smaller female but that larvae would not beg more when they were in close contact with the larger female. These two mechanisms are not mutually exclusive, and it is, therefore, possible that larvae would beg more when they were in close contact with the larger female and spend more time in close contact with the larger female.

MATERIALS AND METHODS
Origin and rearing of experimental beetles

The beetles used in the experiments descended from individuals collected in the wild in the Hermitage of Braid and Blackford Hill Local Nature Reserves, Edinburgh, UK. The beetles had been kept under laboratory conditions for at least three generations. We kept the stock population outbred by breeding a large number of individuals each generation, recruiting only three individuals from each family to the next generation, outcrossing our stock population with wild-caught beetles each summer, and avoiding breeding between close relatives (see Mattey et al. 2018). We maintained our laboratory population at constant temperature (20 °C) and under a 16:8 h light:dark photoperiod. Nonbreeding adult beetles were housed in individual transparent plastic containers (12 × 8 × 2 cm) filled with moist soil and were fed small pieces of organic beef twice a week.

Experimental design

We used a simultaneous choice design, in which larvae could choose between two dead females—one larger and one smaller (Paquet et al. 2018)—because such designs are better at detecting preferences than alternative sequential choice designs (Dougherty and Shuker 2014). This design is biologically realistic for our species given that females will breed communally on large carcasses (Eggert and Müller 1992; Komdeur et al. 2013; Richardson and Smiseth 2020). We used dead females as stimuli to exclude any potential effects of differences in the behavior of larger and smaller females that might affect larval behavior. Prior work shows that larvae beg toward dead parents in a similar way as they do toward live ones (Smiseth and Parker 2008; Mäenpää et al. 2015; Paquet et al. 2018).

Experimental procedures

At the start of the experiment, we generated the smaller and larger females that we later used as stimuli when investigating larval begging toward larger and smaller females. We generated these females following established protocols (Steiger 2013; Pilakouta et al. 2015). To this end, we mated 34 pairs of unrelated males and females from our stock population by providing them with a mouse carcass to initiate breeding. To manipulate female body size, we removed larvae from each of these 34 the carcasses at two different stages in their development: when they had reached a mass of 100–150 mg (mean mass ± standard error [SE] at dispersal: 0.130 g ± 0.004) and when they had reached a mass of 200–250 mg (mean ± SE mass at dispersal: 0.222 g ± 0.003). The former larvae were destined to become smaller adults, whereas the latter were destined to become larger adults. From each brood, we aimed to remove a similar number of larvae that had reached a mass of 100–150 mg and that had reached a mass of 200–250 mg. Given that larval mass at the time of dispersal from the carcass determines adult body size (Lock et al. 2004), these treatments allowed us to generate a difference in size between females. The treatment was effective as larger females had a pronotum width (mean ± SE: 5.08 ± 0.03 mm) that was on average 23% larger than that of the smaller females (mean ± SE: 4.10 ± 0.02 mm).

Once removed from the carcass, larvae destined to become smaller or larger adults were placed in individual containers (12 × 8 × 2 cm) filled with moist soil until they eclosed as adults. After eclosion, we sexed all adults, keeping females only for use in our experiment. We kept all smaller and larger females in individual containers for a minimum of 10 days after eclosion to allow them sufficient time to undergo sexual maturation. During this period, we fed all females small pieces of organic beef twice a week until they were used in our experiment. Insect species that undergo complete metamorphosis cease growing once they reach adulthood. Thus, feeding adult females will have no effect on their body size, although it will have an impact on their adult body mass.

Once the females reached sexual maturity, we randomly selected 32 larger and 32 smaller females for use in our experiments. These females were first used to produce larvae for the experimental broods and, later, as foster parents and stimuli during our behavioral observations (see below). To initiate breeding, we paired each female with an unrelated male from our stock population, placed each pair in a larger container (17 × 12 × 6 cm) with 2 cm of

moist soil, and provided them with a defrosted mouse carcass of a standardized size (20.01–23.64 g; Livefoods Direct, Sheffield, UK). We checked the containers daily for the presence of eggs, defining the onset of egg-laying as the day on which the first eggs were laid. Two days after the outset of egg-laying (corresponding to the day preceding the expected time of hatching; Smiseth et al. 2006), we separated each female from her eggs by transferring females and their carcasses into fresh containers lined with moist soil. We did this to ensure that no other larvae were present when we later allocated females with a donor brood (see below). Females lay their eggs asynchronously (Smiseth et al. 2006). We, therefore, separated females from the eggs 2 days after the start of egg-laying because this coincides with a time in the breeding cycle where females had ceased egg-laying but where the first egg had yet to hatch. At this time, we removed the male to exclude any potential effects of male presence on larval begging. There is no evidence that the removal of the male has any detrimental effects on offspring growth or survival under laboratory conditions (Smiseth et al. 2005).

We generated 32 donor broods by pooling newly hatched larvae from across multiple broods. All such broods were comprised of 10 same-aged larvae of mixed maternity. Once assembled, donor broods were allocated at random to a smaller or a larger female foster parent. Females have a temporal kin discrimination mechanism, culling any larvae that hatch earlier than the expected time of hatching of their own eggs (Müller and Eggert 1990). We, therefore, provided females with a donor brood only after her own eggs had started hatching. Given that donor broods were composed of larvae of mixed maternity, most larvae would be genetically unrelated to their foster female. We used donor brood to generate experimental broods of 10 larvae immediately before the start of our behavioral observations (see below). We used experimental broods to exclude any potential confounding effects due to natural variation in brood size (Ratz and Smiseth 2018), larval age (Smiseth, Lennox, et al. 2007; Smiseth, Ward, et al. 2007), or age composition within the brood (Smiseth and Moore 2007) on larval behavior.

Larval begging

We conducted the behavioral observations 24 h (±15 min) after we had allocated an experimental donor brood to a foster female when larvae had reached their second instar (Smiseth and Moore 2002). We did our observations at this stage in larval development because it coincides with a peak in the amount of time that larvae spend for food from their parents (Smiseth et al. 2003; Smiseth, Lennox, et al. 2007). Approximately 1 h before the start of each behavioral observation session, we sacrificed the females to be used as stimuli by freezing them at −20 °C for 30 min. These females had previously produced larvae for our experimental broods and had, therefore, been caring for larvae during the 24 h preceding the observation. We used breeding females as stimuli because larvae beg more toward breeding females than toward nonbreeding ones (Smiseth et al. 2010). We then thawed the females at ambient temperature for a minimum of 10 min to ensure that we could position their body and legs (see below).

We generated experimental broods comprised of 10 larvae from the donor broods. We always assigned experimental broods to a pair of unfamiliar dead females to exclude any potential confounding effects should larvae behave differently toward familiar and unfamiliar females (Mäenpää et al. 2015). All experimental broods were comprised of five larvae that had been reared by a larger female and five that had been reared by a smaller female. This procedure ensured that all experimental broods comprised an equal number of larvae that had prior exposure to a larger and a smaller female. Thus, if larvae learned a preference for larger or smaller females depending on their prior exposure to a larger and a smaller female, this would be detected as five larvae being in close contact with each of the two females. We confirm that this was not the case as, on average, eight larvae were in close contact with one female and one with the other. We placed the 10 larvae in a small container (11 × 11 × 3 cm) lined with moist paper towel 30 min prior to observation to ensure that the larvae were not fully satiated and, therefore, motivated to beg at the start of the observation. Just before the start of the observation sessions, we pinned the larger and smaller females near the center of the box (Figure 1a) and positioned them such that they mimicked a parent provisioning food (Mäenpää et al. 2015; Paquet et al. 2018; Figure 1b). We then placed all larvae in front of the two females such that they were equidistant from them (Figure 1a). We allowed a 5-min acclimation period before we started the observation session.

We observed larval behavior using instantaneous recording, noting the number of larvae that were begging toward each female and the number of larvae that were in close contact with each female every 60 s for 15 min. We defined larval begging as when larvae touched any body part of a female with their legs

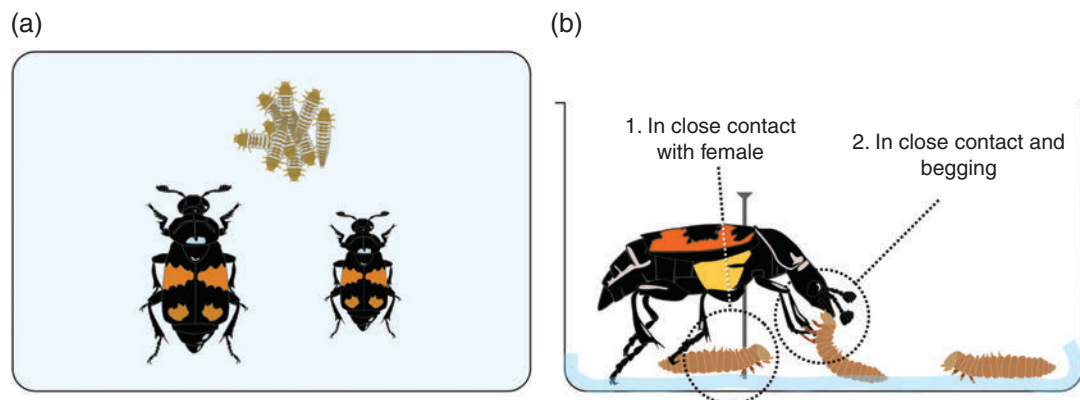(a)   (b)

Figure 1
Experimental design used to test whether larvae spend more time begging toward a larger female over a smaller female. (a) At the beginning of the behavioral observation, we placed the brood of 10 larvae equidistant from the two females. (b) We then recorded the amount of time that larvae spent begging toward each female and the amount of time that larvae spent in close contact with each female (i.e., at a distance less than or equal to the female's pronotum length).

(Smiseth et al. 2003; Figure 1b). We defined larvae as being in close contact with a female as the larvae being closer to the female than the width of her pronotum (Rauter and Moore 1999). We first calculated the mean proportion of time spent begging per larva in the brood toward each female (B) as the number of begging events toward each female across the 15 scans (Σb) divided by the number of sampling scans (15) and the number of larvae in the brood (10) as $B = (\Sigma b/15)/10$. This metric used information of larval begging only and represents the overall mean time spent begging per larva in the brood toward each female.

Behavioral mechanisms

We next investigated two potential behavioral mechanisms for why larvae might beg more toward the larger female. First, larvae may respond directly to female body size by begging more when they were in close contact with the larger female. We calculated the mean proportion of time spent begging per larva when larvae were in close contact with either the larger or the smaller female (B) as the number of begging events toward each female across the 15 scans (Σb) divided by the total number of counts of larvae being in close contact with the female in question across the 15 scans (n) as $B = \Sigma b/n$. This metric differs from the one above because it takes into account potential differences in the amount of time that larvae spent in close contact with each female.

Second, larvae may respond indirectly to female body size by spending more time in close contact with the larger female, thereby having more opportunities to beg toward her. We recorded the total number of counts of larvae being in close contact with the female in question across the 15 scans. Given that the number of larvae that were close to the larger female is inversely related to the number of larvae that were close to the smaller one, we focused on the proportion of larvae in the brood that associated with the larger female. We calculated the proportion of larvae that were in close contact with the larger female (C) as the number of counts of larvae being close to the larger female across the 15 scans (Σc) divided by the number counts of larvae being close to either one of the two females across the 15 scans (n) as $C = \Sigma c/n$.

Statistical analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team 2011) loaded with the packages *car* (Fox et al. 2016), *MASS* (Ripley et al. 2017), and *lme4* (Bates et al. 2014). We first investigated whether larvae begged more toward the larger

female. To do so, we tested whether there was a difference in mean proportion of time spent begging per larva toward larger and smaller females. Given that these data represented the proportion of time that larvae spent begging, we used generalized linear mixed models (GLMMs) with a binomial distribution. We included female body size (larger or smaller) as a fixed effect and brood ID and observation level as random effects to account for repeated measures on the same broods and to handle overdispersion (Harrison 2015). We then tested whether larvae begged more when they were in close contact with the larger female using a GLMM with a binomial distribution. We included female body size (larger or smaller) as a fixed effect and brood ID and observation level as random effects. Finally, we tested whether larvae spent more time in close contact with the larger female. To this end, we used a Wilcoxon signed-rank test comparing the observed proportion of larvae that were in close contact with the larger female against the null expectation of 0.5 as expected if larvae spent an equal amount of time in close contact with the two females (Crawley 2005).

We excluded 10 broods out of 32 broods from our analyses because at least one of the two females later used as stimuli during our experiments had not been observed at the carcass at the time we removed the females from their original container. We excluded these females because such females had deserted their brood. We did this because prior work shows that breeding and nonbreeding females have different cuticular chemical profiles (Müller et al. 2003; Steiger et al. 2007) and that larvae have a strong preference for begging toward breeding females over nonbreeding ones (Smiseth et al. 2010). Thus, we excluded cases where one of the females had deserted the brood prior to the observation to exclude potential confounding effects due to another factor that is likely to influence larvae begging behavior (i.e., female breeding status). The final sample size in our study was, thus, 22 broods.

RESULTS

We first tested for a difference in the overall time spent begging toward the larger and smaller females. Larvae spent on average about three times as much time begging toward the larger female as they did toward the smaller one (Table 1; Figure 2a), confirming that larvae begged more toward the large female. We next tested between two potential behavioral mechanisms underpinning this preference; that is, whether larvae begged more when they were in close contact with the larger female or whether larvae spent more time in close contact with the larger female. We found that larvae spent more time in close contact with the larger female

Table 1

Summary of our statistical approach and our results concerning whether larvae beg more toward larger females and whether this was because larvae begged more toward larger females when in close contact with them or because larvae spent more time in close contact with larger females

	Model		Results		
	GLMM		χ^2	df	*P*
Overall time spent begging per larva in the brood	Fixed effect: female status (larger/smaller)	Random effects: brood ID, observation level	12.7	1	**<0.001**
Time spent begging per larva in the brood when in close contact with the larger or smaller female	Fixed effect: female status (larger/smaller)	Random effects: brood ID, observation level	0.005	1	0.942

Statistically significant *P*-values (<0.05) are shown in boldface.

than expected due to chance (Wilcoxon signed-rank test: $V = 180$, $P = 0.04$; Figure 2b). However, there was no evidence that larvae begged more when they were in close contact with the larger female as opposed to when they were in close contact with the smaller one (Table 1; Figure 2c). Thus, our results show that larvae begged more toward the larger female simply because they spent more time in close contact with her.

DISCUSSION

Here, we show that larvae in the burying beetle *N. vespilloides* begged more toward the larger female when given a simultaneous choice between two dead females: one larger and one smaller. We predicted that larvae would beg more toward the larger female given that larger females provision more food than smaller ones (Steiger 2013) and that begging incurs costs to larvae (Andrews and Smiseth 2013; Takata et al. 2019). Thus, our results support the hypothesis that offspring beg more toward the adult that is likely to provision them with more food, thereby maximizing the offspring's returns on begging. There is support for this hypothesis from prior work on species with biparental care showing that offspring beg more toward parents of the sex that provisions the most food (Kölliker et al. 1998; Roulin and Bersier 2007; Dickens et al. 2008; Suzuki 2015; Paquet et al. 2018). Our study adds to this work by showing that offspring have preferences based on parental attributes other than sex, such as body size.

