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Complex effects of inbreeding on biparental cooperation

Sarah N. Matthey and Per T. Smiseth

Institute of Evolutionary Biology School of Biological Sciences, University of Edinburgh,
West Mains Road, Edinburgh, EH9 3JT, UK

E-mail: s.n.matthey@sms.ed.ac.uk and per.t.smiseth@ed.ac.uk

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ABSTRACT: There is mounting evidence that inbreeding can have detrimental effects on the fitness of outbred individuals that interact with or depend on inbred individuals. However, little is currently known about the behavioral mechanisms by which interactions with inbred individuals induce fitness costs in outbred individuals. Here, we study effects of inbreeding on the behavioral dynamics of biparental cooperation in the burying beetle *Nicrophorus vespilloides*. To this end, we used a two-by-two factorial design, in which an inbred or outbred female was mated to an inbred or an outbred male and tested for effects on cooperation between male and female parents providing care for their joint offspring. We found no evidence that inbred parents provided less care than outbred parents. Nevertheless, partners of inbred parents increased the amount of care they provided, leading to overcompensation. Our study shows that inbreeding can have strong and complex effects on the behavioral dynamics of biparental cooperation, and that these effects are mediated mainly through changes in the partner's behavior. We suggest that similar effects of inbreeding on outbred individuals may extend to other social contexts, such as cooperative breeding and mating.

Introduction

Inbreeding results from matings between related individuals, and attracts interest in evolutionary and conservation biology because it often leads to a reduction in fitness known as inbreeding depression (Crow and Kimura 1970). Inbreeding depression is thought to result from an increase in homozygosity, which in turn, increases the risk that rare deleterious recessive alleles are expressed (Falconer and Mackay 1996; Lynch and Walsh 1998; Charlesworth and Willis 2009). There is ample evidence that inbred individuals suffer fitness costs in terms of reduced growth, survival and reproduction (Miller et al. 1993; DeRose and Roff 1999; Drayton et al. 2010). However, there is also mounting evidence that the detrimental effects of inbreeding can extend to outbred individuals that interact with inbred individuals. For example, studies on birds and beetles show that the fitness of outbred offspring often is reduced when they receive care from inbred parents (Reid et al. 2003; Richardson et al. 2004; Szulkin et al. 2007; Matthey et al. 2013). Furthermore, studies on beetles and flies show that outbred females have reduced fecundity when they mate with inbred males (Okada et al. 2011; Fox et al. 2012), while a study on ants shows that the colony's productivity is reduced when workers are inbred (Vitikainen et al. 2011). However, although these studies show that outbred individuals often pay a fitness cost by interacting with inbred individuals, we have little knowledge about the behavioral mechanisms causing such fitness costs.

Biparental cooperation between male and female parents caring for their joint offspring is an excellent system for studying the behavioral mechanisms mediating fitness costs to outbred individuals due to interactions with inbred individuals. First, it is likely that outbred parents suffer fitness costs from cooperating with an inbred partner (Matthey et al. 2013) because the offspring's fitness depends on the total amount of care by the two parents (Lessells 2012). Second, theoretical models and empirical studies show that the behavioral

mechanisms underlying biparental cooperation have evolved in response to selection due to sexual conflict (Lessells 2012). In species with biparental care, there is sexual conflict over the amount of care each parent should contribute because the benefit of care to each parent depends on the two parents' combined effort, while the cost depends only on each parent's personal effort (Lessells 2012). The resolution of this conflict may be mediated through two mechanisms: (1) a negotiation process, whereby each parent adjusts its level of care in direct response to its partner's contribution (McNamara et al. 1999), or (2) a sealed bid process, whereby each parent makes an initial fixed decision about how much care to provide (Houston and Davies 1985). Negotiation models predict that, if one parent reduces its contribution to care, its partner should respond by increasing its own contribution, but only such that it compensates incompletely for this reduction (McNamara et al. 1999). In contrast, sealed bid models predict that the partner will not compensate for any reductions in the contribution by the other parent, although the partner could alter its initial fixed decision (Houston and Davies 1985). Thus, any effect of inbreeding on biparental care will depend on the mechanism of conflict resolution. If sexual conflict is resolved through negotiation, inbreeding should affect biparental cooperation if inbred parents reduce their contribution to care, thereby causing incomplete compensation by their partner. Meanwhile, if sexual conflict is resolved through sealed bid decisions, inbreeding should affect biparental cooperation if partners of inbred parents alter their initial fixed decision. In other words, negotiation models predict that partners of inbred parents respond to the behavior of inbred parents, while sealed models predict that the responses of partners of inbred parents are independent of the behavior of inbred parents. In the latter situation, partners of inbred parents may respond to non-behavioral cues linked with inbreeding, such as the other parent's overall condition.

