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Sex differences in parental defence against conspecific intruders in the burying beetle *Nicrophorus vespilloides*

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In species with biparental care, females often provide more care than males. Previous work has focused on sex differences in parental food provisioning and defence against predators. However, parents often also defend their offspring against conspecific intruders, which could be male or female. Thus, there is a need for studies examining sex differences in the behaviour of both caring parents and intruders, and whether sex differences in the behaviour of caring parents depend upon the intruder's sex. We conducted an experiment on the burying beetle *Nicrophorus vespilloides* where a single female or male resident caring for a brood was confronted with a male or female intruder. Female residents were more successful in defending their brood and engaged in more fights against an intruder than males. Residents engaged in more fights against male intruders and, among those that successfully defended their brood, residents spent more time provisioning food to larvae when confronted with female intruders. There was no evidence that sex differences in the behaviour of caring parents depended upon the intruder's sex. There were no sex differences in any measures of reproductive success among those residents that successfully defended their brood and no sex differences in the life span or mass gain of either residents or intruders. Our study extends the study of sex differences in parental care to the context of defence against conspecific intruders by demonstrating sex differences in the behaviour of both residents and intruders and sex differences in reproductive success in the presence of conspecific intruders.

In the majority of birds, as well as some mammals, amphibians, fishes and arthropods, male and female parents cooperate to care for their joint offspring (Balshine, 2012; Cockburn, 2006; Lessells, 2012; Trumbo, 2012). In many such species, females provide more care than males (Kokko & Jennions, 2012; West & Capellini, 2016). For example, this is observed in red-winged blackbirds, *Agelaius phoeniceus* (Whittingham, 1989), oldfield mice, *Peromyscus polionotus* (Margulis, 1998), convict cichlids, *Cichlasoma nigrofasciatum* (Lavery & Keenleyside, 1990) and the burying beetles *Nicrophorus vespilloides* (Smiseth & Moore, 2004) and *Nicrophorus orbicollis* (Trumbo, 2006). In general, females are expected to provide more care whenever the benefits of care are higher and/or the costs of care are lower for females than for males (Fromhage & Jennions, 2016; Kokko & Jennions, 2008). This condition may be satisfied when there is sperm competition, which reduces the benefits of care to males by lowering the coefficient of relatedness between them and the offspring (Kokko & Jennions, 2008; Westneat & Sherman, 1993). Furthermore, it may be satisfied when sexual selection introduces disparity in male attractiveness (Kokko & Jennions, 2008). When this is the case, more attractive males incur higher costs of care because they have a higher probability of attracting additional female mates, while less attractive males have lower benefits of care because they sire fewer offspring (Alonzo, 2010; Kokko & Jennions, 2008; Queller, 1997).

Previous work on species with biparental care has focused on sex differences in parental food provisioning (Smiseth & Moore, 2004; Walling, Stamper, Smiseth, & Moore, 2008) and parental defence against predators (Whittingham, 1989). In many such species, parents also defend their offspring or any resources used for breeding against conspecific intruders that may terminate the parent's breeding attempt (Rasa, 1999; Scott, 1998; Sowersby & Lehtonen, 2017; Wilson, 1971). Thus, there is a need for studies examining sex differences in parental defence against such intruders. Furthermore, given that intruders can be male or female (Itzkowitz, Santangelo, & Richter, 2001; Lang & Jaeger, 2000; Palanza, Re, Mainardi, Brain, & Parmigiani, 1996; Sowersby & Lehtonen, 2017; Trumbo, 2007; Trumbo & Valletta, 2007), such studies should also examine sex differences in the behaviour of intruders, and whether any sex differences in the defence of caring parents are conditional upon the sex of the intruder. Such

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interaction effects are expected if the sex of the intruder alters the costs of defence for the resident. For example, losing to a same-sex intruder may imply that the current reproductive attempt is terminated. In contrast, losing to an intruder of the opposite sex may be less costly given that the resident may initiate a second breeding attempt with the intruder (Trumbo, 1990; Trumbo & Valletta, 2007; Wilson, Knollenberg, & Fudge, 1984).