We also show that larvae spent more time in close contact with the larger female than with the smaller one (Table 1; Figure 2a), whereas there was no evidence that larvae begged more when they were in close contact with the larger female (Table 1; Figure 2c). These results provide valuable insights into the behavioral mechanisms for why larvae beg more toward the larger female by showing that larvae do so simply as a consequence of spending more time in close contact with her (Figure 2b). Larvae may spend more time in close contact with the larger female because larger females provision more food, as shown by a prior study on *N. vespilloides* (Steiger 2013). However, there are alternative explanations for why larvae spent more time in close contact with the larger female. First, if larvae move randomly within the container, they may end up more often underneath the larger female simply by chance. This explanation seems unlikely given that there was no evidence that larvae moved randomly within the container. The larvae spent on average 92% of their time underneath one of the two females. Yet, the surface area covered by the two females (including the width of their pronotum around the females to match our criteria for determining whether larvae were in close contact with a given female) made up only 5.5% of the area of the container. We also note that the larvae were already underneath the two females by the time we started our observation session, only 5 min after we had placed the larvae in the container. Keeping in mind that we placed the larvae away from the two females at the start of the experiment, this suggests that the larvae moved quickly in the direction of the two females. Second, larvae might approach females for protection and shelter, spending more time in close contact with the larger female if larger females are better at protecting larvae from potential threats. For example, in our study species, conspecific intruders pose a threat to the larvae as they may commit infanticide in order to attempt to take over the carcass (Trumbo 2007; Trumbo and Valetta 2007; Georgiou Shippi et al. 2018). There is also evidence that larger females are stronger competitors against conspecifics than smaller females (Otronen 1988; Trumbo 2007). Thus, we cannot rule out the potential explanation that larvae may beg more toward the larger female as a consequence of being close to her for protection.

Our results imply that larvae somehow assessed the body size of the two females. As our study species normally breeds underground in complete darkness (Scott 1998), it is unlikely that larvae did so based on visual cues. Instead, larvae might assess differences between females based on behavioral, acoustic, and vibrational cues that reflect body size. Although we cannot rule out that such cues play a role when larvae interact with live females, it seems unlikely that they could explain our results given that we used dead females as stimuli to trigger larval begging. Potentially, larvae may use tactile cues to assess female size once they had approached the two females. There was some indication that larvae moved between the two females (on average, one larva moved between each scan), but there was no evidence that larvae moved in a specific direction, that is, from the smaller toward the larger female. Alternatively, larvae may assess differences in body size between females at a distance based on chemical cues, such as cuticular hydrocarbons (CHCs) and methyl geranate (Steiger et al. 2007; Smiseth et al. 2010; Steiger et al. 2011; Engel et al. 2016). Such chemical cues are present on dead parents and stimulate larval begging (Smiseth et al. 2010), and, although CHCs are not volatile, they break down into volatile organic compounds when exposed to air and water vapor (Hatano et al. 2020). Thus, there is now a need for studies that compare CHC profiles of different-sized females.

We investigated whether offspring beg more toward larger adults in the context of communal breeding for practical reasons because it allowed us to exclude potential confounding effects due to other attributes of adults, such as their sex or breeding status. Nevertheless, we suggest that offspring preferences for larger adults may also be found in other contexts, such as biparental care, cooperative breeding, and uniparental care (Table 1). In the context of biparental care, there is evidence that offspring beg more toward the parent of the sex that provisions the most food (Kölliker et al. 1998; Roulin and Bersier 2007; Dickens et al. 2008; Suzuki 2015; Paquet et al. 2018). Thus, demonstrating that offspring beg more toward the larger parent will require designs that can separate between offspring responses to parental body size and sex. Furthermore, in species where there are sex differences in body size, offspring preferences for larger adults may potentially explain why offspring beg toward parents of a particular sex. This is unlikely to be the case in our species because there are no sex differences in body size in *N. vespilloides* (Paquet et al. 2018). Similarly, offspring may beg more toward larger adults in the context of cooperative breeding, where offspring are provisioned food by parents that are assisted by nonbreeding helpers (Vehrencamp 2000; Koenig and Dickinson 2004). In this context, there is some evidence that offspring beg more toward their parents than toward the helpers (Fortuna 2016), and such preferences may be based on adults' breeding status (breeders or helpers) or body size if there are differences in body size between parents and helpers. Finally, there may be offspring preferences for larger adults in the context of uniparental care, although it is less obvious that this would be the case given that offspring would have no opportunity to choose between different adults. Nevertheless, we suggest that offspring might have such preferences even in this context provided that offspring can choose between obtaining food by begging from their parent or finding food for themselves (Smiseth et al. 2003) and that begging incurs costs to offspring (Redondo and Castro 1992; Haskell 1994; Kilner 2001; Andrews and Smiseth 2013). Thus, offspring preferences for larger adults may occur in the context of uniparental care where offspring are only partially dependent on their parents, as in *N. vespilloides* (Smiseth et al. 2003), or where there is a transition period from
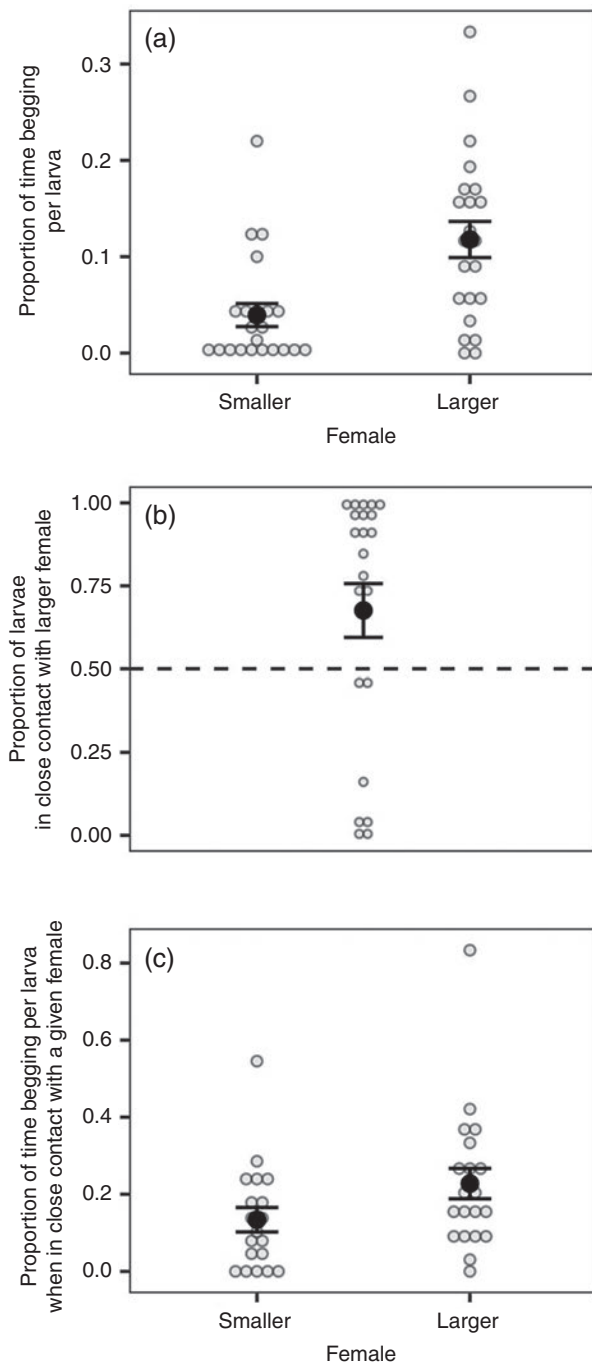
Figure 2

(a) Mean overall proportion of time spent begging per larva in the brood toward the larger and smaller female, (b) mean proportion of time spent in close contact with the larger female relative to time spent in close contact with the smaller female, and (c) mean proportion of time spent begging per larva when in close contact with either the larger female or the smaller female. The dash line in (b) represents the null expectation when larvae associated as much with the larger female as with the smaller one. Black dots and error bars represent means ± SE.

complete dependence on parents for food toward full independence, as in many birds and mammals.

Our study has important implications for our understanding of parent–offspring communication. It is well established that

offspring adjust their begging behavior in response to changes in their own state, such as their hunger state (Kilner and Johnstone 1997; Smiseth and Moore 2004, 2007), their long-term need (Price et al. 1996), or their inbreeding status (Mattey et al. 2018). Here, we show that offspring also adjust their begging behavior in response to female body size, an attribute that predicts the ability of female parents to provision their offspring with food (e.g., Tveera et al. 1998; Landete-Castillejos et al. 2003; Bosch and Vicens 2006; Steiger 2013). Traditionally, parent–offspring communication is described as a process where begging offspring signal their needs, thereby playing the role as senders, and parents respond to the offspring's signals, thereby playing the role as receivers. Our study suggests that parent–offspring communication is more complex as begging offspring also act as receivers by responding to cues from their parents. Thus, it seems more appropriate to describe parent–offspring communication as a two-way process where both parents and offspring act as senders and receivers, adjusting their behavior based on signals or cues produced by each other.

REFERENCES

Andrews CP, Smiseth PT. 2013. Differentiating among alternative models for the resolution of parent-offspring conflict. Behav Ecol. 24:1185–1191.

Bates D, Mächler M, Bolker B. 2014. Fitting linear mixed-effects models using lme4. J Stat Softw. 67:1–48.

Bell MB. 2008. Strategic adjustment of begging effort by banded mongoose pups. Proc Biol Sci. 275:1313–1319.

Bosch J, Vicens N. 2006. Relationship between body size, provisioning rate, longevity and reproductive success in females of the solitary bee *Osmia cornuta*. Behav Ecol Sociobiol. 60:26–33.

Budden AE, Wright J. 2001. Begging in nestling birds. In: Nolan V Jr, Thompson CF, editors. Current ornithology. Boston (MA): Springer. p. 83–118.

Crawley MJ. 2005. Statistics: an introduction using R. Chichester (UK): Wiley.

Dickens M, Berridge D, Hartley IR. 2008. Biparental care and offspring begging strategies: hungry nestling blue tits move towards the father. Anim Behav. 75:167–174.

Dougherty LR, Shuker DM. 2014. The effect of experimental design on the measurement of mate choice: a meta-analysis. Behav Ecol. 26:311–319.

Eggert A-K, Müller JK. 1992. Joint breeding in female burying beetles. Behav Ecol Sociobiol. 31:237–242.

Eggert A-K, Müller JK. 2000. Timing of oviposition and reproductive skew in cobreeding female burying beetles (*Nicrophorus vespilloides*). Behav Ecol. 11:357–366.

Engel KC, Stökl J, Schweizer R, Vogel H, Ayasse M, Ruther J, Steiger S. 2016. A hormone-related female anti-aphrodisiac signals temporary infertility and causes sexual abstinence to synchronize parental care. Nat Commun. 7:11035.

Fortuna RAFG. 2016. Begging for answers: how do parents and helpers respond to offspring begging in a cooperatively breeding system? [MSc thesis]. [Porto (Portugal)]: University of Porto.

Fox J, Weisberg S, Adler D, Bates D, Baud-bovy G, Ellison S, Firth D, Friendly M, Gorjanc G, Graves S, et al. 2016. Package "car." CRAN Repository 171. [cited 2020 March 9]. Available from: https://cran.r-project.org/web/packages/car/car.pdf.

Georgiou Shippi A, Paquet M, Smiseth PT. 2018. Sex differences in parental defence against conspecific intruders in the burying beetle *Nicrophorus vespilloides*. Anim Behav. 136:21–29.

Godfray HCJ. 1991. Signalling of need by offspring to their parents. Nature. 352:328.

Harrison XA. 2015. A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology & evolution. PeerJ. 3:e1114.

Haskell DG. 1994. Experimental evidence that nestling begging behavior incurs a cost due to nest predation. Proc Biol Sci. 257:161–164.

Hatano E, Wada-Katsumata A, Schal C. 2020. Environmental decomposition of olefinic cuticular hydrocarbons of *Periplaneta americana* generates a volatile pheromone that guides social behaviour. Proc Biol Sci. 287:20192466.

Kilner RM. 2001. A growth cost of begging in captive canary chicks. Proc Natl Acad Sci USA. 98:11394–11398.

Kilner R, Johnstone RA. 1997. Begging the question: are offspring solicitation behaviours signals of need? Trends Ecol Evol. 12:11–15.

Koenig WD, Dickinson JL. 2004. Ecology and evolution of cooperative breeding in birds. Cambridge (UK): Cambridge University Press.

Kölliker M, Richner H, Werner I, Heeb P. 1998. Begging signals and biparental care: nestling choice between parental feeding locations. Anim Behav. 55:215–222.

Komdeur J, Schrama MJ, Meijer K, Moore AJ, Beukeboom LW. 2013. Cobreeding in the burying beetle, *Nicrophorus vespilloides*: tolerance rather than cooperation. Ethology. 119:1138–1148.

Landete-Castillejos T, García A, Gómez JA, Molina A, Gallego L. 2003. Subspecies and body size allometry affect milk production and composition, and calf growth in red deer: comparison of *Cervus elaphus hispanicus* and *Cervus elaphus scoticus*. Physiol Biochem Zool. 76:594–602.

Lock JE, Smiseth PT, Moore AJ. 2004. Selection, inheritance, and the evolution of parent-offspring interactions. Am Nat. 164:13–24.

Madden JR, Kunc HJP, English S, Manser MB, Clutton-Brock TH. 2009. Do meerkat (*Suricata suricatta*) pups exhibit strategic begging behaviour and so exploit adults that feed at relatively high rates? Behav Ecol Sociobiol. 63:1259–1268.

Mäenpää MI, Andrews CP, Collette D, Leigh D, Smiseth PT. 2015. Burying beetle larvae discriminate between individual parents and between some classes of adults. Ethology. 121:395–402.

Mattey SN, Richardson J, Ratz T, Smiseth PT. 2018. Effects of offspring and parental inbreeding on parent-offspring communication. Am Nat. 191:716–725.

Müller JK, Eggert AK. 1990. Time-dependent shifts between infanticidal and parental behavior in female burying beetles a mechanism of indirect mother-offspring recognition. Behav Ecol Sociobiol. 27:11–16.

Müller JK, Eggert A-K, Elsner T. 2003. Nestmate recognition in burying beetles: the "breeder's badge" as a cue used by females to distinguish their mates from male intruders. Behav Ecol. 14:212–220.

Otronen M. 1988. The effect of body size on the outcome of fights in burying beetles (*Nicrophorus*). Ann Zool Fenn. 25:191–201.

Paquet M, Drummond H, Smiseth PT. 2018. Offspring are predisposed to beg more towards females in the burying beetle *Nicrophorus vespilloides*. Anim Behav. 141:195–201.

Parker GA, Royle NJ, Hartley IR. 2002. Intrafamilial conflict and parental investment: a synthesis. Philos Trans R Soc Lond B Biol Sci. 357:295–307.

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. J Evol Biol. 28:1965–1974.

Price K, Harvey H, Ydenberg R. 1996. Begging tactics of yellow-headed blackbirds, *Xanthocephalus xanthocephalus*, in relation to need. Anim Behav. 51:421–435.

R Development Core Team. 2011. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing.

Ratz T, Smiseth PT. 2018. Flexible parents: joint effects of handicapping and brood size manipulation on female parental care in *Nicrophorus vespilloides*. J Evol Biol. 31:646–656.

Ratz T, Stenson S, Smiseth PT. 2020. Offspring beg more towards larger females in a burying beetle. Behav Ecol. doi:10.5061/dryad.0zpc866vv.

Rauter CM, Moore AJ. 1999. Do honest-signalling models of offspring solicitation apply to insects? Proc Biol Sci. 266:1691–1696.

Redondo T, Castro F. 1992. The increase in risk of predation with begging activity in broods of Magpies, *Pica pica*. Ibis. 134:180–187.

Richardson J, Smiseth PT. Forthcoming 2020. Maternity uncertainty in cobreeding beetles: females lay more and larger eggs and provide less care. Behav Ecol. 31:641–650.

Ripley B, Venables B, Bates DM, Hornik K, Gebhardt A, Firth D. 2017. Package "MASS." [cited 2020 March 9]. Available from: http://cran.r-projectorg/web/ packages/MASS/MASS.pdf.

Roulin A, Bersier L-F. 2007. Nestling barn owls beg more intensely in the presence of their mother than in the presence of their father. Anim Behav. 74:1099–1106.

Scott MP. 1998. The ecology and behavior of burying beetles. Annu Rev Entomol. 43:595–618.