Here, we investigate the effects of inbreeding on biparental cooperation in the burying beetle *N. vespilloides*. This species is an excellent study system because it is one of the few

insects where both parents provide care for the offspring (Eggert et al. 1998; Smiseth and Moore 2004). Like all members of the genus *Nicrophorus*, *N. vespilloides* breeds on the carcasses of small vertebrates (Scott 1998). Both parents cooperate to prepare the carcass, protect it and the brood from predators and conspecifics, apply antimicrobials to the carcass, and provision the larvae with pre-digested carrion (Eggert et al. 1998; Smiseth et al. 2005; Rozen et al. 2008; Arce et al. 2012). Females spend more time provisioning food for the larvae and stay on the carcass for longer than males (Eggert et al. 1998; Smiseth and Moore 2002; Smiseth et al. 2005), whilst males spend more time maintaining the carcass (Smiseth et al. 2005). There is evidence that parents adjust their contribution to the amount of care provided by the other parent (Smiseth and Moore 2004), although only males compensate (incompletely) when the partner is experimentally removed (Smiseth et al. 2005). A recent study found that outbred offspring suffered significant fitness costs when they received care from inbred parents (Mattey et al. 2013). Furthermore, there was an effect of the interaction between inbreeding in male and female parents on offspring during the parental care period, suggesting that inbreeding affects biparental cooperation (Mattey et al. 2013). However, there is no information on the behavioral mechanisms by which inbreeding might affect biparental cooperation.

To test for effects of inbreeding on biparental cooperation, we used a two-by-two factorial design in which we paired an inbred or an outbred male to an inbred or an outbred female. This experimental design allowed us to test for differences in the amount of care provided by inbred and outbred parents and test for differences in the amount provided by partners of inbred and outbred parents. In our analyses, we refer to the parent whose inbreeding status (inbred or outbred) was treated as a predictor variable as the ‘focal parent’, and the individual paired with the focal parent as its ‘partner’. First, we tested for effects of the focal parent’s inbreeding status on the amount of care it provided. We predict that inbred

parents should provide less care than outbred parents given that parental care is costly and inbreeding should reduce an individual's ability to perform costly behaviors (Falconer and Mackay 1996; Lynch and Walsh 1998; DeRose and Roff 1999). Second, we tested for effects of the focal parent's inbreeding status on the amount of care provided by its partner. If inbred parents provide less care than outbred parents, we predict that partners of inbred parents should increase their contribution and thus provide more care than partners of outbred parents. If this response is mediated through negotiation, we predict that any differences in the amount of care provided by partners of inbred or outbred parents should be explained by variation in the amount care provided by inbred and outbred parents. Negotiation models predict that biparental care is evolutionary stable only if the partner responds by incomplete compensation (McNamara et al. 1999). To test this prediction, we compared the total amount of care provided by the two parents across the different treatment groups. Meanwhile, if sexual conflict is resolved through sealed bid decisions, we predict that any differences in the amount of care by partners of inbred parents should be independent of variation in the amount of care provided by inbred and outbred focal parents. Third, our design allowed us to test for effects of the interaction between the inbreeding status of the focal parent and its partner. Such interaction effects might be expected if inbred partners of inbred parents are more constrained in their ability to compensate than outbred partners of inbred parents. Finally, we tested for effects of inbreeding on the growth and survival of the offspring, which were always outbred in our experiment. Based on previous results from the same species (Mattey et al. 2013), we predict offspring to have lower survival when they receive care from inbred parents.

Material and Methods

General Methods

All beetles were maintained under laboratory conditions at 20°C and under constant light. Non-breeding adults were housed individually in transparent plastic containers (12cm x 8cm and 2cm high) and fed organic beef twice a week. Experimental pairs were bred in transparent plastic boxes (17cm x 12cm and 6cm high) by providing them with 1cm of moist soil and a mouse carcass (supplied from Livefoods Direct Ltd, Sheffield, UK) with a mass range of 20–22g. The stock population comprised of beetles originally collected at Corstorphine Hill and The Hermitage of Braid, Edinburgh; Jodrell Bank, Manchester; Kennel Vale, Cornwall; and Madingley Woods, Cambridge. We bred the population to ensure that it was outbred and not subject to purging or selection that might bias any estimates of inbreeding. To this end, we mated pairs that were not closely related in the sense that they did not share a common grandparent (or a closer relative). We only used virgin beetles in the experiments. The outbred individuals in the experiments were bred according to the protocol used for the stock population, while the inbred individuals derived from matings between two full siblings. Once adult, inbred and outbred individuals were maintained under standard laboratory conditions.

Experimental Design

To investigate the effects of inbreeding on biparental cooperation, we used a blocked two-by-two factorial design in which parents of each sex were either inbred or outbred (Mattey et al. 2013). The experimental pairs were set up in 18 blocks, each of which comprised of four pairs (one from each treatment), yielding a total of 72 pairs. We ensured that all pairs comprised of individuals that were not closely related (i.e., did not share a common grandparent or a closer relative). We excluded 13 pairs from further analyses because we did not have sufficient number of larvae to generate a brood, one pair because one of the parents died, and one pair because the larvae were hidden underneath the carcass, thus preventing us

from collecting accurate data on the amount of care provided by the parents. This latter pair was included in our analyses on offspring growth and survival. The sample sizes in our analyses on parental behaviors were as follows: (i) an outbred male mated to an outbred female (n=16); (ii) an outbred male mated to an inbred female (n=15); (iii) an inbred male mated to an outbred female (n=14); and (iv) an inbred male mated to an inbred female (n=12).