Burying beetles of the genus *Nicrophorus* (Coleoptera: Silphidae) are ideal model organisms for studying sex differences in parental defence against conspecific intruders and sex differences in the behaviour of intruders. Burying beetles breed on small vertebrate carcasses, which are highly valuable and unpredictable in time and space (Scott, 1998). Burying beetles have facultative biparental care, and males and females cooperate to defend, bury and prepare the carcass (Smiseth & Moore, 2004; Trumbo, 1992; Walling et al., 2008), and provision the larvae with predigested carrion after hatching (Scott, 1998). There are sex differences in parental behaviours when both parents provide care as females spend more time provisioning food than males (Smiseth & Moore, 2004; Walling et al., 2008). However, little is known about the ultimate causation of these sex differences. For example, it seems unlikely that sperm competition, although well documented in this species (Müller, Braunisch, Hwang, & Eggert, 2007), can account for the observed sex differences in care. The reason for this is that there is also substantial intraspecific brood parasitism, which reduces the benefits of care to females to a similar degree to how sperm competition reduces the benefits of care to males (Müller et al., 2007). Prior work shows that the removal of the male has no impact on the parental behaviour or reproductive success of females, whereas males compensate for the removal of the female by investing as much in direct care as a female (Rauter & Moore, 2004; Smiseth & Moore, 2004; Smiseth, Dawson, Varley, & Moore, 2005; Trumbo, 1991, 2006). The ability of both sexes to care for the larvae on their own is useful as it enables direct comparison of the two sexes, thereby allowing us to disentangle the effects on the focal parent's behaviour from any potential effects on the social partner's behaviour (Smiseth & Moore, 2004; Trumbo, 2007). Infanticidal take-overs in burying beetles by conspecific intruders occur regularly in the wild (Koulianos & Schwarz, 2000; Robertson, 1993; Suzuki, 2000; Trumbo, 1990; Wilson et al., 1984). When an intruder discovers a carcass, he or she will attempt to expel the same-sex resident, commit infanticide and mate with the opposite-sex resident to produce a replacement brood (Scott, 2006; Trumbo & Valletta, 2007; Trumbo, 1991, 2006). Subsequent reproductive opportunities for the expelled resident will depend on the probability of finding a new carcass, which is generally assumed to be very low (Trumbo, 2006; Trumbo & Valletta, 2007).

The aim of this study was to examine parental defence of single male or female residents exposed to a conspecific male or female intruder, and the fitness consequences of such interactions, using the burying beetle *N. vespilloides* as the study system. To this end, we used a two-by-two fully factorial design where a single female or male parent caring for a brood of first-instar larvae (hereafter, termed 'resident') was confronted by a potentially infanticidal male or female conspecific (hereafter, termed 'intruder'). We tested whether the sex of the resident, the sex of the intruder, or the interaction between the two, influenced the outcome of the encounter (i.e. successful defence by resident or take-over by intruder), number of fights, time spent provisioning food to the larvae, mean larval mass, brood size, change in the resident's and the intruder's mass, and life span. Given evidence that females spend more time provisioning food than males (Smiseth & Moore, 2004; Walling et al., 2008), we expected female residents also to put more effort into defence. Thus, female residents should win more often, fight more often, spend more time provisioning food and have higher current reproductive success than male residents. We also expected female residents to have a lower mass gain and a shorter postbreeding life span than resident males due to their higher effort in defence. Moreover, the cost of defence for the resident should be lower when exposed to an intruder of the opposite sex, due to the opportunity of producing a replacement brood (Scott, 2006; Trumbo, 1991, 2006; Trumbo & Valletta, 2007). Thus, female residents should win and fight more often when exposed to a female intruder than when exposed to a male intruder, while male residents should win and fight more often when exposed to a male intruder than when exposed to a female intruder.

METHODS

General Procedures

We used beetles derived from our laboratory population maintained at the University of Edinburgh. Beetles were kept under constant light and at 21 ± 1 °C. Nonbreeding adults were kept in individual plastic boxes (12×8 cm and 2 cm deep) filled with moist soil. Beetles were fed twice a week with organic beef for at least 10 days after eclosion before they were used in experimental trials to allow time for sexual maturation.

Experimental Design

To investigate sex differences in the behaviours of male and female residents and the behaviours of male and female intruders, we used a two-by-two fully factorial design where a single resident (female or male) was exposed to a single intruder (female or male). Thus, our experimental design included the following treatments: (1) a female resident challenged by a female intruder ($N = 20$), (2) a female resident challenged by a male intruder ($N = 21$), (3) a male resident challenged by a female intruder ($N = 21$) and (4) a male resident challenged by a male intruder ($N = 21$). These numbers refer to the final sample sizes upon which we based our analyses and exclude any cases where either no egg hatched by day 5 ($N = 27$) or one of the residents died before the introduction of the intruder ($N = 2$).

At the start of the experiment, we weighed each experimental beetle to obtain information on its pretrial mass. We also measured the pronotum width of each beetle using digital callipers to obtain information on its body size (Müller & Eggert, 1990). We used this information to match residents and intruders for body size by ensuring that they had a pronotum width within ±16%. There was no difference in the pronotum width of residents and intruders (mean ± SE; residents: 5.25 ± 0.04 mm; intruders: 5.24 ± 0.04 mm; paired *t* test: $t_{82} = 0.32$, $P = 0.75$). We did this to exclude any effects due to differences in body size given that body size is a main determinant of the outcome of aggressive interactions in this species (Otronen, 1988; Trumbo, 2007). We also marked all residents and intruders on the elytra with either one or two spots of correction fluid to ensure that we could identify the two beetles during experimental trials (Richardson & Smiseth, 2017; Suzuki, Nagano, & Trumbo, 2005). Correction fluid is short lasting, nontoxic and has no detectable effects on behaviour (Hagler & Jackson, 2001; Wineriter & Walker, 1984). We alternated which beetle (resident or intruder) was given two spots between experimental trials to exclude any potential effects of marking. We marked the beetles at least 1 h before the introduction of the intruder to avoid any effects due to handling. We ensured that the beetles were of the same age to avoid any effects due to age on either fighting effort or parental care (mean ± SE: residents: 21.06 ± 0.32 days; intruders: 21.43 ± 0.37 days; paired *t* test: $t_{82} = -0.84$, $P = 0.40$). All experimental subjects were virgins at the