Smiseth PT, Andrews C, Brown E, Prentice PM. 2010. Chemical stimuli from parents trigger larval begging in burying beetles. Beha Ecol. 21:526–531.

Smiseth PT, Darwell CT, Moore AJ. 2003. Partial begging: an empirical model for the early evolution of offspring signalling. Proc Biol Sci. 270:1773–1777.

Smiseth PT, Dawson C, Varley E, Moore AJ. 2005. How do caring parents respond to mate loss? Differential response by males and females. Anim Behav. 69:551–559.

Smiseth PT, Lennox L, Moore AJ. 2007. Interaction between parental care and sibling competition: parents enhance offspring growth and exacerbate sibling competition. Evolution. 61:2331–2339.

Smiseth PT, Lorentsen SH. 2001. Begging and parent–offspring conflict in grey seals. Anim Behav. 62:273–279.

Smiseth PT, Moore AJ. 2002. Does resource availability affect offspring begging and parental provisioning in a partially begging species? Anim Behav. 63:577–585.

Smiseth PT, Moore AJ. 2004. Behavioral dynamics between caring males and females in a beetle with facultative biparental care. Behav Ecol. 15:621–628.

Smiseth PT, Moore AJ. 2007. Signalling of hunger by senior and junior larvae in asynchronous broods of a burying beetle. Anim Behav. 74:699–705.

Smiseth PT, Parker HJ. 2008. Is there a cost to larval begging in the burying beetle *Nicrophorus vespilloides*? Behav Ecol. 19:1111–1115.

Smiseth PT, Ward RSJ, Moore AJ. 2006. Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. Funct Ecol. 20:151–156.

Smiseth PT, Ward RJ, Moore AJ. 2007. Parents influence asymmetric sibling competition: experimental evidence with partially dependent young. Ecology. 88:3174–3182.

Steiger S. 2013. Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. Proc Biol Sci. 280:20131225.

Steiger S, Haberer W, Müller JK. 2011. Social environment determines degree of chemical signalling. Biol Lett. 7:822–824.

Steiger S, Peschke K, Francke W, Müller JK. 2007. The smell of parents: breeding status influences cuticular hydrocarbon pattern in the burying beetle *Nicrophorus vespilloides*. Proc Biol Sci. 274:2211–2220.

Suzuki S. 2015. Recognition of the sex of the parents by young: effects of hunger and biparental care on the begging behavior of *Nicrophorus quadripunctatus* larvae. J Insect Behav. 28:38–344.

Takata M, Mitaka Y, Steiger S, Mori N. 2019. A parental volatile pheromone triggers offspring begging in a burying beetle. iScience. 19:1260–1278.

Trumbo ST. 1992. Monogamy to communal breeding: exploitation of a broad resource base by burying beetles (*Nicrophorus*). Ecol Entomol. 17:289–298.

Trumbo ST. 2007. Defending young biparentally: female risk-taking with and without a male in the burying beetle, *Nicrophorus pustulatus*. Behav Ecol Sociobiol. 61:1717–1723.

Trumbo ST, Valletta RC. 2007. The costs of confronting infanticidal intruders in a burying beetle. Ethology. 113:386–393.

Tveera T, Saether BE, Aanes R, Erikstad KE. 1998. Regulation of food provisioning in the Antarctic petrel; the importance of parental body condition and chick body mass. J Anim Ecol. 67:699–704.

Vehrencamp SL. 1978. The adaptive significance of communal nesting in groove-billed anis (*Crotophaga sulcirostris*). Behav Ecol Sociobiol. 4:1–33.

Vehrencamp SL. 2000. Evolutionary routes to joint-female nesting in birds. Behav Ecol. 11:334–344.

Yoshioka M, Meeks C, Summers K. 2016. Evidence for begging as an honest signal of offspring need in the biparental mimic poison frog. Anim Behav. 113:1–11.

RESEARCH PAPER

Effects of inbreeding on behavioural plasticity of parent–offspring interactions in a burying beetle

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Abstract

Inbreeding depression is defined as a fitness decline in progeny resulting from mating between related individuals, the severity of which may vary across environmental conditions. Such inbreeding-by-environment interactions might reflect that inbred individuals have a lower capacity for adjusting their phenotype to match different environmental conditions better, as shown in prior studies on developmental plasticity. Behavioural plasticity is more flexible than developmental plasticity because it is reversible and relatively quick, but little is known about its sensitivity to inbreeding. Here, we investigate effects of inbreeding on behavioural plasticity in the context of parent–offspring interactions in the burying beetle *Nicrophorus vespilloides*. Larvae increase begging with the level of hunger, and parents increase their level of care when brood sizes increase. Here, we find that inbreeding increased behavioural plasticity in larvae: inbred larvae reduced their time spent associating with a parent in response to the length of food deprivation more than outbred larvae. However, inbreeding had no effect on the behavioural plasticity of offspring begging or any parental behaviour. Overall, our results show that inbreeding can *increase* behavioural plasticity. We suggest that inbreeding-by-environment interactions might arise when inbreeding is associated with too little or too much plasticity in response to changing environmental conditions.

KEYWORDS
begging, inbreeding-by-environment interactions, *Nicrophorus vespilloides*, parental care, phenotypic plasticity

1 | INTRODUCTION

Inbreeding, or mating between related individuals, is a key issue in ecology and evolution because of its impact on the persistence of populations and on their ability to evolve in response to changing environments (Charlesworth, 2003; Keller & Waller, 2002). Inbreeding is often associated with a decline in fitness of any resulting progeny, a phenomenon known as inbreeding depression (Davenport, 1908; East, 1908). Inbreeding depression is caused by greater homozygosity associated with inbreeding, which reduces fitness by increasing the risk that rare, deleterious and recessive alleles are expressed and exposed to selection (dominance hypothesis; Davenport, 1908)

or by reducing any potential benefits due to heterozygote advantage (overdominance hypothesis; East, 1908). The severity of inbreeding depression can vary across environments (Armbruster & Reed, 2005; Cheptou & Donohue, 2011; Fox & Reed, 2011), and sources of environmental stress, such as intense intraspecific competition (Haag, Hottinger, Riek, & Ebert, 2002; Meagher, Penn, & Potts, 2000), extreme temperatures (Bijlsma, Bundgaard, & van Putten, 1999; Fox, Stillwell, Wallin, Curtis, & Reed, 2011), parasitic infection (Haag, Sakwinska, & Ebert, 2003) and nutrient deprivation (Auld & Henkel, 2014; Schou, Loeschcke, & Kristensen, 2015), are known to exacerbate inbreeding depression. However, little is known about the mechanisms for these inbreeding-by-environmental stress interactions (Reed, Fox, Enders, & Kristensen, 2012). Potentially, environmental stress might exacerbate inbreeding depression by increasing the intensity of selection acting against deleterious alleles (Laffafian, King, & Agrawal, 2010) or by increasing the amount of phenotypic variation induced by stress, and thereby fitness differences between inbred and outbred individuals (Waller, Dole, & Bersch, 2008). A plausible underlying mechanism is that inbreeding is associated with reduced phenotypic plasticity (Bijlsma & Loeschcke, 2012; Fowler & Whitlock, 1999; Reed et al., 2012; Reed, Lowe, Briscoe, & Frankham, 2003). This mechanism requires that inbred individuals have a lower capacity for adjusting their phenotype to match different environmental conditions than outbred ones.

There is good empirical evidence that inbreeding alters developmental plasticity. For example, inbreeding reduces the duration of developmental growth in response to changing temperatures in *Drosophila subobscura* (e.g. Maynard Smith, Clarke, & Hollingsworth, 1955) and the development of morphological defences in response to the presence of predators in the freshwater snail *Physa acuta* (e.g. Auld & Relyea, 2010). Inbreeding also reduces plasticity in life history traits, such as laying date in response to advancing spring temperatures in red-cockaded woodpeckers (Schiegg, Pasinelli, Walters, & Daniels, 2002) and brood size in response to changes in resource availability in the burying beetle *Nicrophorus vespilloides* (Richardson, Comin, & Smiseth, 2018). On the other hand, inbreeding increases plasticity in the development of wing shape in response to changing temperatures in *Drosophila melanogaster* (Schou, Kristensen, & Loeschcke, 2015). However, little is known about the effects of inbreeding on behavioural plasticity; that is, how an individual adjusts its behaviour in response to changing environmental conditions. Unlike developmental traits, behaviours can change relatively quickly in response to variation in the social and physical environment. These changes are also reversible, allowing an individual to match its behavioural phenotype rapidly to environmental changes that occur within its lifetimes (Candolin & Wong, 2012; Piersma & Drent, 2003; Snell-Rood, 2013). Behavioural plasticity is likely to be linked to an individual's reproductive success and survival given that many behaviours play a key role during mating (e.g., Rodríguez, Rebar, & Fowlere-Finn, 2013), parenting (e.g. Royle, Russell, & Wilson, 2014), foraging (e.g., Sol, Timmermans, & Lefebvre, 2002) and avoidance of predators or pathogens (e.g., Benard, 2004). Understanding the interplay between behavioural plasticity and inbreeding is now an

important challenge given that anthropogenic environmental change is expected to cause a reduction in population sizes, thereby increasing the risk of inbreeding, and induce changes in environmental conditions, such as resources required for breeding due to advancing spring temperatures (Schiegg et al., 2002). Thus, there is now a need for studies that investigate the effects of inbreeding on behavioural plasticity.

We investigate the effects of inbreeding on behavioural plasticity, focusing on behaviours expressed in social interactions between individuals. We examine these behaviours because the social environment is usually highly variable and social interactions often involve highly plastic behaviours (Foster, 2013). This is because individuals often adjust their behaviour in response to characteristics of the conspecifics with which they interact, such as their behaviour, body size or state, as well as the number of individuals in the group or the population. For instance, individuals often adjust aggression to the competitive ability of competitors (Simmons, 1986), mating behaviour to the availability or quality of mating partners (Kokko & Rankin, 2006; Kvarnemo & Ahnesjo, 1996), and parental behaviour to the presence of and/or the amount of care provided by their partner (Johnstone & Hinde, 2006) or the offspring's begging behaviour (Kacelnik, Cotton, Stirling, & Wright, 1995). Furthermore, there is evidence that inbreeding affects social interactions (e.g. Richardson & Smiseth, 2017; Mattey, Richardson, Ratz, & Smiseth, 2018), suggesting that inbreeding impacts how individuals respond to variation in their social environment. Inbreeding might alter behavioural plasticity in social interactions if inbred individuals invest less in costly mechanisms required for adaptive behavioural plasticity (Dingemanse & Wolf, 2013; Snell-Rood, 2013). These might include the necessary sensory and cognitive systems to perceive variation in the social environment, process the relevant information and mount a plastic behavioural response (Auld, Agrawal, & Relyea, 2010; Coppens, De Boer, & Koolhaas, 2010; DeWitt, Sih, & Wilson, 1998; Mathot, Wright, Kempenaers, & Dingemanse, 2012). If so, we expect inbred individuals to adjust their behaviour to the social context (requiring high cognitive abilities; Humphrey, 1976) less well than outbred ones. Altogether, we might expect behaviours expressed in social interactions to be particularly sensitive to the effects of inbreeding due to the key role of behavioural plasticity in social interactions and the potential impact of inbreeding on the necessary sensory and cognitive systems of such behaviours.

In this study, we investigate whether inbreeding alters the behavioural plasticity of offspring and parental behaviours expressed in parent–offspring interactions in the burying beetle *Nicrophorus vespilloides*. We focus specifically on parent–offspring interactions because both offspring and parental behaviours are highly flexible (Kilner & Johnstone 1997; Smiseth, Wright, & Kölliker, 2008; Royle et al., 2014). Larvae beg to obtain food from their parents, and parent provisioning predigested food to larvae (Eggert, Reinking, & Müller, 1998; Smiseth, Darwell, & Moore, 2003). Larvae adjust begging behaviour to their hunger state (which reflects the amount of food provisioned by parents in the recent past), spending more time begging when subject to food deprivation (Smiseth

& Moore, 2004, 2007). This plasticity in larval begging behaviour is likely to be adaptive given that begging is associated with both fitness benefits and fitness costs (Andrews & Smiseth, 2013; Takata, Mitaka, Steiger, & Mori, 2019). Likewise, parents adjust their parental behaviour in response to brood size, providing more care towards larger broods (Ratz & Smiseth, 2018; Smiseth, Lennox, & Moore, 2007; Smiseth, Ward, & Moore, 2007). This plasticity in parental behaviour is also likely to be adaptive given that parents caring for larger broods incur a fitness cost from providing more care (Ratz & Smiseth, 2018). Thus, assuming that larval and parental responses are adaptive, any changes in plasticity in larval behaviour in response to food deprivation and parental behaviour in response to brood size are likely to have detrimental fitness consequences. In addition, previous work shows that inbreeding affects larval begging behaviour (Mattey et al., 2018; Mattey & Smiseth, 2015), and offspring inbreeding affects the amount of care provided by outbred parents (Mattey et al., 2018; Mattey, Strutt, & Smiseth, 2013; Ratz, Castel, & Smiseth, 2018). Thus, inbreeding alters trait values of behaviours involved in parent–offspring interactions.

Our aim was to test for effects of inbreeding on behavioural plasticity by focusing on the interactions between inbreeding status and larval and parental behaviours across two environmental gradients. In the first experiment, we manipulated the inbreeding status of larvae (inbred or outbred) and monitored larval responses to variable lengths of food deprivation. In the second experiment, we manipulated the inbreeding status of parents (inbred or outbred) and monitored parental responses to variable brood sizes. If inbreeding reduced the ability of individuals to respond to variation in their environment, we predicted an effect of the interaction between the inbreeding status of larvae and food deprivation on the amount of time spent begging and/or associating with the parent by larvae. Likewise, we predicted an effect of the interaction between the inbreeding status of the parent and brood size on time spent provisioning food and/or associating with the brood by parents.

2 | MATERIALS AND METHODS

2.1 | Origin and rearing of experimental beetles

We used beetles from the 7–9th generations of an outbred laboratory population descending from individuals collected in Corstorphine Hill, Edinburgh, UK. The population was maintained under 20°C and a 16:8 hr light:dark photoperiod. Nonbreeding adult beetles were kept in individual transparent plastic containers (12 cm × 8 cm × 2 cm) filled with moist soil and fed organic beef twice a week. We minimized inbreeding in our stock population by avoiding breeding between closely related individuals (defined as individuals sharing at least one common grandparent), by maintaining a large stock population comprised of 100–150 breeding pairs per generation (Mattey et al., 2018), and by supplementing the stock population annually with wild-caught beetles from our collection

site in Blackford Hill, Edinburgh, UK. We produced inbred individuals by pairing full-sibling beetles from the stock population in the previous generation (Mattey et al., 2018). Given the negligible level of inbreeding in our stock population (see Mattey et al., 2018), inbred and outbred individuals had a coefficient of inbreeding of $F ≈ 0.25$ and 0, respectively, when referenced to the local wild population from our collection site.

2.2 | Larval behaviour

In our first experiment, we manipulated the inbreeding status of larvae and monitored their response to three different levels of food deprivation. We manipulated the inbreeding status of larvae by assembling experimental broods where all larvae in the brood were either outbred ($N = 26$) or inbred ($N = 28$). To this end, we set up pairs of virgin outbred parents at the start of the experiment by placing a male and a female in a large plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass weighing 20.1–25.0 g. We generated inbred offspring by mating females to their full-sibling brothers and produced outbred offspring by mating other females to unrelated males. On the day before we anticipated the eggs to hatch (i.e. two days after the onset of egg-laying; Smiseth, Ward, & Moore, 2006), we moved females and their carcasses to new containers lined with fresh soil (the males were discarded; Figure 1a) while leaving their eggs behind in the old container. These separations were done so that we could allocate an experimental brood made up of 15 same-aged larvae of mixed maternal origin to each female (Smiseth, Lennox, et al., 2007). We standardized our brood sizes in order to avoid potential confounding effects due to variation in brood size and larval age on larval behaviour (Paquet & Smiseth, 2017; Smiseth et al., 2003; Smiseth, Lennox, et al., 2007). We only allocated experimental broods to each female once her own eggs had hatched because parents will kill any larvae that emerge on the carcass before their own eggs have hatched (Müller & Eggert, 1990).