Immediately prior to hatching, we moved the parents and the prepared carcass to a new box with fresh soil. Once the eggs left in the original box had started hatching, we began generating experimental broods of ten larvae by mixing larvae from eggs laid by different females from across treatments. We used this cross-fostering design because it excludes any confounding effects on the two parents' behavior mediated through effects of inbreeding on larval begging or brood size. Thus, our design ensures that any effect of the focal parent's inbreeding status on its partner's behavior is attributed to interaction between the parents. All experimental pairs comprised of individuals that were not closely related, and the larvae used to set up the broods were therefore always outbred. We recorded the initial brood mass of experimental broods to calculate offspring growth from hatching to subsequent stages of larval development. Because parents kill all larvae that arrive before their own eggs hatch (Müller and Eggert 1990), we only provided a given pair with an experimental brood once their own eggs had started hatching.

We conducted behavioral observations of inbred and outbred parents exactly 24 hours after they had been provided with an experimental brood. We did the observation at this stage because it corresponds to the peak in parental care in *N. vespilloides* (Smiseth et al. 2003, 2007). We used instantaneous sampling, scoring behaviors every 1 min for 30 min in accordance with established protocols (Smiseth and Moore 2002). We scored the number of scans each parent spent providing (1) direct care, defined as when the parent was

provisioning food to larvae (engaging in mouth-to-mouth contact with at least one larva) or interacting with larvae (standing still and allowing larvae to beg); and (2) indirect care, defined as when the parent was guarding (standing still in a position where it could defend the brood from predators and conspecifics) or maintaining the carcass (depositing anal or oral secretions to the carcass, manipulating the carcass from below or excavating the crypt). We excluded time spent consuming carrion from our definition of direct care, because males (but not females) often consumed carrion without later provisioning food to the larvae. This suggests that males consumed carrion to replenish their own energy reserves. Our definition of direct care differ slightly from that used by Walling et al. (2008). We note that, due to constraints on the parents' time budget, it is unlikely that the amount of time spent at direct and indirect care are completely independent.

Immediately after the observation, we counted the number of larvae in the brood and weighed the brood. From this information, we determined larval growth and survival until the time of observation (24 h after hatching). Once counted and weighed, we returned the larvae to the carcass. Parents were then allowed to raise the brood undisturbed until the larvae dispersed from the carcass (approximately 8-10 days after hatching), at which point we recorded the number of larvae and weighed the brood. We used this information to estimate larval growth and survival until dispersal. After dispersal, we placed the larvae into individual containers to allow them to pupate and eclose as adults. Once the pupae had eclosed, we counted the number of adults to measure survival to eclosion.

Statistical Analysis

We analyzed the data using R version 2.13. We used linear mixed-effects models (lme) for traits that had a normal error distribution (larval body mass at hatching, at the time of observation and at dispersal) and generalized linear mixed models (lmer) for traits that had a

Poisson error structure (direct care and indirect care) or a binomial error structure (larval survival to observation, survival to dispersal and survival to eclosion). We assigned block as a random factor in all models. For parents of each sex, we built separate mixed effects models for effects of inbreeding on the time it spent providing direct and indirect care, and for effects of inbreeding on body mass of larvae at hatching, at the time of observation and at dispersal, and larval survival until the time of observation, at dispersal and at eclosion (table 1).

We conducted separate models for the amount of direct and indirect care provided by inbred and outbred parents of each sex. In all such models, we assigned the focal parent's inbreeding status (inbred or outbred) as the first main fixed effect and the partner's inbreeding status (inbred or outbred) as the second main fixed effect. These two terms were always included in final models (table 1). We next added the interaction term between the focal parent's inbreeding status and that of its partner (table 1). The inclusion of this interaction term was based on the lowest AIC score (see below). We also tested for effects of brood size and the focal parent's behavior on the amount of care provided by the partner (table 1). We added brood size as an additional fixed effect because there was some variation between broods in the number of surviving larvae at the time of observation. Females spent more time providing direct ($Z = 1.98$, $P = 0.048$) and indirect care ($Z = 2.02$, $P = 0.043$) when caring for a larger brood, while there were no significant effects of brood size on male care (direct care: $Z = -0.54$, $P = 0.59$; indirect care: $Z = -0.86$, $P = 0.39$). We included brood size in the final models on female care, while we excluded it from all models on male care. We added the focal parent's behavior as additional fixed effect to test whether each parent adjusted its contribution to care in response to the amount of care provided by the other parent (see Results). The exclusion of additional fixed effects in final models was based on the lowest AIC score, and in all cases, excluded terms were also non-significant. We used the simplest model whenever the difference in the AIC score was less than two.

