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

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Abstract

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

Introduction

Parents of many animals, including the majority of birds (Cockburn, 2006) as well as some mammals, fishes, and insects (Balshine, 2012; Trumbo, 2012), cooperate to provide care for their joint offspring. Until now, most work on biparental cooperation has focused on how a focal parent adjusts its contribution based on the amount of care provided by its partner (Lessells, 2012). This focus is motivated by theoretical models for the evolutionary resolution of sexual conflict between caring parents (Houston *et al.*, 2005; Lessells, 2012).

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Sexual conflict arises because the benefit of care to each parent depends on the parents' combined effort, whereas the cost depends only on the parent's personal effort (Lessells, 2012). Thus, each parent will be under selection to reduce its personal cost by shifting as much of the workload as possible over to its partner. Theoretical models suggest that this conflict can be resolved through three behavioural mechanisms: negotiation, matching, and sealed-bid decisions. Negotiation and matching occur when each parent adjusts its level of care in direct response to its partner's contribution. When there is negotiation, the focal parent responds to a reduction in amount of care provided by its partner by increasing its contribution, though only such that it compensates incompletely for the partner's reduction (McNamara *et al.*, 1999). In contrast, when there is matching, the focal parent responds by matching any increase or reduction in its partner's contribution

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

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

Nicrophorus burying beetles are well suited as a system for investigating these issues because parental care by both parents is very flexible (Eggert *et al.*, 1998; Smiseth & Moore, 2004). Burying beetles breed on carcasses of small vertebrates, which provide the sole source of food for the developing larvae (Scott, 1998). Both parents help prepare the carcass, protect it and the brood from predators and conspecifics, apply antimicrobials to the carcass, and provision the larvae with predigested carrion (Eggert *et al.*, 1998; Rozen

et al., 2008; Walling *et al.*, 2008; Arce *et al.*, 2012). Females often spend more time provisioning food for the larvae and stay on the carcass for longer than males, whereas males spend more time maintaining the carcass (Fetherston *et al.*, 1994; Eggert *et al.*, 1998; Smiseth & Moore, 2002; Rauter & Moore, 2004; Smiseth *et al.*, 2005; Walling *et al.*, 2008). Previous studies based on mate removal, handicapping, or random-pairing designs provide mixed evidence with some support for both negotiation (Fetherston *et al.*, 1994; Rauter & Moore, 2004; Smiseth & Moore, 2004; Smiseth *et al.*, 2005; Suzuki & Nagano, 2009; Creighton *et al.*, 2015) and sealed-bid models (Jenkins *et al.*, 2000; Rauter & Moore, 2004; Smiseth *et al.*, 2005; Suzuki & Nagano, 2009). A recent study on the effects of inbreeding on biparental care found evidence for both negotiation and sealed-bid models, suggesting that these two mechanisms are not mutually exclusive (Mattey & Smiseth, 2015).

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

partner. In contrast, if such adjustments were mediated through sealed-bid decisions, we predicted they would occur in direct response to the partner's state and thus be independent of the amount of care by the partner.

Materials and methods

General methodology

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

Experimental design

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

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

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

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

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

Statistical analyses

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

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

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

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

Results

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

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

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

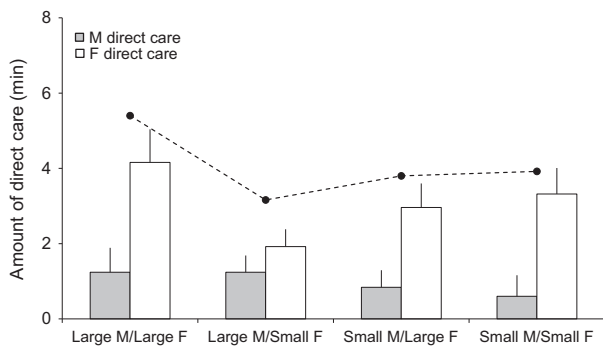| Type of care	Focal parent's size				Partner's size				Interaction			
	Est	SE	z	P	Est	SE	z	P	Est	SE	z	P
M direct care	−1.06	0.63	−1.7	0.093	−1.10	0.49	−2.2	**0.026**	1.52	0.83	1.4	0.066
F direct care	−0.51	0.14	−3.5	**< 0.001**	−0.32	0.14	−2.3	**0.022**	0.75	0.20	3.8	**< 0.001**
M indirect care	−0.23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

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

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

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

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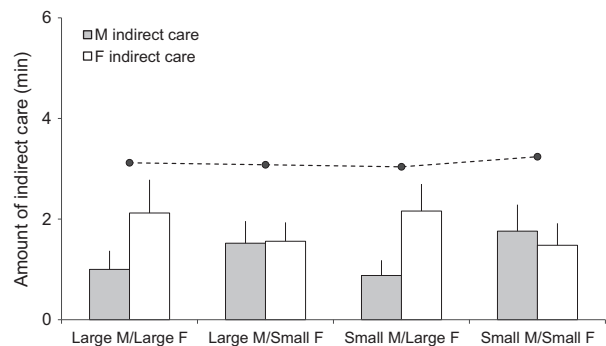
parents was not affected by the parents' size (Table 2; Fig. 2).