For each brood, we collected data on larval behaviour at three different lengths of food deprivation: 0, 90 and 180 min. To this end, we performed three consecutive 15-min observation sessions on each brood over a 195-min period, starting 24 hr (±15 min) after a given brood was placed on a carcass. We recorded larval behaviour away from the mouse carcasses using a dead female parent as a stimulus. We did so to ensure that larvae had no access to food during the experiment, which otherwise would have interfered with our food deprivation treatment. Using a dead female as a stimulus also allowed us to exclude any potential effect of variation in female behaviour on larval behaviour (Smiseth, Andrews, Brown, & Prentice, 2010; Smiseth & Parker, 2008), and larvae beg towards a dead female in a similar way as towards a live female (Smiseth et al., 2010; Smiseth & Parker, 2008). We used dead female parents that had bred and produced a brood to ensure that larvae perceive them as caring parents (Smiseth et al., 2010).

We killed females used as a stimulus approximately 1 hr before the start of each behavioural session by freezing them for 30 min and
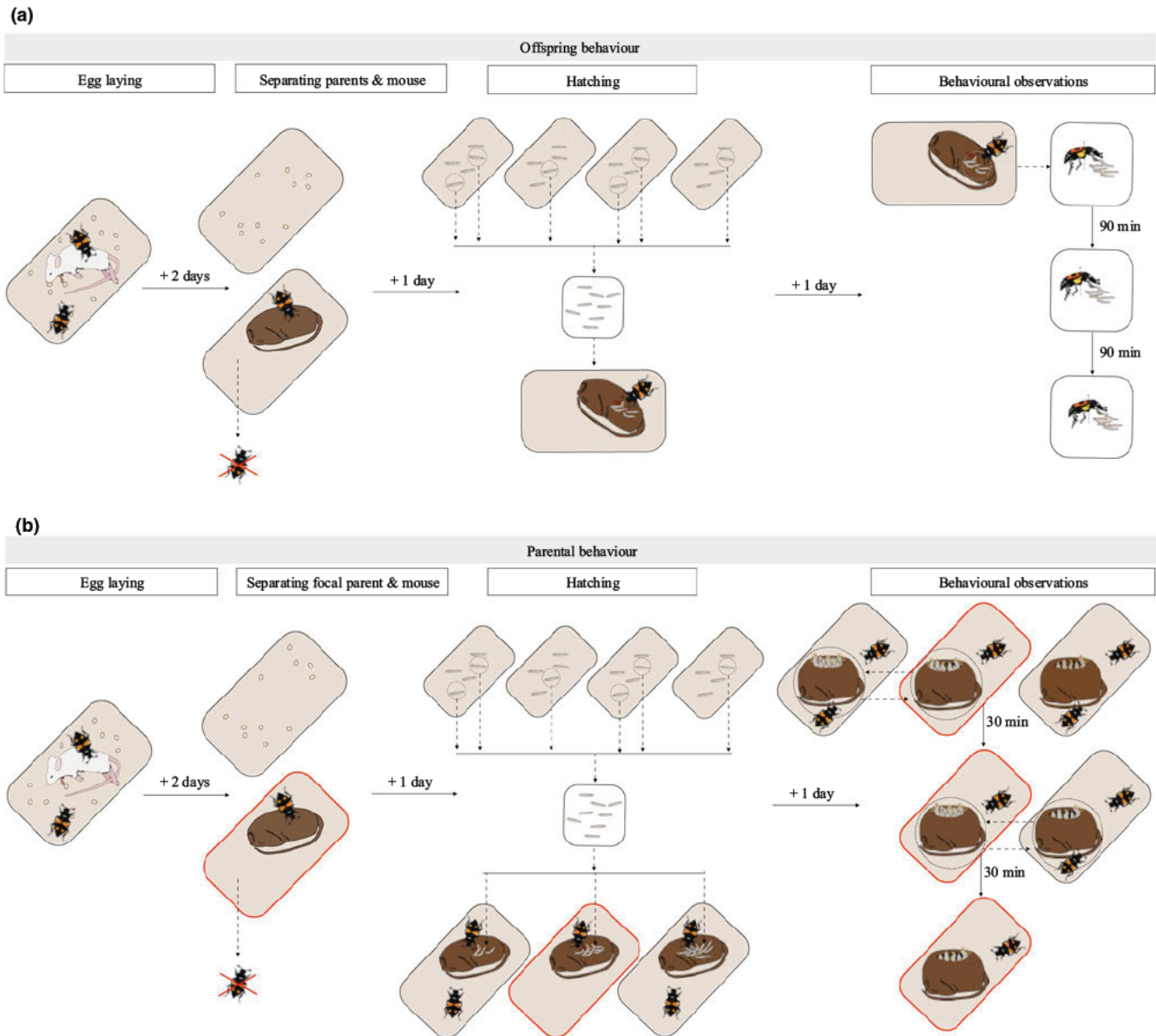
FIGURE 1 Diagram of the experimental design to investigate offspring response to the length of food deprivation (a) and parental response to variation in brood size (b)

then thawing them for another 30 min. Once thawed, we pinned each dead female to the centre of a small container (12 cm × 8 cm × 2 cm) lined with moist paper and in a position mimicking that of a parent provisioning food to the brood (Mäenpää, Andrews, Collette, Leigh, & Smiseth, 2015). We placed the experimental brood away from the female and left the larvae to acclimatize for 5 min before starting the first observation (see details below). Thus, in order to beg for food from the female, larvae first had to move towards the female to associate with her. Larvae might later move away from the female to search for other sources of food given that the female was dead and that larvae would receive no returns on their begging effort. Larvae were often observed to remain cohesive as a group, regardless of whether they were associating with the female or away from her. When away from the female, larvae would sometimes split into multiple groups and move around the container at a slow pace

either individually or in as a group. Note that each brood was placed with its caring female, and that larvae therefore always were exposed to a familiar female during the observation. After the first observation, the female was removed, and the larvae were kept in the container for another 75 min to give a total of 90 min of food deprivation. For the second observation, we again pinned the female in the centre of the container and returned the experimental brood to where it was placed at the start of the first observation. We repeated this procedure once more by removing the female at the end of the second observation and keeping the larvae in the container for another 75 min for a total of 180 min of food deprivation. Although larvae may not experience this level of food deprivation in natural situations, there will be natural variation in hunger level due to the time elapsed since they were last provisioned food by a parent (Smiseth et al., 2003). Larvae beg more and are hungrier when they

cannot receive food from a parent, suggesting that larvae are less efficient at obtaining food by self-feeding and have greater benefits when they obtain food from their parents (Smiseth & Moore, 2004). Furthermore, larvae may have limited access to those parts of the carcass that are most easily processed, especially when larvae are young and have relatively small mandibles (Eggert et al., 1998; Jarrett et al., 2018). We used our food deprivation treatments for pragmatic reasons, because it provides a straightforward procedure for generating variation in larval hunger levels (Smiseth & Moore, 2004, 2007).

During each observation session, we monitored larval behaviour every 60 s over a 15-min period. We recorded larval begging as the number of larvae that were touching any part of the female's body with their legs (Smiseth et al., 2003). We also recorded larval association with the female as the number of larvae that were within reaching distance from the female (i.e. a distance equal to or less than the pronotom length of the female). Based on these measures, we calculated the average time spent begging per larva in the brood (B) as the number of begging events cumulated across the 15 scans (Σb) divided by the cumulated number of larvae near the female (n), or $B = \Sigma b/n$. We also calculated the average time per larva in the brood spent associating with the female (A) as the number of larvae that were near the female across the 15 scans (Σa) divided by the total number of larvae in the brood (n), or $A = \Sigma a/n$.

2.3 | Parental behaviour

In our second experiment, we manipulated the inbreeding status of parents and monitored their response to small and large broods. In the previous generation, we generated inbred parents by mating their mother to her full-sibling brother, and we generated outbred parents by mating their mother to an unrelated male. We used both male and female parents in this experiment, allowing us to detect potential sex differences in behavioural plasticity of parents (Royle & Hopwood, 2017; Royle et al., 2014). Thus, we used a 2 × 2 factorial design in which we recorded the behaviour of 313 adult beetles. As we were interested in how parents adjust care in response to brood size, we excluded 175 individuals that were not observed providing care at least once to any one of the two broods. The final sample included 36 inbred males, 31 outbred males, 36 inbred females and 35 outbred females. To initiate breeding, we paired each experimental parent to an unrelated outbred partner. We placed the breeding pair into a larger plastic container (17 cm × 12 cm × 6 cm) filled with 1 cm of moist soil and containing a previously frozen mouse carcass of a standardized size (20.3–23.9 g) (Livefoods Direct, Sheffield). We separated the parents from their eggs two days after the first egg was laid by moving the parents and their carcass to a new container containing fresh soil (Figure 1b). We discarded the partner at the same time to ensure that any effect of brood size on parental behaviour was not confounded by the presence of the partner. Once the eggs had started hatching, each experimental parent was allocated a brood of ten larvae (hereafter referred to as the baseline brood) to whom they provided care until being allocated the first experimental broods

24 hr later (see below). To avoid filial cannibalism, we allocated baseline broods to parents only once their own larvae had hatched.

In parallel with setting up the experimental parents, we set up additional pairs of unrelated males and females. We did this to produce additional larvae that were used to generate both baseline and experimental broods. The additional pairs also functioned as foster parents for the small and large experimental broods until they were allocated to an experimental parent 24 hr after it had been allocated its initial baseline brood. As described for the experimental parents above, we separated foster parents from their eggs two days after the first egg was laid by moving the parents and their carcass to a new container containing fresh soil. However, we left both foster parents with the broods to ensure that all experimental broods had encountered both a male and a female parent. Once eggs had started hatching, we allocated each foster pair either a small brood of five larvae or a large brood of 20 larvae, which fall well within the range of natural brood sizes for this species (Smiseth & Moore, 2002). We used these brood sizes because prior studies have shown that parents provide double the amount of time spent caring towards a brood of 20 compared to a brood of five larvae (Ratz & Smiseth, 2018; Smiseth, Lennox, et al., 2007).

For each parent, we collected data on their parental behaviour towards two different brood sizes: 5 and 20 larvae. We performed two consecutive 30-min observation sessions for each parent, starting 24 hr (±15 min) after the parent had been provided with the initial baseline brood. We randomized the order in which experimental parents were provided with broods of different sizes. We first removed the original mouse carcass containing the baseline brood of 10 larvae and immediately replaced it with a carcass from a foster pair containing an experimental brood of either 5 or 20 larvae. We allowed the larvae to settle for 30 min before starting the first observation. Immediately after the first observation was completed, we replaced this carcass with a carcass from a different foster pair containing an experimental brood of the opposite treatment (five larvae if the first experimental brood had 20 larvae and vice versa). We again allowed the larvae to settle 30 min before starting the second observation.

During each observation session, we monitored the behaviour of experimental parents every 60 s over a 30-min period. We recorded parental provisioning of food to the brood as a mouth-to-mouth contact between the parent and at least one larva. We also recorded parental association with the brood as the parent being present on the carcass or within the crypt (the depression in the soil immediately surrounding the carcass). We calculated the percentage of time spent provisioning food to the brood and associating with the brood as the total number of scans the parents were performing the behaviour of interest (i.e. 0–30) divided by the number of scans in the observation session (i.e. 30).

2.4 | Statistical analysis

All statistical analyses were conducted using R version 3.6.0 (R Development Core Team, 2019) with the packages *car* (Fox

TABLE 1 Effects of the level of food deprivation, inbreeding status and the interaction between food deprivation and inbreeding on average larval begging and average time spent associating with the female by the larvae. Effects of brood size, inbreeding status and the interaction between brood size and inbreeding on the time spent provisioning and associating with the brood by the carrying parent. Values are obtained from GLMMs

	Environmental variable				Inbreeding status (outbred versus. inbred)				Environment:Inbreeding			
	Estimate	SE	z-value	P	Estimate	SE	z-value	P	Estimate	SE	z-value	P
Food deprivation (0, 90 or 180 min)												
Offspring Begging	0.850	0.095	8.99	**<.0001**	0.031	0.298	0.103	.918	0.046	0.131	0.351	.725
Offspring association with the parent	−0.891	0.236	−3.99	**<.0001**	1.70	0.762	2.23	**.026**	−0.891	0.330	−2.70	**.007**
Brood size (5 or 20 larvae)												
Time spent provisioning	1.37	0.109	12.6	**<.0001**	0.032	0.295	1.07	.284	−0.215	0.148	−1.46	.146
Time spent associating with the brood	1.00	0.261	3.85	**.0001**	0.394	0.607	0.650	.516	−0.127	0.367	−0.346	.730

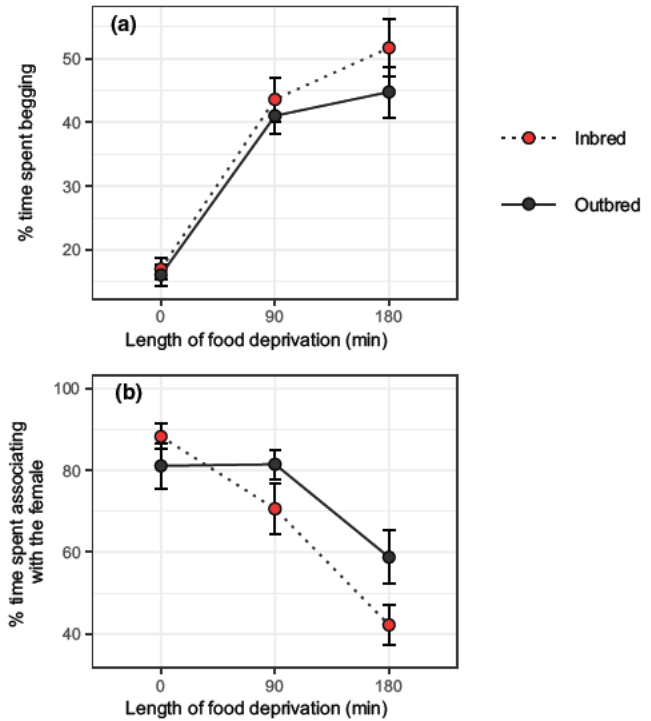*Note:* Statistically significant p values (<.05) are shown in boldface.

FIGURE 2 Effects of increasing length of food deprivation on the amount of time (percentage) larvae spent begging towards (a) and associating with (b) a female parent. Mean ± *SE*

et al., 2016) and *lme4* (Bates, Mächler, & Bolker, 2014). We quantified differences in behavioural plasticity between inbred and outbred larvae by estimating the effect of the interaction between the inbreeding status of larvae and the length of food deprivation on larval behaviour. We used general linear mixed models that assumed a binomial error structure to analyse larval behaviours (i.e. time spent begging towards and associating with the female). These models included the length of food deprivation (0, 90 and 180 min) as a continuous fixed effect and inbreeding status of larvae (inbred or outbred) as a categorical fixed effect, as well as the interaction between the two. We included brood size at the time of observation as covariate in the models to account for potential effects of brood size on larval behaviour. We also included brood ID and observation level as random effects to account for repeated observations on each brood and overdispersion of the data (Harrison, 2015), respectively.

To quantify differences in behavioural plasticity between inbred and outbred parents, we estimated the effect of the interaction between the inbreeding status of parents and brood size on parental behaviour. We used generalized linear mixed models that assumed a binomial error structure to analyse parental behaviours (i.e. time spent provisioning food and associating with the brood). These models included brood size (5 and 20 larvae) as a continuous fixed effect, inbreeding status of the parent (inbred or outbred) as a categorical fixed effect, and an effect of the interaction between the two. We also included sex of the parent as covariate to test for potential sex differences in the behavioural plasticity of parental behaviour. To account for repeated observations on the same focal individuals, we

included parental ID as random effects in both models. To account for overdispersion, we also included observation level as additional random effects in the model testing for effects on time spent associating with the brood.