In order to assess whether any response by partners of inbred parents was mediated through a negotiation process, we compared models where the amount of time that the focal parent spent providing care was either added or removed as an additional fixed effect. If such responses were mediated through a negotiation process, we predicted that any differences in the amount of care by partners of inbred or outbred parents should be explained by variation in the amount care by inbred and outbred parents. In other words, including the focal parent's behavior should remove or significantly reduce the effect of the focal parent's inbreeding status on the amount of care by the partner. We also conducted separate analyses to examine the level of compensation. To this end, we conducted separate analyses for the total amount of direct and indirect care by the two parents. These models included the inbreeding status of males and females as main fixed effects as well as the interaction between the two (table 1).

For models on growth and survival, we always assigned the male's inbreeding status as the first main fixed effect and the female's inbreeding status as the second main fixed effect. These main fixed effects were included in all final models. We then added the interaction term between the inbreeding status of male and female parents. Finally, we added carcass size (for survival traits) or brood size (for growth traits) as additional fixed effects (table 1). The inclusion of the interaction effects and additional fixed effects was based on the lowest AIC score (see above for further details).

Results

Effect on Focal Parent

We found no evidence that inbred parents provided less care than outbred parents (table 2: Focal parent's IS; figure 1). Inbred females spent a similar amount of time providing direct and indirect care as outbred females (figure 1A). Inbred males spent significantly more time

providing indirect care than outbred males, while there was no significant difference in the amount of time spent providing direct care between inbred and outbred males (figure 1).

Response by the Partner

There were significant differences in the amount of care provided by partners of inbred and outbred parents (table 2: Partner's IS; figure 1A). Male partners of inbred females spent significantly more time providing direct care than male partners of outbred females, and female partners of inbred males spent significantly more time providing direct care than female partners of outbred males (figure 1A). Female partners of inbred males spent significantly less time providing indirect care than female partners of outbred males, while there was no significant difference in the time spent providing indirect care by male partners of inbred or outbred females (figure 1B). Thus, partners of inbred parents increased the amount of direct care they provided despite that there was no difference in the amount of direct care by inbred and outbred parents (figure 1A). As a consequence, the total amount of direct care by the two parents was higher when at least one parent was inbred than when both parents were outbred (table 3; figure 1A), indicating that partners of inbred parents responded by overcompensation rather than incomplete or no compensation. Concerning indirect care, there was evidence for complete compensation as the total amount of indirect care was similar regardless of whether parents were inbred or outbred (figure 1B).

In both sexes, the amount of direct care by the focal parent had a highly significant effect on the amount of direct care by partners (table 2: Partner's behavior). This finding suggests that each parent adjusted its contribution to care in response to the amount of care provided by the other parent, as predicted by negotiation models. To determine whether such a negotiation process accounted for the effect of the inbreeding status of the focal parent on the amount of care by its partner, we compared models in which we either included or

excluded the amount of care by the focal parent as an additional fixed effect. Including or excluding the focal parent's behavior did not alter the effect that the focal parent's inbreeding status (inbred or outbred) had on the amount of direct or indirect care by the partner. Indeed, partners of inbred parents still spent significantly more time providing direct care than partners of outbred parents if we excluded the focal parent's behavior in the models (male direct care: $Z = 6.09$, $P < 0.0001$; female direct care: $Z = 3.36$, $P = 0.0008$). Female partners of inbred males also spent significantly less time providing indirect care than female partners of outbred males when the male's behavior was included in the model ($Z = -2.94$, $P = 0.003$), and there was still no significant difference in the time spent providing indirect care by male partners of inbred or outbred females when the female's behavior was included in the model ($Z = 0.091$, $P = 0.93$). These results suggest that the effect of inbreeding on biparental care was not mediated through a negotiation process. Instead, the partners' response was independent of the actual amount of care by inbred or outbred parents, suggesting that the effect of inbreeding on biparental cooperation was mediated through a sealed bid process.

Interaction Between Inbreeding in Focal Parent and its Partner

Our design allowed us to test for effects of the interaction between inbreeding in female and male parents. We found that this interaction had a significant effect on the amount of care by both females and males (table 2). The significant interaction effects for females reflected that outbred females spent more time providing direct care when they were mated to an inbred male, whilst inbred females provided a similar amount of care regardless of whether they were mated to an outbred or an inbred male (figure 1A). Furthermore, inbred females spent more time providing indirect care when they were mated to an outbred male, whilst outbred females provided a similar amount of indirect care regardless of whether they were mated to an inbred or outbred male (figure 1B). The significant interaction effect for males reflected

that inbred males spent more time providing indirect care when they were mated to an inbred female, whilst outbred males provided a similar amount of indirect care regardless of whether they were mated to an inbred or outbred female (figure 1B).