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

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

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

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

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

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

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

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

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

Does the parents' size affect offspring fitness?

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

Discussion

Here, we report evidence from a burying beetle with biparental care showing that individual parents adjust

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

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

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

A key aim of our study was to identify the potential mechanisms whereby the focal parent adjusted its contribution based on its partner's size. We predicted that such responses would be mediated through negotiation, matching, or sealed-bid responses. We found evidence for negotiation as both males and females provided more direct care when their partner provided less direct care (see also Smiseth & Moore, 2004; Mattey & Smiseth, 2015). Nevertheless, including the partner's behaviour in the models did not remove or reduce the initial effect of the partner's size on the amount of care provided by the focal parent. This suggests that the way in which parents responded to their partner's size was not mediated through a response to the amount of care provided by the partner as predicted by negotiation or matching models (McNamara *et al.*, 1999; Johnstone & Hinde, 2006) but rather that it was independent of the partner's behaviour as predicted by sealed-bid models (Houston & Davies, 1985). This finding has important implications for our understanding of the behavioural

mechanisms mediating the resolution of sexual conflict over parental care. Negotiation, matching, and sealed-bid responses have been traditionally considered as mutually exclusive mechanisms. However, our study provides evidence for both negotiation, as parents adjusted the amount of care that they provided based on the amount provided by their partners, and sealed-bid responses, as the focal parent's adjustment based on its partner's state was independent of the partner's behaviour. These results are consistent with those of a previous study investigating the effects of inbreeding on biparental cooperation in the same species (Mattey & Smiseth, 2015).

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

We also found evidence for an effect of the interaction between the parent's own state and the state of its partner on the amount of direct care provided by females. Such an interaction effect might reflect that
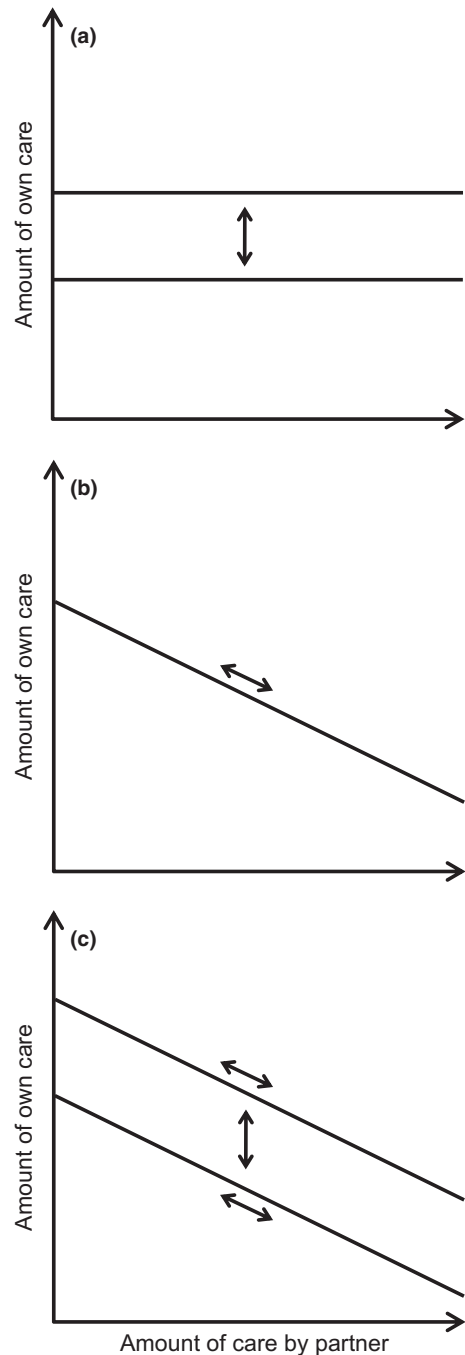

Fig. 3 Graphical model illustrating sealed-bid decisions (a), negotiation (b), and a combination of sealed-bid decisions and negotiation (c). In all cases, the intercept represents a fixed initial decision that is independent of the amount of care provided by the partner as assumed by sealed-bid models,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

the focal parent's ability to adjust its contribution to its partner's state is dependent on its own state. For example, if small parents are working closer to their maxi-

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

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

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

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