3 | RESULTS

3.1 | Larval behaviour

Our main aim was to test for differences in behavioural plasticity between inbred and outbred individuals, and we therefore focused first on the interaction between the inbreeding status of larvae and the length of food deprivation on larval behaviour. There was no effect of this interaction on time spent begging (Table 1). Thus, for larval begging, there was no difference between inbred and outbred larvae with respect to behavioural plasticity in response to a change in hunger state (Figure 2a). However, there was a significant effect of this interaction on the amount of time spent associating with the female (Table 1), indicating that inbred larvae spent less time associating with the female as they became hungrier compared to outbred ones (Figure 2b). Thus, for time spent associating with the female, inbreeding increased behavioural plasticity exhibited by larvae in response to a change in their hunger state.

The length of food deprivation had a significant positive main effect on time spent begging and a negative main effect on time spent associating with the female (Figure 2a; Table 1). There was no main effect of the inbreeding status on time spent begging or associating with the female (Table 1). Finally, there was a negative main effect of brood size at the time of observation on time spent begging (estimate = −0.117, SE = 0.047, z = −2.47, p = .014), but brood size had no effect on time spent associating with the female (estimate = 0.012, SE = 0.120, z-value = 1.01, p = .314).

3.2 | Parental behaviour

For reasons explained above, we first focused on the interaction between the inbreeding status of the parent (inbred vs. outbred) and brood size (5 and 20 larvae) to test for potential differences in behavioural plasticity between inbred and outbred parents. There was no effect of this interaction on time spent provisioning food or associating with the brood (Figure 3a,b; Table 1). Thus, inbreeding did not appear to change behavioural plasticity exhibited by parents in response to changes in brood size.

As expected, brood size had a significant positive main effect on time spent provisioning food and associating with the brood (Table 1), confirming that parents spent more time provisioning food and associating with the brood when brood size increased. Finally, there were no main effects of parental inbreeding status (Table 1) or sex (estimate = −0.229, SE = 0.153, z-value = −1.50, p-value = .133) on time spent provisioning food. Likewise, there were no main effects of parental inbreeding status (Table 1) or sex (estimate = −0.255,
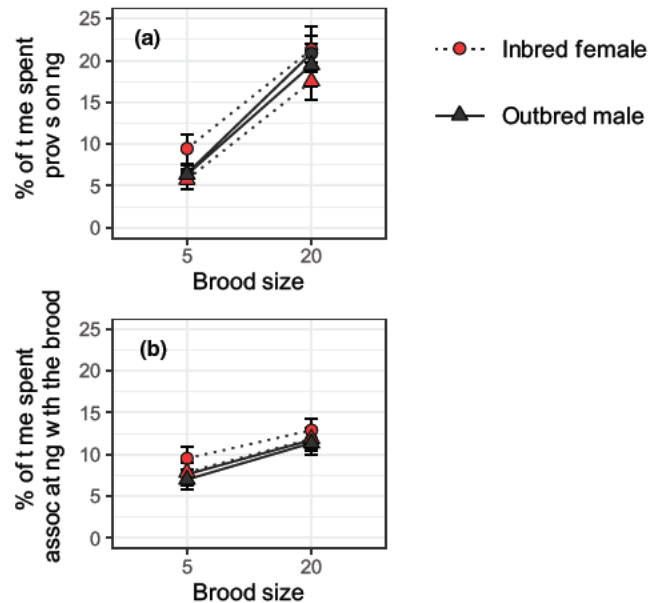

FIGURE 3 Effects of brood size on the amount of time (percentage) male and female parents spent provisioning food to (a) and associating with (b) the brood. Mean ± SE

SE = 0.244, z-value = −1.05, p-value = .296) on time spent associating with the brood.

4 | DISCUSSION

We show that inbreeding in larvae of *N. vespilloides* was associated with increased behavioural plasticity for time spent associating with the female parent. However, inbreeding was not associated with a change in behavioural plasticity in time spent begging or in the time that parents of either sex spent provisioning food or associating with the larvae. Our results derive from two experiments, in which we monitored behavioural plasticity in larvae in response to experimental variation in the length of food deprivation and behavioural plasticity in parents in response to experimental variation in brood size. We generated variation across two environmental stress gradients experimentally in order to remove confounding effects on plasticity in larval and parental behaviours. Furthermore, our study focused on behavioural plasticity in environmental gradients that larvae and parents are exposed to and respond to under natural conditions. Below, we discuss the wider implications of our results for our understanding of the effects of inbreeding on behavioural plasticity and how such effects may provide a mechanism for inbreeding-by-environment interactions affecting fitness.

Our study shows that larvae spent less time associating with the female as they became hungrier and that this decline was more pronounced in inbred larvae than in outbred ones. Currently, little is known about the potential adaptive value of behavioural plasticity in larval association with the female. Larvae associate with parents because they need to be in close proximity to them in order to beg for food (Smiseth & Moore, 2002). In our experimental design,

larvae had to move towards the female in order to be in close proximity to her. Larvae may later move away from the female because they would receive no returns on their begging given that we used a dead female as a standardized stimulus. Our results suggest that inbred and outbred larvae spent a similar amount of time associating with the female at the start of the experiment, but inbred larvae spent more time away from the female as the length of food deprivation increased (Figure 2b). Thus, our results show that inbred larvae had a greater degree of behavioural plasticity than outbred ones. Nevertheless, we urge caution when interpreting our results given that we monitored larval behaviour towards a dead parent in the absence of a carcass. We used a dead parent as a stimulus to ensure that larvae had no access to food during the experiment (which would otherwise interfere with our experimental treatment) and to control for confounding effects caused by parental behaviour (e.g. Smiseth et al., 2010; Smiseth & Parker, 2008). Prior work shows that the presence of a dead parent stimulates high levels of larval begging for at least 180 min (Smiseth & Parker, 2008). Yet, a consequence of this design is that larvae were exposed to an unresponsive parent for a considerable amount of time, which might explain why hungrier larvae spent *less* time associating with the female. In natural situations, where larvae interact with a live female on a carcass, we would expect hungrier larvae to spend more time associating with the female because larvae must stay in close proximity to her in order to have the opportunity to beg for food (Smiseth & Moore, 2002). In such situations, larvae face a choice between self-feeding from within the crater of the carcass (i.e. the cavity prepared by the parents) and leaving the crater to associate with a caring parent (Smiseth et al., 2003). Given that the larvae in our experiment could not get access to food from the dead female, and that there was no carcass from which to self-feed, larvae may have responded to food deprivation by associating less with the female and by searching for opportunities to obtain food by self-feeding (Smiseth et al., 2003). In light of this, we would not necessarily expect larvae to respond in a similar way to food deprivation when interacting with a live parent (Smiseth et al., 2003).

One potential explanation for our finding that inbreeding was associated with increased behavioural plasticity in larvae is that inbred larvae have higher nutritional needs than outbred ones. Offspring begging is thought to be an honest signal that reliably reflects the offspring's nutritional needs (Godfray, 1995), and there is good evidence that begging reflects larval hunger in our study species (Smiseth & Moore, 2004). Thus, if inbred larvae did have higher nutritional needs than outbred ones, we would expect inbred larvae to spend more time begging and to show greater plasticity in this behaviour. However, we found no evidence that this was the case as there was no effect of the interaction between larval inbreeding status and length of food deprivation on time spent begging. Furthermore, prior work on this species shows that inbred larvae spend *less* time begging to a live parent than outbred ones (Mattey et al., 2018). An alternative explanation is that inbred larvae were less able to sustain the costs of begging with an increase in the length of food deprivation than outbred ones.

This explanation, however, seems unlikely given that we found that inbred and outbred larvae increased their begging to similar degrees in response to an increase in the length of food deprivation. Thus, there is no evidence that our results can be explained as a consequence of inbred larvae having higher nutritional needs or greater costs of begging. A final explanation is that inbreeding constrains an individual's ability to invest in costly cognitive and/ or sensory mechanisms required for adaptive behavioural plasticity (Dingemanse & Wolf, 2013; Snell-Rood, 2013). In this case, inbred individuals may not be able to adjust their behaviour as effectively to match changing conditions (e.g. Schiegg et al., 2002). For example, a recent study on our study species found that inbred females are less able than outbred females to adjust brood size when the size of the carcass is changed experimentally just prior to hatching (Richardson et al., 2018). Thus, inbreeding undermines the ability of burying beetles to make sensible life decisions, suggesting that our results may reflect that inbred larvae were less able to make an appropriate decision between staying near the female and searching for opportunities to self-feed.

Our finding that inbred larvae showed greater behavioural plasticity has important implications for our understanding of the mechanism for inbreeding-by-environment interactions. Inbreeding is often associated with an increased sensitivity to environmental stress (Armbruster & Reed, 2005; Cheptou & Donohue, 2011; Fox & Reed, 2011), and prior work suggests that such inbreeding-by-environment interactions may arise if inbreeding is associated with reduced phenotypic plasticity (Bijlsma & Loeschcke, 2012; Fowler & Whitlock, 1999; Reed et al., 2003, 2012). The rationale for this explanation is that inbred individuals are less able to adjust their phenotype to cope with stressful environmental conditions than outbred individuals. However, our results show that inbreeding can be associated with *increased* phenotypic plasticity. Increased behavioural plasticity may cause inbreeding-by-environment interactions for traits that are canalized because, for some traits, there may be selection that favours resistance to phenotypic plasticity (Schou, Kristensen, et al., 2015). For example, Schou, Kristensen, et al. (2015) found that inbred lines of *Drosophila melanogaster* had higher plasticity in the developmental response of wing size in response to high temperatures. This may come at a fitness cost as small wings may reduce flight performance in warmer environments more in inbred individuals (Frazier, Harrison, Kirkton, & Roberts, 2008). Just as there can be detrimental effects from too much developmental plasticity, stabilizing selection may also favour the evolution of intermediate levels of behavioural plasticity. Thus, if there is an optimal behavioural response, we might expect inbreeding-by-environment interactions if inbred individuals show either too much or too little behavioural plasticity. Furthermore, inbreeding-by-environment interactions could occur under stabilizing selection if inbred individuals show greater variance in behavioural plasticity, even if there is no difference in mean plasticity between inbred and outbred individuals. This would be the case if some inbred individuals show reduced behavioural plasticity whereas others show increased behavioural plasticity compared to outbred individuals. Thus, there is a need

for further work focusing on how selection works on behavioural plasticity.

In summary, we found that inbreeding affects behavioural plasticity of some larval behaviours (time spent associating with a parent), whereas inbreeding had no effect on behavioural plasticity of other larval behaviours (time spent begging) or any parental behaviours (time spent provisioning food and associating with the brood). To our knowledge, this is the first study investigating how inbreeding affects plasticity of social behaviours. Our findings suggest that effects of inbreeding on behavioural plasticity may be one of the potential mechanisms underlying the effects of inbreeding on social interactions among individuals (e.g. Richardson & Smiseth, 2017; Mattey et al., 2018). More generally, our findings have important implications for our knowledge about inbreeding depression by showing that inbred individuals can show *greater* behavioural plasticity in response to environmental variation than outbred ones. We suggest that effects of inbreeding on behavioural plasticity may cause inbreeding-by-environment interactions for traits where there are negative fitness consequences of showing either too much or too little plasticity in response to changing environmental conditions. We encourage more work on the interplay between inbreeding and adaptive behavioural plasticity given that inbreeding and stress due to environmental change are growing conservation concerns in many natural populations (e.g. Hamilton & Miller, 2016; Reed et al., 2012). Understanding the interplay between them will now be critical in our understanding of how natural populations respond to environmental change, such as climate change and population decline.

AUTHORS' CONTRIBUTION

T.R. and P.T.S. conceived and designed the experiment. T.R. and A.P. performed the experiment. T.R. analysed the data and wrote the first draft of the manuscript. All authors discussed the results and contributed to the final manuscript.

DATA AVAILABILITY STATEMENT

Data deposited at dryad: https://doi.org/10.5061/dryad.c2fqz615b.

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REFERENCES

Andrews, C. P., & Smiseth, P. T. (2013). Differentiating among alternative models for the resolution of parent-offspring conflict. *Behavioral Ecology, 24*, 1185–1191.

Armbruster, P., & Reed, D. H. (2005). Inbreeding depression in benign and stressful environments. *Heredity, 95*, 235–242. https://doi.org/10.1038/sj.hdy.6800721

Auld, J. R., Agrawal, A. A., & Relyea, R. A. (2010). Re-evaluating the costs and limits of adaptive phenotypic plasticity. *Proceedings of the Royal Society of London B: Biological Sciences 277*(1681), 503–511.

Auld, J. R., & Henkel, J. F. (2014). Diet alters delayed selfing, inbreeding depression, and reproductive senescence in a freshwater snail. *Ecology & Evolution, 4*, 2968–2977.

Auld, J. R., & Relyea, R. A. (2010). Inbreeding depression in adaptive plasticity under predation in a freshwater snail. *Biology Letters, 6*, 222–224.

Bates, D., Mächler, M., & Bolker, B. (2014). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* arXiv, *67*, 1–48.

Benard, M. F. (2004). Predator-induced phenotypic plasticity in organisms with complex life histories. *Annual Review of Ecology, Evolution, and Systematics, 35*, 651–673.

Bijlsma, R., Bundgaard, J., & van Putten, W. F. (1999). Environmental dependence of inbreeding depression and purging in *Drosophila melanogaster*. *Journal of Evolutionary Biology, 12*, 1125–1137. https://doi.org/10.1046/j.1420-9101.1999.00113.x

Bijlsma, R., & Loeschcke, V. (2012). Genetic erosion impedes adaptive responses to stressful environments. *Evolutionary Applications, 5*, 117–129. https://doi.org/10.1111/j.1752-4571.2011.00214.x

Candolin, U., & B. B. M. Wong (Eds.) (2012). *Behavioural responses to a changing world: Mechanisms and consequences*. Oxford: Oxford University Press.

Charlesworth, D. (2003). Effects of inbreeding on the genetic diversity of populations. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 358*, 1051–1070. https://doi.org/10.1098/rstb.2003.1296

Cheptou, P. O., & Donohue, K. (2011). Environment-dependent inbreeding depression: Its ecological and evolutionary significance. *New Phytologist, 189*(2), 395–407.

Coppens, C. M., De Boer, S. F., & Koolhaas, J. M. (2010). Coping styles and behavioural flexibility: Towards underlying mechanisms. *Philosophical Transactions of the Royal Society B, 365*, 4021–4028. https://doi.org/10.1098/rstb.2010.0217

Davenport, B. (1908). Degeneration, a binism and inbreeding. *Science, 28*, 454–455.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends Ecology & Evolution, 13*, 77–81. https://doi.org/10.1016/S0169-5347(97)01274-3

Dingemanse, N. J., & Wolf, M. (2013). Between-individual differences in behavioural plasticity within populations: Causes and consequences. *Animal Behaviour, 85*, 1031–1039. https://doi.org/10.1016/j.anbehav.2012.12.032

East, E. M. (1908). *Inbreeding in corn. In Report of the Connecticut Agricultural Experiment Station for the Year 1907*. New Haven: Conn. Agric. Exp. Stn. pp. 419–428.

Eggert, A.-K., Reinking, M., & Müller, J. K. (1998). Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour, 55*, 97–107. https://doi.org/10.1006/anbe.1997.0588

Foster, S. A. (2013). Evolution of behavioural phenotypes: Influences of ancestry and expression. *Animal Behaviour, 85*, 1061–1075. https://doi.org/10.1016/j.anbehav.2013.02.008

Fowler, K., & Whitlock, M. C. (1999). The distribution of phenotypic variance with inbreeding. *Evolution, 53*, 1143–1156. https://doi.org/10.1111/j.1558-5646.1999.tb04528.x

Fox, C. W., & Reed, D. H. (2011). Inbreeding depression increases with environmental stress: And experimental study and meta-analysis. *Evolution, 65*, 246–258.