Larval Growth and Survival

Finally, we tested for the effects of inbreeding in parents on the growth and survival of outbred offspring. In contrast to what we predicted, we found no evidence that inbreeding in parents affected offspring fitness (table 4). However, there were strong effects of brood size on larval growth and of carcass size on larval survival (table 4). These effects reflected that larvae grew better in larger broods, and that they survived less well from hatching to dispersal but better from dispersal to eclosion on larger carcasses.

Discussion

Here, we present evidence that inbreeding can have complex effects on biparental cooperation. We found that inbred parents provided as much direct care as outbred parents, but that partners of inbred parents nevertheless provided more direct care than partners of outbred parents. As a consequence, the total amount of direct care provided by the two parents was greater when at least one parent was inbred. There was no evidence that the difference in care between partners of inbred and outbred parents could be explained by variation in the amount of care provided by inbred and outbred focal parents. This finding suggests that the response of partners of inbred parents was mediated through a sealed bid response rather than negotiation. We found effects due to the interaction between inbreeding in males and females as outbred females provided more direct care when mated to an inbred male, whilst inbred females provided a similar amount of direct care regardless of whether they were mated to an outbred or an inbred male. Given that biparental care is taxonomically

widespread and the predominant pattern of care in birds (Clutton-Brock 1991; Royle et al. 2012), our results highlight the need for further work to advance our understanding of potential effects of inbreeding in species with biparental care. Below we discuss the wider implications of our results in more detail.

We found that inbred parents provided as much, or more, care than outbred parents. Our results contradict the prediction that inbred parents should provide less care than outbred parents, and differ somewhat from those of a previous study on oldfield mice, *Peromyscus polionotus*. In this latter study, inbred males provided less care than outbred males, although inbred females provided as much care as outbred females (Margulis 1998). There are two reasons why inbred parents are expected to provide less care than outbred ones. First, inbreeding depression often is particularly strong for fitness-related traits, including life-history traits, reflecting that mutations affecting these traits often are recessive and highly deleterious (Lynch and Walsh 1998; DeRose and Roff 1999). Thus, given that parental care is a major life-history trait that determines both parental and offspring fitness (Clutton-Brock 1991; Royle et al. 2012), inbreeding should have strong effects on parental care. Second, parental care often incurs significant energetic costs to parents (Clutton-Brock 1991; Royle et al. 2012; Trumbo and Rauter 2014), and inbreeding may reduce an individual's ability to cope with such costs because it often induces physiological stress (Kristensen et al. 2006; Ayroles et al. 2009). Our results call into question whether these two scenarios are met, at least for *N. vespilloides*. However, this seems unlikely given that previous work on this and closely related species suggests that parental care is both costly and strongly linked with parental and offspring fitness (Eggert et al. 1998; Smiseth et al. 2003; Trumbo 2007; Creighton et al. 2009; Cotter and Kilner 2010). A potential explanation for why inbreeding may have little or no net effect on parental care is that any increase in the energetic costs of providing parental care to inbred parents is counteracted by a reduction in the opportunity

costs of care (Kokko and Jennions 2008). Inbred parents might have lower opportunity costs if they have a lower future reproductive potential than outbred ones (Miller et al. 1993; Aspi 2000; Drayton et al. 2010). This offers an explanation for why we found that inbred males provided more indirect care than outbred males. In *N. vespilloides*, males normally provide less care than females, suggesting that males have higher opportunity costs of care, presumably because they can gain some reproductive success by mating away from carcasses (Müller and Eggert 1989). If so, inbreeding might have a stronger effect on the opportunity costs of males, thereby causing inbred males to increase their investment in the current brood. We suggest that further work needs to consider whether inbreeding has differential effects on the energetic and opportunity costs of parental care.

Our results show that partners of inbred parents provided more direct care than partners of outbred parents, while female partners of inbred males spent less time providing indirect care than female partners of outbred males. These findings are surprising given that we predicted such effects only if inbred parents provided less care, causing a subsequent compensatory response by the partner. Our results show that partners of inbred parents increased the amount of direct care they provided despite that inbred parents provided as much direct care as outbred parents. As a consequence, there was an increase in the amount of total direct care when at least one of the parents was inbred (table 3; figure 1). Thus, partners of inbred parents responded by overcompensation rather than by incomplete or no compensation as we predicted. Previous studies on species with biparental care provide little evidence for overcompensation, although it has been reported in a small number of mate removal experiments on birds (Royle et al. 2002; Griggio and Pilastro 2007). Theoretical considerations suggest that there should be strong selection against overcompensation, as it allows the other parent to exploit its partner, thereby rendering biparental care evolutionarily unstable (Lessells 2012; Lessells and McNamara 2012). Our evidence for overcompensation