Fox, C. W., Stillwell, R. C., Wallin, W. G., Curtis, C. L., & Reed, D. H. (2011). Inbreeding-environment interactions for fitness: Complex relationships between inbreeding depression and temperature stress in a seed-feeding beetle. *Evolutionary Ecology, 25*, 25–43. https://doi.org/10.1007/s10682-010-9376-3

Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-bovy, G., Ellison, S., ...Monette, G. (2016). Package "car." CRAN Repos.:171.

Frazier, M. R., Harrison, J. F., Kirkton, S. D., & Roberts, S. P. (2008). Cold rearing improves cold-flight performance in Drosophila via changes in wing morphology. *Journal of Experimental Biology, 211*, 2116–2122. https://doi.org/10.1242/jeb.019422

Godfray, C. H. J. (1995). Signaling of need between parents and offspring: Parent-offspring conflict and sibling rivalry. *American Naturalist, 146*, 1–24.

Haag, C. R., Hottinger, J. W., Riek, M., & Ebert, D. (2002). Strong inbreeding depression in a Daphnia metapopulation. *Evolution, 56*, 518–526. https://doi.org/10.1111/j.0014-3820.2002.tb01363.x

Haag, C. R., Sakwinska, O., & Ebert, D. (2003). Test of synergistic interaction between infection and inbreeding in Daphnia magna. *Evolution, 57*, 777–783. https://doi.org/10.1111/j.0014-3820.2003.tb00289.x

Hamilton, J. A., & Miller, J. M. (2016). Adaptive introgression as a resource for management and genetic conservation in a changing climate. *Conservation Biology, 30*, 33–41.

Harrison, X. A. (2015). A comparison of observation-level random effect and beta-binomial models for modelling overdispersion in binomial data in ecology & evolution. *PeerJ, 3*, e1114.

Humphrey, N. K. (1976). The social function of intellect. In P. P. G. Bateson, & R. A. Hinde (Eds.), *Growing points in ethology* (pp. 303–317). Cambridge: Cambridge University Press.

Jarrett, B. J., Evans, E., Haynes, H. B., Leaf, M. R., Rebar, D., Duarte, A., ... Kilner, R. M. (2018). A sustained change in the supply of parental care causes adaptive evolution of offspring morphology. *Nature Communications, 9*, 1–8. https://doi.org/10.1038/s41467-018-06513-6

Johnstone, R. A., & Hinde, C. A. (2006). Negotiation over offspring care–how should parents respond to each other's effort? *Behavioral Ecology, 17*, 818–827.

Kacelnik, A., Cotton, P. A., Stirling, L., & Wright, J. (1995). Food allocation among nestling starlings—sibling competition and the scope of parental choice. *Proceedings of the Royal Society of London B: Biological Sciences 259*: 259–263.

Keller, L. F., & Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends in Ecology & Evolution, 17*, 19–23. https://doi.org/10.1016/S0169-5347(02)02489-8

Kilner, R., & Johnstone, R. A. (1997). Begging the question: are offspring solicitation behaviours signals of need? *T rends in Ecology and Evolution, 12*(1), 11–15.

Kokko, H., & Rankin, D. J. (2006). Lonely hearts or sex in the city? Density-dependent effects in mating systems. *Philosophical Transactions of the Royal Society B: Biological Sciences, 361*, 319–334. https://doi.org/10.1098/rstb.2005.1784

Kvarnemo, C., & Ahnesjo, I. (1996). The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution, 11*, 404–408. https://doi.org/10.1016/0169-5347(96)10056-2

Laffafian, A., King, J. D., & Agrawal, A. F. (2010). Variation in the strength and softness of selection on deleterious mutations. *Evolution, 64*, 3232–3241. https://doi.org/10.1111/j.1558-5646.2010.01062.x

Mäenpää, M. I., Andrews, C. P., Collette, D., Leigh, D., & Smiseth, P. T. (2015). Burying beetle larvae discriminate between individual parents and between some classes of adults. *Ethology, 121*, 395–402.

Mathot, K. J., Wright, J., Kempenaers, B., & Dingemanse, N. J. (2012). Adaptive strategies for managing uncertainty may explain personality-related differences in behavioural plasticity. *Oikos, 121*, 1009–1020. https://doi.org/10.1111/j.1600-0706.2012.20339.x

Mattey, S. N., Richardson, J., Ratz, T., & Smiseth, P. T. (2018). Effects of offspring and parental inbreeding on parent-offspring communication. *American Naturalist, 191*, 716–725. https://doi.org/10.1086/697236

Mattey, S. N., & Smiseth, P. T. (2015). Complex effects of inbreeding on biparental cooperation. *American Naturalist, 185*, 1–12. https://doi.org/10.1086/679067

Mattey, S. N., Strutt, L., & Smiseth, P. T. (2013). Intergenerational effects of inbreeding in Nicrophorus vespilloides: Offspring suffer fitness costs when either they or their parents are inbred. *Journal of Evolutionary Biology, 26*, 843–853.

Maynard Smith, J., Clarke, J. M., & Hollingsworth, M. J. (1955). The expression of hybrid vigour in Drosophila subobscura. *Proceedings of the Royal Society London B: Biological Sciences 144*: 159–171.

Meagher, S., Penn, D., & Potts, W. (2000). Male-male competition magnifies inbreeding depression in wild house mice. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 3324–3329. https://doi.org/10.1073/pnas.97.7.3324

Müller, J. K., & Eggert, A.-K. (1990). Time-dependent shifts between infanticidal and parental behavior in female burying beetles a mechanism of indirect mother-offspring recognition. *Behavioral Ecology & Sociobiology, 27*, 11–16. https://doi.org/10.1007/BF00183307

Paquet, M., & Smiseth, P. T. (2017). Females manipulate behavior of caring males via prenatal maternal effects. *Proceedings of the National Academy of Sciences of the United States of America, 114*, 6800–6805. https://doi.org/10.1073/pnas.1619759114

Piersma, T., & Drent, J. (2003). Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology & Evolution, 18*, 228–233. https://doi.org/10.1016/S0169-5347(03)00036-3

R Development Core Team. (2019). *R: A language and environment for statistical computing*. Vol. 1. Vienna, Austria: R Foundation for Statistical Computing. 409.

Ratz, T., Castel, E., & Smiseth, P. T. (2018). Male assistance in parental care does not buffer against detrimental effects of maternal inbreeding on offspring. *Frontiers in Ecology & Evolution, 6*, 196. https://doi.org/10.3389/fevo.2018.00196

Ratz, T., & Smiseth, P. T. (2018). Flexible parents: Joint effects of handicapping and brood size manipulation on female parental care in Nicrophorus vespilloides. *Journal of Evolutionary Biology, 31*, 646–656.

Reed, D. H., Fox, C. W., Enders, L. S., & Kristensen, T. N. (2012). Inbreeding-stress interactions: Evolutionary and conservation consequences. *Annals of the New York Academy of Sciences, 1256*, 33–48. https://doi.org/10.1111/j.1749-6632.2012.06548.x

Reed, D. H., Lowe, E. H., Briscoe, D. A., & Frankham, R. (2003). Fitness and adaptation in a novel environment: Effect of inbreeding, prior environment, and lineage. *Evolution, 57*, 1822–1828.

Richardson, J., Comin, P., & Smiseth, P. T. (2018). Inbred burying beetles suffer fitness costs from making poor decisions. *Proceedings of the Royal Society of London B: Biological Sciences 285*: 20180419.

Richardson, J., & Smiseth, P. T. (2017). Intraspecific Competition and Inbreeding Depression: Increased Competitive Effort by Inbred Males Is Costly to Outbred Opponents. *American Naturalist, 189*(5), 539–548.

Rodríguez, R. L., Rebar, D., & Fowlere-Finn, K. D. (2013). The evolution and evolutionary consequences of social plasticity in mate preferences. *Animal Behaviour, 85*, 1041–1047. https://doi.org/10.1016/j.anbehav.2013.01.006

Royle, N. J., Hopwood, P. E. (2017). Covetable corpses and plastic beetles—The socioecological behavior of burying beetles. In M. Naguib (Ed.), *Advances in the study of behavior* (pp. 101–146). Cambridge, UK: Academic Press.

Royle, N. J., Russell, A. F., & Wilson, A. J. (2014). The evolution of flexible parenting. *Science, 345*, 776–781. https://doi.org/10.1126/science.1253294

Schiegg, K., Pasinelli, G., Walters, J. R., & Daniels, S. J. (2002). Inbreeding and experience affect response to climate change by endangered woodpeckers. *Proceedings of the Royal Society of London B: Biological Sciences 269*: 1153–1159.

Schou, M. F., Kristensen, T. N., & Loeschcke, V. (2015). Trait-specific consequences of inbreeding on adaptive phenotypic plasticity. *Ecology & Evolution, 5*, 1–6. https://doi.org/10.1002/ece3.1339

Schou, M. F., Loeschcke, V., & Kristensen, T. N. (2015). Inbreeding depression across a nutritional stress continuum. *Heredity, 115*, 56–62. https://doi.org/10.1038/hdy.2015.16

Simmons, L. W. (1986). Inter-male competition and mating success in the field cricket, *Gryllus bimaculatus* (De Geer). *Animal Behaviour, 34*, 567–579.

Smiseth, P. T., Andrews, C., Brown, E., & Prentice, P. M. (2010). Chemical stimuli from parents trigger larval begging in burying beetles. *Behavioral Ecology, 21*, 526–531. https://doi.org/10.1093/beheco/arq019

Smiseth, P. T., Darwell, C. T., & Moore, A. J. 2003. Partial begging: an empirical model for the early evolution of offspring signalling. *Proceedings of the Royal Society of London B: Biological Sciences 270*: 1773–1777.

Smiseth, P. T., Lennox, L., & Moore, A. J. (2007). Interaction between parental care and sibling competition: Parents enhance offspring growth and exacerbate sibling competition. *Evolution, 61*, 2331–2339. https://doi.org/10.1111/j.1558-5646.2007.00192.x

Smiseth, P. T., & Moore, A. J. (2002). Does resource availability affect offspring begging and parental provisioning in a partially begging species? *Animal Behaviour, 63*, 577–585. https://doi.org/10.1006/anbe.2001.1944

Smiseth, P. T., & Moore, A. J. (2004). Signalling of hunger when offspring forage by both begging and self-feeding. *Animal Behaviour, 67*, 1083–1088. https://doi.org/10.1016/j.anbehav.2003.10.012

Smiseth, P. T., & Moore, A. J. (2007). Signalling of hunger by senior and junior larvae in asynchronous broods of a burying beetle. *Animal Behaviour, 7*, 699–705. https://doi.org/10.1016/j.anbehav.2006.09.022

Smiseth, P. T., & Parker, H. J. (2008). Is there a cost of begging in the burying beetle *Nicrophorus vespilloides*? *Behavioral Ecology, 19*, 1111–1119.

Smiseth, P. T., Ward, R. J. S., & Moore, A. J. (2006). Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. *Functional Ecology, 20*, 151–156.

Smiseth, P. T., Ward, R. S. J., & Moore, A. J. (2007). How do parents influence asymmetric sibling competition? Experimental evidence from a species with partially dependent young. *Ecology, 88*, 3174–3182.

Smiseth, P. T., Wright, J., & Kölliker, M. (2008). Parent–offspring conflict and co–adaptation: behavioural ecology meets quantitative genetics. *Proceedings of the Royal Society of London B: Biological Sciences 275*, 1823–1830.

Snell-Rood, E. C. (2013). An overview of the evolutionary causes and consequences of behavioural plasticity. *Animal Behaviour, 8*, 1004–1011. https://doi.org/10.1016/j.anbehav.2012.12.031

Sol, D., Timmermans, S., & Lefebvre, L. (2002). Behavioral flexibility and invasion success in birds. *Animal Behaviour, 63*, 495–502.

Takata, M., Mitaka, Y., Steiger, S., & Mori, N. (2019). A parental volatile pheromone triggers offspring begging in a burying beetle. *iScience, 19*: 1260–1278.

Waller, D. M., Dole, J., & Bersch, A. J. (2008). Effects of stress and phenotypic variation on inbreeding depression in *Brassica rapa. Evolution, 62*, 917–931.

Male Assistance in Parental Care Does Not Buffer Against Detrimental Effects of Maternal Inbreeding on Offspring

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The severity of inbreeding depression often varies across environments and recent work suggests that social interactions can aggravate or reduce inbreeding depression. For example, stressful interactions such as competition can exacerbate inbreeding depression, whereas benign interactions such as parental care can buffer against inbreeding depression in offspring. Here, we test whether male assistance in parental care can buffer against the detrimental effects of maternal inbreeding on offspring fitness in the burying beetle *Nicrophorus vespilloides*. Our results confirm that maternal inbreeding had detrimental effects on offspring survival. However, we found no evidence that male assistance in parental care buffered against those effects on offspring fitness. Outbred females benefitted from male assistance, gaining more weight over the breeding attempt when assisted by a male. In contrast, inbred females did not benefit from male assistance, gaining as much weight regardless of whether they were assisted by a male or not. Surprisingly, we find that males gained more weight during the breeding attempt when mated to an inbred female, suggesting that males benefitted from assisting an inbred female partner in terms of their weight gain. Overall, our findings suggest that parental care or other benign social interactions may not always buffer against detrimental effects of inbreeding depression.

Keywords: inbreeding depression, inbreeding–environment interaction, indirect genetic effects, *Nicrophorus vespilloides*, parental care

INTRODUCTION

Inbreeding depression, defined as the reduction in fitness of progeny produced as a consequence of mating between relatives, has been reported in a broad diversity of animals and plants (reviewed in Charlesworth and Charlesworth, 1987; Keller and Waller, 2002; Charlesworth and Willis, 2009). There is ample evidence for inbreeding depression in fitness-related traits, including fecundity, offspring growth and survival, and longevity, from studies conducted under both laboratory and natural conditions (Keller, 1998; Slate et al., 2000; Keller and Waller, 2002). There is growing awareness that the magnitude of inbreeding depression often varies between species or studies on the same species (e.g., Fox and Scheibly, 2006). This may reflect that inbreeding depression is often more severe under more stressful environmental conditions (Hoffmann and Parsons, 1991; Armbruster and Reed, 2005; Cheptou and Donohue, 2011). The social environment may play an

important role in this context because social interactions can amplify or alleviate stress, thereby exacerbating or buffering against inbreeding depression. For example, direct competition between inbred and outbred males exacerbates inbreeding depression in house mice (*Mus domesticus*) (Meagher et al., 2000). Meanwhile, parental care buffers against inbreeding depression in offspring in the burying beetle *Nicrophorus vespilloides* (Pilakouta et al., 2015).

The examples provided above illustrate that social interactions with other individuals can have an important impact on the fitness of those individuals that are themselves inbred (e.g., Meagher et al., 2000; Pilakouta et al., 2015). However, there is mounting evidence for indirect genetic effects associated with inbreeding whereby outbred individuals suffer fitness costs as a result of interacting with or depending upon inbred ones (Mattey et al., 2013; Richardson and Smiseth, 2017). For example, in species where parents care for their offspring, parental inbreeding can have detrimental effects on the offspring's fitness. Recent studies on the burying beetle *Nicrophorus vespilloides* and red deer (*Cervus elaphus*) show that maternal inbreeding is associated with lower offspring survival (Mattey et al., 2013; Huisman et al., 2016). Such effects of maternal inbreeding on offspring fitness may result from inbred females providing less or lower-quality care than outbred ones (Mattey et al., 2013). Currently, it is unclear whether interactions with third-party individuals may buffer against the detrimental effects of maternal inbreeding on offspring fitness. For example, in species with biparental care, the presence of a male partner may offset some of the detrimental effects of maternal inbreeding on offspring. In support for this hypothesis, a study on zebra finches (*Taeniopygia guttata*) found that maternal inbreeding had no detectable effect on offspring fitness even though inbred mothers spent less time incubating their eggs (Pooley et al., 2014). In this study, males always assisted with parental care. Thus, there is now a need for studies that examine whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness by manipulating the presence and absence of male assistance.

The burying beetle *Nicrophorus vespilloides* is well-suited to test whether male assistance in care buffers against the detrimental effects of maternal inbreeding on offspring fitness. In this species, both parents cooperate to bury, maintain and guard the vertebrate carcass, which serves as the sole food source for both larvae and parents during breeding. Both parents also care for the larvae after hatching, though females spend more time provisioning food than males and males desert the brood earlier than females (Bartlett and Ashworth, 1988; Smiseth et al., 2005). Males respond to the removal or desertion of the female, and to the reduced amount of care of handicapped females, by increasing their time spent on paternal care (Smiseth et al., 2005; Royle et al., 2014; Creighton et al., 2015). Furthermore, males spend more time providing care when paired with an inbred female, suggesting that males respond to the inbreeding status of their female partner (Mattey and Smiseth, 2015). There is good evidence that maternal inbreeding has a detrimental impact on the survival of outbred offspring (Mattey et al., 2013, 2018; but see Mattey and Smiseth, 2015; Ford et al., 2018). However, there is no information as to whether male assistance in parental care

buffers against the detrimental effects of maternal inbreeding on offspring fitness.

Here, we use a 2 × 3 factorial design to test whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness in the burying beetle *N. vespilloides*. We paired an inbred or outbred female with an unrelated inbred or outbred male that assisted the female with parental care during larval development. We also added additional treatments where the male was experimentally removed before larval hatching such that the inbred or outbred female received no assistance in parental care. Our first aim was to test whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness. If so, we predicted effects of the interaction between maternal inbreeding (inbred or outbred) and male status (inbred, outbred, or absent) on offspring fitness (i.e., mean offspring survival and/or weight), reflecting that maternal inbreeding had a greater negative impact on offspring fitness when the male was absent than when the female received assistance from a male. Furthermore, if inbred males have a reduced capacity to buffer against the detrimental effects of maternal inbreeding, we predicted that maternal inbreeding would have a greater negative impact on offspring fitness when the female was assisted by an inbred male rather than an outbred male. We next tested whether male assistance in parental care had an impact on female and male weight change whilst providing care. In this species, the amount of carrion consumed by a parent reflects parental investment in future reproduction (Creighton et al., 2009). Thus, if male assistance in parental care buffered against the detrimental effects of maternal inbreeding, thereby allowing females to save more resources for investment in future reproduction, we predicted females to gain more weight when assisted by a male than when the male was absent. If outbred males were better able to buffer for the effects of maternal inbreeding than inbred ones, we predicted that females assisted by an outbred male would gain more weight than those assisted by an inbred male. Finally, as inbred females are expected to provide lower quality care than outbred ones, we predicted that males paired with an inbred female would gain less weight than males paired with an outbred female, reflecting that the former increase their investment in current reproduction (Mattey and Smiseth, 2015).

MATERIALS AND METHODS
Origin and Rearing of Experimental Beetles
The beetles used in these experiments originated from wild beetles originally collected in Corstorphine Hill, Edinburgh, U.K. In order to avoid unintended inbreeding, we maintained a large outbred laboratory population (Mattey et al., 2018). To this end, we bred 200–300 individuals each generation, recruiting three offspring from each brood to the next generation. Non-breeding adult beetles were kept in individual transparent plastic containers (12 × 8 × 2 cm) filled with moist soil, and fed small pieces of organic beef twice a week. The beetles were kept under constant temperature (20–22°C) and photoperiod (16:8 h light:dark).

Experimental Design

To test whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness, we used a 2 × 3 factorial design in which an inbred or an outbred female was mated with an inbred or outbred male that later assisted the female in parental care (inbred female mated to an inbred male: $N = 51$; inbred female mated to an outbred male: $N = 36$; outbred female mated to an inbred male: $N = 48$; outbred female mated to an outbred male: $N = 35$). Our design also included two additional treatments where an inbred or an outbred female was mated with a male that was removed before the larvae hatched ($N = 40$ and $N = 38$ for inbred and outbred females, respectively).

We generated inbred females and males for use as parents in this experiment by paring their mother with her full-sibling brother in the previous generation (Mattey et al., 2018). Meanwhile, we generated outbred females and males by paring their mother with an unrelated male (i.e., a male with which the mother did not share an ancestor for at least two generations; Mattey et al., 2018). Once the inbred and outbred females and males had reached sexual maturity, we randomly assigned each individual to one of the six treatments. At the start of the experiment, we weighed each female and male. We then paired inbred and outbred females with an unrelated inbred or outbred male partner depending on the treatment to which they had been assigned, and transferred them into a larger transparent plastic container (17 × 12 × 6 cm) filled with 1 cm of moist soil. We provided each pair with a previously frozen mouse carcass of a standardized size (22.33–26.89 g) (supplied by Livefoods Direct, Sheffield, UK) to initiate breeding. We checked each container for the presence of eggs daily and recorded the date at which the first eggs appeared as the start of egg laying. Two days after the start of egg laying, we recorded clutch size as the number of eggs visible through the bottom of the transparent containers (Monteith et al., 2012). In the limited amount of soil that we used, the number of eggs visible at the bottom of the container is strongly correlated with the actual clutch size (Monteith et al., 2012).

In those treatments where the male was absent during larval development, we removed the male from the container 2 days after the outset of egg laying as this corresponds to the day before hatching (Smiseth et al., 2006). In the remaining treatments, we left the inbred or outbred male within the container thereby allowing him to assist the female in providing parental care until the larvae completed development and dispersed from the mouse carcass (hereafter referred to as larval dispersal). At larval dispersal, we recorded brood size by counting the number of larvae in the brood and weighed the total mass of the brood. We estimated the proportion of offspring surviving until dispersal as the brood size at dispersal divided by the clutch size. We calculated mean larval mass as the total mass of the brood divided by the brood size. At larval dispersal, we also weighed each female and male parent. We then estimated the percentage of weight gain of females and males during breeding as the relative difference in body mass measured at mating (W_m) and the body mass at larval dispersal (W_d) using the following equation: $\frac{W_d - W_m}{W_m} \times 100$. We also

calculated the absolute weight gain in females and males (i.e., $W_d - W_m$).

Statistical Analysis

All statistical analyses were conducted using R v 3.3.3 (R Development Core Team, 2011) loaded with the package *car* (Fox et al., 2016). To analyze our data on offspring survival until dispersal, we used a Poisson generalized linear model. We analyzed our data on mean larval mass at dispersal and weight gain by females and males using general linear models fitted with a Gaussian error structure. These models always included maternal inbreeding (inbred or outbred) and male status (inbred, outbred, or absent) as fixed factors. We included female relative weight gain as an additional fixed factor in the model on male relative weight gain as male carrion consumption and weight change has been shown to depend on female carrion consumption and weight gain (Pilakouta et al., 2016). In the models on absolute weight gain by females and males, we also included the parent's initial mass as a fixed factor to control for potential differences in body size across individuals given that inbred females were significantly lighter than outbred females at the start of the experiment (LR$\chi^2 = 4.43$, $df = 1$, $P = 0.035$). We excluded carcass size from our analyses given that we used mouse carcasses within a narrow, standardized size range (22.33–26.89 g) in our experiment. Furthermore, there was no significant effect of carcass size in any of our analyses when we included it as a fixed factor. We assessed and evaluated whether the structure of all models was appropriate for each variable by plotting the residuals from the models. Whenever there was a significant effect of male status (inbred, outbred or, absent), we tested for differences between each treatment using Tukey contrasts reporting p-values based on the Bonferroni correction for multiple testing. The complete dataset and R code used for the analyses are provided in **Data Sheet 1** and **Data Sheet 2** (Supplementary Material).

RESULTS

Offspring Fitness

There was no significant effect of the interaction between maternal inbreeding (inbred or outbred) and male status (inbred, outbred, or absent) on the proportion of offspring surviving until dispersal (Maternal inbreeding:Male status, **Table 1**, **Figure 1A**). Thus, there was no evidence that male assistance in parental care buffered against the detrimental effects of maternal inbreeding on offspring fitness.
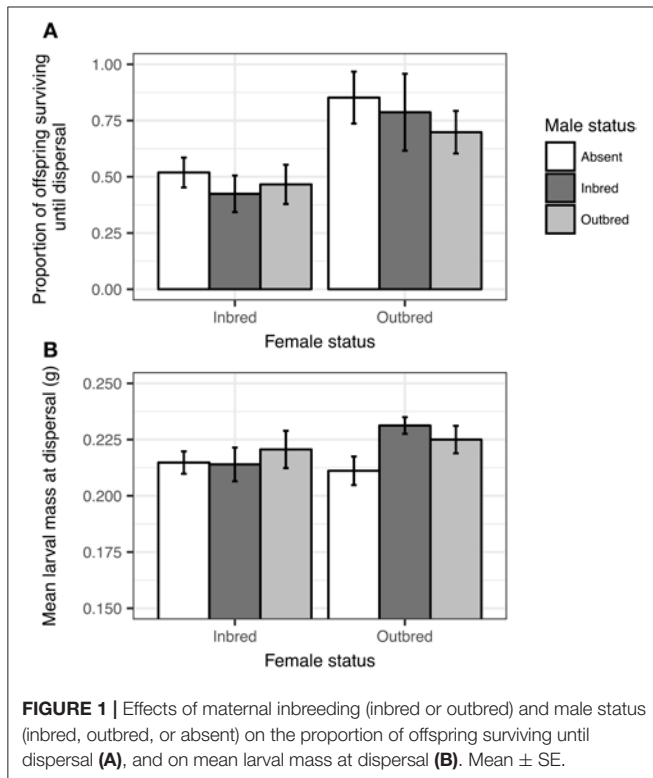
We next explored potential main effects of maternal inbreeding and male status on offspring fitness. As expected, broods reared by outbred females had a larger proportion of offspring surviving until dispersal than broods reared by inbred females (**Table 1** and **Figure 1A**), thus confirming that there were detrimental effects of maternal inbreeding on offspring survival. There was no difference in the proportion of offspring surviving until dispersal depending on whether the male was inbred, outbred or absent (**Table 1**, **Figure 1A**). There was no difference in mean larval mass at dispersal between inbred and outbred females (**Table 1** and **Figure 1B**). Likewise, there were

TABLE 1 | Effects of maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) on offspring survival until dispersal and mean larval mass at larval dispersal.

	Offspring survival			Mean larval mass		
	LRχ^2	df	P	LRχ^2	df	P
Maternal inbreeding:Male status	0.275	2	0.871	2.85	2	0.239
Maternal inbreeding	9.55	1	**0.002**	1.21	1	0.270
Male status	0.711	2	0.700	3.52	2	0.172

Values are obtained from GLMs.
LR, likelihood ratio. Statistically significant P values (<0.05) are shown in boldface.

FIGURE 1 | Effects of maternal inbreeding (inbred or outbred) and male status (inbred, outbred, or absent) on the proportion of offspring surviving until dispersal **(A)**, and on mean larval mass at dispersal **(B)**. Mean ± SE.

no significant main effects of male status on mean larval mass at dispersal (**Table 1** and **Figure 1B**). These findings suggest that there was no detrimental effect of maternal or paternal inbreeding on mean offspring weight and that male assistance had no positive main effects on mean offspring weight.

Female and Male Weight Gain

There was a significant effect of the interaction between maternal inbreeding and male status on both relative and absolute weight gain of females (**Table 2**). This interaction effect reflected that outbred females gained more weight when a male assisted in parental care than when the male was removed (**Figure 2A**), while inbred females gained a similar amount of mass regardless of whether the male assisted with parental care or not. There was no difference in either relative or absolute weight gain of females depending on whether the male was inbred or outbred (Female inbred; Male outbred vs. Male inbred: Estimate = −0.010 ±

0.007, $Z = -1.34$, $P > 0.999$; Female outbred; Male outbred vs. Male inbred: Estimate = 0.007 ± 0.008, $Z = 0.891$, $P > 0.999$). Thus, there was no evidence that females gained more weight when assisted by an outbred rather than an inbred male.

There was no significant effect of the interaction between maternal inbreeding and male status on either relative or absolute weight gain of males (**Table 2** and **Figure 2B**). There were no significant differences in either relative or absolute weight gain between inbred and outbred males (**Table 2** and **Figure 2A**). However, males gained less relative and less absolute weight when paired with an outbred female than when paired with an inbred female (**Figure 2A**), suggesting that males benefitted from having an inbred partner.

DISCUSSION

Here, we tested whether male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness in *N. vespilloides*. We found that maternal inbreeding had detrimental effects on offspring fitness in terms of reduced offspring survival, confirming the results of prior work on this species (Mattey et al., 2013, 2018; but see Mattey and Smiseth, 2015; Ford et al., 2018) and consistent with evidence from studies on vertebrate systems (Keller, 1998; Bérénos et al., 2016; Huisman et al., 2016). However, we found no evidence that male assistance in parental care buffered against these detrimental effects. Male assistance in care had a positive effect on female weight gain during breeding, showing that male assistance was beneficial to females. However, this was only the case when females were outbred, suggesting that outbred females benefitted more from male assistance than inbred ones. Finally, males paired with an inbred female gained more weight than those paired with an outbred female. This finding is opposite to what we predicted if males paired with an inbred female increased their investment in current reproduction. Instead, this result may reflect that males paired with an inbred female spent more time provisioning food to the larvae, thereby gaining better access to consume food from the carcass (Pilakouta et al., 2016). Overall, our results provide no evidence that male assistance in parental care buffers against the detrimental effects of maternal inbreeding on offspring fitness. Below, we provide a more detailed discussion of our results and their implications for our understanding of the consequences of inbreeding in populations where social interactions are prevalent.
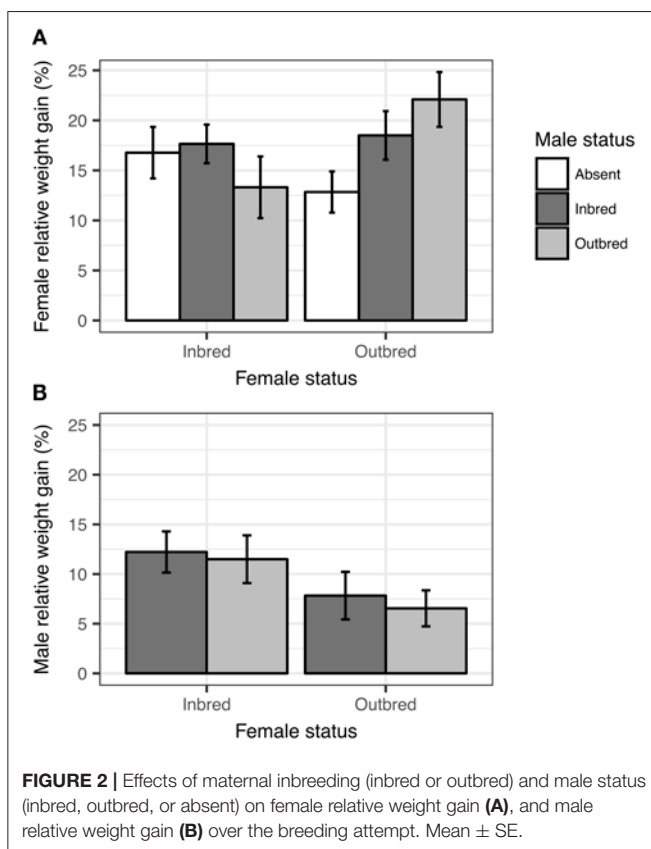
Our first key finding was that maternal inbreeding had detrimental effects on offspring fitness in terms of reduced larval survival from egg laying until dispersal, but that this effect was independent of whether the male was absent or present, and when the male was present, whether the male was inbred or outbred. Thus, our results provide no evidence that male assistance in parental care buffered against the detrimental effects of maternal inbreeding on offspring fitness. This finding contrasts with experimental evidence from a recent study on zebra finches, suggesting that male assistance in parental care buffers against the detrimental effects of maternal inbreeding. In zebra finches, inbred females spend less time incubating eggs, and buffering

TABLE 2 | Effects of maternal inbreeding (inbred or outbred) and male status (inbred, outbred or absent) on female and male relative and absolute weight gains over the breeding attempt.