contradicts previous work on *Nicrophorus* beetles. Mate removal experiments report that males respond by incomplete compensation to female removal, while females show no compensation in response to male removal (Rauter and Moore 2004; Smiseth et al. 2005; Suzuki and Nagano 2009). Meanwhile, handicapping experiments show that, although handicapped parents provide less care, there is no compensation by the partner (Suzuki and Nagano 2009). Further work is needed to examine why partner responses seemingly lead to overcompensation when a partner interacts with an inbred parent (this study) but not when a partner interacts with a handicapped parent or when the other parent is removed (Rauter and Moore 2004; Smiseth et al. 2005; Suzuki and Nagano 2009). One potential explanation for these diverging partner responses is that inbred parents provide lower-quality care (e.g., effectiveness per unit time devoted to care), while handicapped parents reduce their quantity of care (e.g., overall amount of time devoted to care). An alternative explanation is that the partners of inbred parents respond to changes in the phenotypic condition of inbred parents, while partners of handicapped parents respond specifically to changes in the amount of care by handicapped parents.

Our study shows that each parent adjusted its contribution to care in response to the amount of care by the other parent, as predicted by negotiation models (McNamara et al. 1999). However, if the increased contribution to care by partners of inbred parents is mediated through negotiation, we predicted that the inclusion of the amount of care by the focal parent should remove or reduce the effect of the focal parent's inbreeding status. We found that the focal parent's inbreeding status had a significant effect on the amount of care provided by the partner regardless of whether or not the amount of care provided by inbred and outbred focal parent was included in the model. Thus, the increase in the amount of care by partners of inbred parents was independent of the amount of care provided by the other parent, suggesting that this response was mediated through a sealed bid response rather than

negotiation. Our results therefore suggest that, although negotiation occurs, it is independent of the observed increase in care by partners of inbred parents. This finding contradicts the traditional view that sealed bid and negotiation models are mutually exclusive. Instead, our results suggest that biparental cooperation may be shaped by both negotiation and sealed bid decisions. For example, sealed bid decisions may determine the overall level of care that a parent provides while negotiation may serve to coordinate short-term adjustments in care based on the other parent's current behavior. This might explain why previous work on *Nicrophorus* beetles report mixed evidence for negotiation and sealed bid models. There is evidence for negotiation from studies showing that there is a negative correlation between male and female parental care (Smiseth and Moore 2004), and that males adjust their care in response to the experimental removal of the female. Meanwhile, there is evidence for sealed bid decisions from studies showing that females do not adjust their care in response to male removal (Rauter and Moore 2004; Smiseth et al. 2005; Suzuki and Nagano 2009), and that partners of handicapped parents do not compensate for the reduction in the amount of care by handicapped parents (Suzuki and Nagano 2009).

The finding that partners of inbred parents altered their contribution independently of the amount of care provided by the focal parent suggests that partners responded to unknown cues that differ between inbred and outbred parents. Our study provides no information on the potential nature of such cues, but potential candidates are cuticular hydrocarbons (CHCs) or other chemical cues (Howard and Blomquist 2005). Such cues could vary between inbred and outbred parents as a direct consequence of inbreeding or as an indirect consequence of changes in correlated traits such as overall condition. There is some evidence females can discriminate between inbred and outbred males (Zajitschek and Brooks 2010; Okada et al. 2011) based on chemical cues (Ilmonen et al. 2009). Furthermore, previous work on *N. vespilloides* shows that CHCs play an important role in partner recognition between parents

that cooperate to care for their joint offspring (Müller et al. 2003; Steiger et al. 2009). Further work is needed to test whether chemical cues can provide information about inbreeding status in *N. vespilloides*, and whether such cues can mediate how partners respond to the focal parent's inbreeding status.

We also found effects of the interaction between the inbreeding status of the focal parent and that of its partner. We expected such interaction effects if inbred partners of inbred parents were more constrained in their ability to compensate than outbred partners of inbred parents. If so, inbred partners should provide less care overall and be less responsive to the other parent's inbreeding status than outbred partners. In contrast, we found that inbred females provided as much direct and indirect care as outbred females. Furthermore, although inbred partners were less responsive to the focal parent's inbreeding status than outbred partners with respect to direct care, inbred partners were more responsive to the focal parent's inbreeding status with respect to indirect care. We are unaware of any theoretical work that might explain the specific patterns of these interaction effects. However, we note that the amount of care by a given parent was influenced both by its own inbreeding status and that of its partner. Thus, inbred partners might provide less direct care when mated to inbred parents because they also responded to their partner's inbreeding status. For example, given that inbred males provided more care when mated to inbred females than when mated to outbred females, inbred females may have been able to reduce their direct care when mated to inbred males. These considerations highlight that these interaction effects reflect the outcome of complex dynamics between decisions made by either sex based on their own inbreeding status, their partner's inbreeding status and the amount of care by the partner. We suggest that our fully factorial design is applied to handicapping experiments, in which a focal parent's ability to provide care is manipulated by for example adding weights to the individual, thereby increasing energetic costs of providing care (Harrison et al. 2009). Handicapping

experiments target only one parent (Harrison et al. 2009), and as a consequence, it is unclear whether such interaction effects are specific to the context of inbreeding or a general attribute of biparental cooperation.

Finally, we found no evidence that inbreeding in male or female parents had detrimental effects on offspring fitness. Our results contrast with a recent study on the same species, which found that offspring survival was reduced when their parents were inbred (Mattey et al. 2013). The different results between the two studies might reflect differences in experimental design. In this experiment, we assigned parents with experimental broods of 10 larvae, while Mattey et al. (2013) allowed parents to raise natural brood sizes. Given that natural broods are larger on average (about 25 larvae; Smiseth and Moore 2002) than our experimental broods, one explanation for why we failed to detect effects of inbreeding is that there were more resources per individual offspring in our experiment. There is mounting evidence that inbreeding depression is more severe under harsh environmental conditions (Armbruster and Reed 2005). If so, our results might reflect that inbreeding in parents has a weaker effect on offspring fitness when sibling competition is less intense.

In conclusion, our study provides the first evidence that inbreeding can moderate biparental cooperation among parents. Surprisingly, we found that partners of inbred parents increased the amount of care they provided despite that inbred parents provided as much care as outbred parents. Thus, future work on the effects of inbreeding in species with biparental care, including birds, needs to consider potential effects mediated through the response of the partner of inbred parents. Our study identifies sealed bid decisions as the behavioral mechanisms mediating the increase in care by partners of inbred parents. Sealed bid decisions are thought to have evolved in response to sexual conflict over how much care each parent should contribute (Lessells 2012). Given that inbreeding is uncommon in nature, it is unlikely that this mechanism evolved in the specific context of inbreeding. Instead, we suggest that,

when the behavior or fitness of outbred individuals is altered due to interactions with inbred individuals, such effects are mediated through mechanisms that evolved to serve an adaptive function in another context. If a previously outbred population becomes subject to inbreeding, for example due to a population bottleneck or habitat fragmentation, these behavioral mechanisms may alter the behavior or fitness of outbred individuals that interact with inbred individuals. Our study reports complex effects of inbreeding on biparental cooperation, but similarly effects of inbreeding could occur in other social contexts, including cooperative breeding, resource competition and mating. For example, in cooperatively breeding species, effects of inbreeding could be mediated through offspring decisions on whether to breed or help the breeding parents. If offspring base such decision on their own condition, and inbred offspring are in poorer condition, inbreeding might increase the probability that offspring become helpers. In this case, inbreeding among helpers might benefit breeders as they receive additional help. However, effects of inbreeding on cooperative breeding could also be mediated by how breeders respond to an increased number of helpers. If breeders adjust their initial investment in eggs to the number of helpers (Russell et al., 2007), and inbred offspring are more likely to become helpers, inbreeding among helpers might cause breeders to lay smaller eggs. This might have negative fitness consequences for breeders if inbred helpers provide less help than outbred helpers, as it would generate a mismatch between the anticipated and actual amounts of care that helpers provide after hatching.

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Figure legend

Figure 1: Effects of inbreeding on biparental cooperation in *Nicrophorus vespilloides*.

Comparison of the amount of time spent providing direct care (A) and indirect care (B) by inbred or outbred male (open bars) and inbred and outbred female (grey bars) parents caring for outbred offspring during a 30min observation (mean \pm SE). The total amount of care offspring received from both parents (filled circles) is shown for each treatment (mean).

Tables

Table 1: Design of statistical models to test for effects of inbreeding on biparental cooperation.

Response variable	Main fixed effects	Interaction	Additional fixed effects	Random effect
Male direct care	Male IS + Female IS	Male IS * Female IS	Female direct care + brood size	Block
Male indirect care	Male IS + Female IS	Male IS * Female IS	Female indirect care + brood size	Block
Female direct care	Female IS + Male IS	Female IS * Male IS	Male direct care + brood size	Block
Female indirect care	Female IS + Male IS	Female IS * Male IS	Male indirect care + brood size	Block
Total direct care	Male IS + Female IS	Male IS * Female IS	Brood size	Block
Total indirect care	Male IS + Female IS	Male IS * Female IS	Brood size	Block
Offspring growth	Male IS + Female IS	Male IS * Female IS	Brood size	Block
Offspring survival	Male IS + Female IS	Male IS * Female IS	Carcass size	Block

Note: In all models, we always included the main effects of the inbreeding status (IS) of the focal parent and its partner. Note that Male IS represents the focal parent's IS and Female IS represents the partner's IS for male behaviors, while Female IS represents the focal parent's IS and Male IS represents the partner's IS for female behaviors. For each model, decisions on whether or not to include interaction effects and

additional fixed effects were based on the lowest AIC score. The order of terms in each column represents the order by which each term was added in the models. Block was included as a random effect in all models.

Table 2: Summary of statistical tests for the effects of inbreeding on biparental cooperation in *Nicrophorus vespilloides*.