	Female relative weight gain			Female absolute weight gain			Male relative weight gain			Male absolute weight gain		
	$LR\chi^2$	df	P	$LR\chi^2$	df	P	$LR\chi^2$	df	P	$LR\chi^2$	df	P
Maternal inbreeding:Male status	6.59	2	**0.037**	7.80	2	**0.020**	0.067	1	0.795	0.064	1	0.799
Maternal inbreeding	0.737	1	0.390	1.61	1	0.203	5.11	1	**0.023**	3.91	1	**0.047**
Male status	2.16	2	0.339	3.46	2	0.176	0.131	1	0.717	0.039	1	0.842
Parent's initial weight	–	–	–	0.006	1	0.938	–	–	–	1.26	1	0.261
Female relative weight gain	–	–	–	–	–	–	2.31	1	0.127	–	–	–
Female absolute weight gain	–	–	–	–	–	–	–	–	–	2.31	1	0.127

Values are obtained from GLMs.

LR, likelihood ratio. Statistically significant P values (<0.05) are shown in boldface. For male relative weight gain, male status had only two level (Inbred or outbred) because the weight was not recorded for males of the "absent" treatment group.

FIGURE 2 | Effects of maternal inbreeding (inbred or outbred) and male status (inbred, outbred, or absent) on female relative weight gain **(A)**, and male relative weight gain **(B)** over the breeding attempt. Mean ± SE.

may also apply to other systems as detrimental effects of maternal inbreeding on hatching success have also been reported for example in song sparrows (Keller, 1998). An alternative explanation for why male assistance in parental care did not buffer against the detrimental effects of maternal inbreeding is that male assistance in care in this species does not increase larval survival from hatching until dispersal under laboratory conditions (Smiseth et al., 2005). This presumably reflects that males contribute far less toward parental care than females in this species (Smiseth et al., 2005). This sex difference in parental care may also explain why there were detrimental effects of maternal inbreeding on offspring fitness, whilst there were no detrimental effects of paternal inbreeding. We note that male assistance in guarding and defending the brood against predators or conspecific intruders plays an important role under natural conditions in burying beetles (Scott, 1990). Thus, it is possible that male assistance in parental care could buffer against detrimental effects of maternal inbreeding on offspring fitness under natural conditions where competitors or conspecific intruders may reduce offspring survival. Further studies are now needed to investigate whether male assistance in care might buffer against detrimental effects of maternal inbreeding when there is a risk of predation or takeovers by conspecific intruders.

Our second main finding was that there was an effect of the interaction between maternal inbreeding and male status on female weight gain with male assistance in parental care having a positive effect on relative mass gain of outbred females only. We anticipated that females would benefit from male assistance regardless of their own inbreeding status, and that this would lead to an increase in their mass gain indicative of a shift toward investment in future reproduction (Creighton et al., 2009). Thus, this finding suggests that outbred females benefitted more from male assistance than inbred ones. One potential explanation for why this might be the case is that inbreeding is associated with terminal investment and that inbred females therefore always invest more effort into current reproduction. There is some evidence that inbreeding is associated with terminal investment from previous studies on *N. vespilloides* (Mattey and Smiseth, 2015; Richardson and Smiseth, 2017; Mattey et al., 2018). In light

is thought to reflect that males compensate for the reduced incubation by inbred females (Pooley et al., 2014). Prior work on *N. vespilloides* shows that maternal inbreeding can reduce offspring fitness both before hatching (i.e., hatching success of eggs; Mattey and Smiseth, 2015; Ford et al., 2018) and after (i.e., survival at the larval stage; Mattey et al., 2013, 2018). Thus, one potential explanation for why male assistance did not buffer against the detrimental effects of maternal inbreeding on offspring is that post-hatching male care cannot buffer against effects on hatching success of eggs. This explanation

of our finding that the presence of a male had a positive effect on the weight gain of outbred females only, future work should now test for a differential effect of male assistance in care on the subsequence breeding performance by outbred and inbred females. Presumably, outbred females would perform better in subsequent breeding attempts when assisted by a male during a first breeding attempt, while inbred females would perform equally well regardless of whether they were assisted by a male or not.

We found no evidence that females gained more mass when assisted by an outbred male, suggesting that females did not benefit more from assistance by outbred males as compared to inbred ones. We predicted that females would gain more mass when assisted by an outbred male if outbred males are better parents than inbred ones. Our results show that that this was not case, which might explain why outbred males were not better able to buffer against the detrimental effects of maternal inbreeding on offspring fitness. Our finding echoes previous work in this species showing that characteristics of the male, such as body size, have little influence on carrion consumption or weight gain of the female, whereas females adjust their consumption and weight gain to match their male partner's weight gain (Pilakouta et al., 2016). In light of this evidence, and keeping in mind that there was no difference in weight gain by inbred and outbred males in our experiment (see discussion below), it seems unlikely that females adjusted their carrion consumption and weight change to the status of their male partner.

The final main result of our study was that males paired with inbred females gained more weight over the breeding attempt than males paired with outbred females. We predicted that males paired with inbred females would gain *less* weight over the breeding attempt. The reason for this is that males paired with inbred females should be expected to increase their investment into current reproduction to compensate for the detrimental effects of maternal inbreeding. Thus, our finding suggests that males instead might increase their investment into future reproduction when their partner is inbred. However, this seems unlikely given that a previous study on *N. vespilloides* found that males paired with inbred females provided more care than males paired with outbred females (Mattey and Smiseth, 2015). An alternative explanation is that males paired with an inbred female gained more weight over the breeding attempt *because* they provided more care than males paired with an outbred female. In this species, parents feed from the carcass whilst breeding and males might gain better access to the carcass if they provide more care (Pilakouta et al., 2016). If so, we might expect a positive correlation between male food provisioning and male weight gain in this species. Altogether, our findings suggest that males benefitted in terms of gaining more weight during the breeding attempt when assisting an inbred partner. Given that male weight gain serves as a proxy for investment in future reproduction (Creighton et al., 2009), one avenue for future work is to compare the subsequent reproductive performance of males paired with an outbred or inbred female during a previous breeding attempt.

Our results have broader implications for understanding how social interactions shape the severity of inbreeding depression. There is increasing evidence that social interactions can alter the severity of inbreeding depression, with stressful interactions aggravating the severity of inbreeding depression (e.g., Meagher et al., 2000) and benign interactions buffering against inbreeding depression (e.g., Pilakouta et al., 2015). It is well documented that maternal care enhances larval survival and growth in burying beetle (e.g., Eggert et al., 1998; Trumbo, 2007; Arce et al., 2012). Thus, maternal care may buffer against inbreeding depression in offspring by reducing environmental stresses to offspring, such as the risk of death due to starvation, infanticide by conspecific intruders, and predation. In contrast, as discussed above, there is mixed evidence as to whether male assistance in care enhances offspring fitness (Pooley et al., 2014; our study). Thus, our results suggest that parental care or other benign social interactions will not always buffer against inbreeding depression. There is now a need for further work on the buffering effects of male assistance in parental care against the detrimental effects of maternal inbreeding on offspring fitness in systems where males contribute more toward care. For example, such experiments could be conducted on bird species where males and females contribute more equally toward parental care (Clutton-Brock, 1991).

ETHICS STATEMENT

Our study adheres to the 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. To minimize disturbance during breeding, the beetles were only handled at mating and dispersal.

AUTHOR CONTRIBUTIONS

TR and PS conceived and designed the experiments. TR and EC collected the data. TR analyzed the data. TR and PS wrote the first draft of the manuscript, with contributions from all authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2018.00196/full#supplementary-material

REFERENCES

Arce, A. N., Johnston, P. R., Smiseth, P. T., and Rozen, D. E. (2012). Mechanisms and fitness effects of antibacterial defences in a carrion beetle. *J. Evol. Biol.* 25, 930–937. doi: 10.1111/j.1420-9101.2012.02486.x

Armbruster, P., and Reed, D. H. (2005). Inbreeding depression in benign and stressful environments. *Heredity* 95, 235–242. doi: 10.1038/sj.hdy.6800721

Bartlett, J., and Ashworth, C. M. (1988). Brood size and fitness in *Nicrophorus vespilloides* (Coleoptera, Silphidae). *Behav. Ecol. Sociobiol.* 22, 429–434. doi: 10.1007/BF00294981

Bérénos, C., Ellis, P. A., Pilkington, J. G., and Pemberton, J. M. (2016). Genomic analysis reveals depression due to both individual and maternal inbreeding in a free-living mammal population. *Mol. Ecol.* 25, 3152–3168. doi: 10.1111/mec.13681

Charlesworth, D., and Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annu. Rev. Ecol. Syst.* 18, 237–268. doi: 10.1146/annurev.es.18.110187.001321

Charlesworth, D., and Willis, J. H. (2009). The genetics of inbreeding depression. *Annu. Rev. Ecol. Syst.* 10, 783–796. doi: 10.1038/nrg2664

Cheptou, P. O., and Donohue, K. (2011). Environment-dependent inbreeding depression: its ecological and evolutionary significance. *New Phytol.* 189, 395–407. doi: 10.1111/j.1469-8137.2010.03541.x

Clutton-Brock, T. H. (1991). *The Evolution of Parental Care*. Princeton, NJ: Princeton University Press.

Creighton, C. J., Smith, A. N., Komendat, A., and Belk, M. C. (2015). Dynamics of biparental care in a burying beetle: experimental handicapping results in partner compensation. *Behav. Ecol. Sociobiol.* 69, 265–271. doi: 10.1007/s00265-014-1839-z

Creighton, J. C., Heflin, N. D., and Belk, M. C. (2009). Cost of reproduction, resource quality, and terminal investment in a burying beetle. *Am. Nat.* 174, 673–684. doi: 10.1086/605963

Eggert, A. K., Reinking, M., and Muller, J. K. (1998). Parental care improves offspring survival and growth in burying beetles. *Anim. Behav.* 55, 97–107. doi: 10.1006/anbe.1997.0588

Ford, L. E., Henderson, K. J., and Smiseth, P. T. (2018). Differential effects of offspring and maternal inbreeding on egg laying and offspring performance in the burying beetle *Nicrophorus vespilloides*. *J. Evol. Biol.* 31, 1047–1057. doi: 10.1111/jeb.13285

Fox, C. W., and Scheibly, K. L. (2006). Variation in inbreeding depression among populations of seed beetle, *Stator limbatus*. *Entomol. Exp. Appl.* 121, 137–144. doi: 10.1111/j.1570-8703.2006.00465.x

Fox, J., Weisberg, S., Bates, D., and Fox, M. J. (2016). *Package "Car."* Avaliable online at: http://cran.r-project.org/web/packages/car/car.pdf

Hoffmann, A. A., and Parsons, P. A. (1991). *Evolutionary Genetics and Environmental Stress*. Oxford: Oxford University Press.

Huisman, J., Kruuk, L. E. B., Ellis, P. A., Clutton-Brock, T. H., and Pemberton, J. M. (2016). Inbreeding depression across the lifespan in a wild mammal population. *Proc. Natl. Acad. Sci. U. S. A.* 113, 3585–3590. doi: 10.1073/pnas.1518046113

Keller, L. F. (1998). Inbreeding and its fitness effects in an insular population of song sparrows (*Melospiza melodia*). *Evolution* 52, 240–250.

Keller, L. F., and Waller, D. M. (2002). Inbreeding effects in wild populations. *Trends Ecol. Evol.* 17, 230–241. doi: 10.1016/S0169-5347(02)02489-8

Mattey, S. N., Richardson, J., Ratz, T., and Smiseth, P. T. (2018). Effects of offspring and parental inbreeding on parent-offspring communication. *Am. Nat.* 191, 716–725. doi: 10.1086/697236

Mattey, S. N., and Smiseth, P. T. (2015). Complex effects of inbreeding on biparental cooperation. *Am. Nat.* 185, 1–12. doi: 10.1086/679067

Mattey, S. N., Strutt, L., and Smiseth, P. T. (2013). Intergenerational effects of inbreeding in *Nicrophorus vespilloides*: Offspring suffer fitness costs when either they or their parents are inbred. *J. Evol. Biol.* 26, 843–853. doi: 10.1111/jeb.12102

Meagher, S., Penn, D. J., and Potts, W. K. (2000). Male-male competition magnifies inbreeding depression in wild house mice. *Proc. Natl. Acad. Sci. U. S. A.* 97, 3324–3329. doi: 10.1073/pnas.97.7.3324

Monteith, K. M., Andrews, C., and Smiseth, P. T. (2012). Post-hatching parental care masks the effects of egg size on offspring fitness: a removal experiment on burying beetles. *J. Evol. Biol.* 25, 1815–1822. doi: 10.1111/j.1420-9101.2012.02567.x

Pilakouta, N., Jamieson, S., Moorad, J. A., and Smiseth, P. T. (2015). Parental care buffers against inbreeding depression in burying beetles. *Proc. Natl. Acad. Sci. U. S. A.* 112, 8031–8035. doi: 10.1073/pnas.1500658112

Pilakouta, N., Richardson, J., and Smiseth, P. T. (2016). If you eat, i eat: resolution of sexual conflict over consumption from a shared resource. *Anim. Behav.* 111, 175–180. doi: 10.1016/j.anbehav.2015.10.016

Pooley, E. L., Kennedy, M. W., and Nager, R. G. (2014). Maternal inbreeding reduces parental care in the zebra finch, *Taeniopygia guttata*. *Anim. Behav.* 97, 153–163. doi: 10.1016/j.anbehav.2014.09.012

R Development Core Team (2011). *R: A Language and Environment for Statistical Computing*. Vienna: The R Foundation for Statistical Computing. Avaliable online at: http://www.R-project.org/

Richardson, J., and Smiseth, P. T. (2017). Intraspecific competition and inbreeding depression: increased competitive effort by inbred males is costly to outbred opponents. *Am. Nat.* 189, 539–548. doi: 10.1086/691328

Royle, N. J., Russell, A. F., and Wilson, A. J. (2014). The evolution of flexible parenting. *Science* 345, 776–781. doi: 10.1126/science.1253294

Scott, M. P. (1990). Brood guarding and the evolution of male parental care in burying beetles. *Behav. Ecol. Sociobiol.* 26, 31–39 doi: 10.1007/BF00174022

Slate, J., Kruuk, L. E., Marshall, T. C., Pemberton, J. M., and Clutton-Brock, T. H. (2000). Inbreeding depression influences lifetime breeding success in a wild population of red deer (*Cervus elaphus*). *Proc. R. Soc. B Biol. Sci.* 267, 1657–1662. doi: 10.1098/rspb.2000.1192

Smiseth, P. T., Dawson, C., Varley, E., and Moore, A. J. (2005). How do caring parents respond to mate loss? Differential response by males and females. *Anim. Behav.* 69, 551–559. doi: 10.1016/j.anbehav.2004.06.004

Smiseth, P. T., Ward, R. J. S., and Moore, A. J. (2006). Asynchronous hatching in *Nicrophorus vespilloides*, an insect in which parents provide food for their offspring. *Funct. Ecol.* 20, 151–156. doi: 10.1111/j.1365-2435.2006.01072.x

Trumbo, S. T. (2007). Defending young biparentally: female risk-taking with and without a male in the burying beetle, *Nicrophorus pustulatus*. *Behav. Ecol. Sociobiol.* 61, 1717–1723. doi: 10.1007/s00265-007-0403-5