Type of care	Focal parent's IS				Partner's IS				Interaction				Partner's behavior			
	Par	SE	Z-value	P-value	Par	SE	Z-value	P-value	Par	SE	Z-value	P-value	Par	SE	Z-value	P-value
Male direct	-0.36	0.3	-1.21	0.23	1.27	0.33	3.9	<0.0001	-0.14	0.69	-0.2	0.84	-0.37	0.09	-3.95	<0.0001
Male indirect	0.47	0.19	2.44	0.015	0.02	0.19	0.12	0.9	0.79	0.39	2.05	0.041	-0.02	0.03	-0.6	0.55
Female direct	-0.0002	0.18	-0.001	0.99	0.67	0.16	4.08	<0.0001	-1.26	0.38	-3.41	0.0008	-0.54	0.17	-3.19	0.001
Female indirect	-0.04	0.15	-0.23	0.817	-0.5	0.16	-3.06	0.002	-0.92	0.36	-2.56	0.01	-0.013	0.03	-0.437	0.66

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Note: Each row represents the amount of time spent providing direct and indirect care provided by male and female parents during a 30 min observation period. For each row, there is information on parameter estimates (Par), standard errors (SE), test statistics (Z-values), and *P*-values for the effects of the focal parent's own inbreeding status (IS), that of its partner, and the interaction between the two. For each row, there is also information on the effects of the amount of care provided by the partner. For information on effects on the focal parent, we refer to the column on Focal parent's IS. For information on responses by the partner, we refer to the column on Partner's IS. The data were analyzed using generalized linear mixed models (fitted with a Poisson error structure), in which experimental block was assigned as a random factor.

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Table 3: Summary of statistical tests for the effects of inbreeding on the total care by the two parents in *Nicrophorus vespilloides*.

	Male IS				Female IS				Interaction			
	Par	SE	Z-value	P-value	Par	SE	Z-value	P-value	Par	SE	Z-value	P-value
Total Direct	0.31	0.12	2.57	0.01	0.23	0.13	1.77	0.07	-0.99	0.26	-3.78	0.0002
Total Indirect	-0.11	0.12	-0.95	0.34	-0.02	0.12	-0.13	0.898	-0.23	0.24	-0.959	0.337

Note: Each row represents the total amount of time spent providing direct and indirect care provided by male and female parents during a 30 min observation period. For each row, there is information on parameter estimates (Par), standard errors (SE), test statistics (Z-values), and *P*-values for the effects of the male's inbreeding status (IS), the female's IS, and the interaction between the two. The data were analyzed using generalized linear mixed models (fitted with a Poisson error structure), in which experimental block was assigned as a random factor.

Table 4: Effects of inbreeding in male and female parents on the growth and survival of outbred offspring in *Nicrophorus vespilloides*.

Trait	Male IS				Female IS				Interaction				Additional fixed effect			
	Par	SE	<i>t</i> / <i>Z</i> -value	P-value	Par	SE	<i>t</i> / <i>Z</i> -value	P-value	Par	SE	<i>t</i> / <i>Z</i> -value	P-value	Par	SE	<i>t</i> / <i>Z</i> -value	P-value
Hatching mass	3x10 ⁻⁵	7.9x10 ⁻⁵	0.44	0.66	4x10 ⁻⁵	7.9x10 ⁻⁵	0.53	0.6	-4.6x10 ⁻⁵	1.6x10 ⁻⁴	-0.29	0.77	-	-	-	-
Growth at observation	0.001	0.0008	1.24	0.22	0.0001	0.0008	0.16	0.87	0.001	0.002	0.66	0.52	0.0003	0.0002	1.34	0.19
Growth at dispersal	0.01	0.08	0.17	0.1	0.01	0.01	1.44	0.16	0.02	0.02	1.25	0.22	0.004	0.002	2.24	0.03
Survival to observation	-0.17	0.21	-0.84	0.40	-0.22	0.21	-1.063	0.29	-0.69	0.44	-1.59	0.11	-0.5	0.21	-2.32	0.02
Survival to dispersal	0.28	0.23	1.19	0.23	0.42	0.24	1.75	0.08	-0.24	0.50	-0.47	0.64	-0.53	0.22	-2.42	0.016
Survival to eclosion	-0.4	0.36	-1.17	0.24	0.05	0.35	0.14	0.88	0.96	0.77	1.25	0.21	1.29	0.4	0.59	0.0013

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Note: For each trait, there is information on parameter estimates (Par), standard errors (SE), test statistic (*t*-values for hatching mass and growth and *Z*-values for survival), and *P*-values for the effects of the male's inbreeding status (IS), the female's IS, and the interaction between the two.

There is also information for additional fixed effects (brood size for growth and carcass size for the three survival measures). The data were analyzed using linear mixed effects models for hatching mass and growth and generalized linear mixed models for survival (fitted with a Poisson

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error structure). Experimental block was assigned as a random factor.