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Difference in parenting in two species of burying beetle, *Nicrophorus orbicollis* and *Nicrophorus vespilloides*

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Abstract

Burying beetles (*Nicrophorus*) are model parents among insects, with all studied species known to regurgitate flesh from vertebrate carcasses to their offspring. However, most studies focus on a very few species, yet the interpretation of the function and importance of care is typically generalized to all burying beetles. Here we characterize subtle variation within and between individuals and sexes, and how this variation differs between two species of burying beetle. We find that *Nicrophorus orbicollis* exhibits low variance, with a normal distribution of parental care provided during peak care periods. In *N. vespilloides*, however, the distribution is more uniform as values of care are spread across the possible phenotypic spectrum. This suggests that there is stabilizing selection on care in *N. orbicollis*, but relaxed or disruptive selection in *N. vespilloides*. Although repeatability was similar between both species, transitions from other care behaviors into feeding were more common in *N. orbicollis* than *N. vespilloides*. Thus, while parenting is coarsely similar across the genus, variation in its expression should not be extrapolated to all *Nicrophorus*. We suggest that subtle variation both within and among species merits greater attention, and could inform us about the factors that lead to different distributions of care.

Keywords

Coleoptera; repeatability; social behavior; variance; behavioral transitionss

Introduction

Burying beetles (*Nicrophorus* spp.) are model organisms for studying parenting behavior in insects (Eggert and Müller 1997; Scott 1998; Trumbo 2012; Sikes and Venables 2013; Smiseth 2014). All known burying beetle species are subsocial, with highly developed parental care including direct interactions with offspring (Scott 1998; Sikes and Venables 2013). Parents find a vertebrate carcass and subsequently bury it, process the carcass into a ball, and coat the flesh with defensive secretions (Eggert and Müller 1997). Females lay eggs in the soil around the carcass, and upon hatching, the larvae crawl to the carcass and reside in a small depression created by the parents. More unusual and remarkable, they also

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provide direct care to their offspring, predigesting the carcass for the young to eat and regurgitating predigested food directly into the mouths of begging young (Eggert and Müller 1998). This direct care results in very rapid weight gain and development; for example, removing parents for just two hours results in significantly less mass gain in *N. vespilloides* young (Smiseth and Moore 2004). Offspring gain twice as much weight, increasing in mass by 600%, in the first 24 h when parents are present and feeding compared to young that feed on their own, which display a gain in mass of only 300% (Smiseth et al. 2003).

Burying beetle behavior and especially parenting is relatively well-studied, but focused on very few species. Most studies have been conducted on *N. vespilloides*, *N. orbicollis*, *N. pustulatus*, *N. defodiens*, and *N. quadripunctatus* with the first two by far the most commonly studied (Eggert and Müller 1997; Scott 1998; Trumbo 2012; Suzuki 2015). The documented differences typically involve ecology and resource use, rather than behavior (Eggert and Müller 1997; Scott 1998) and often species are characterized as, for example, biparental when there is clear evidence that they adopt other patterns of parental care (Müller et al. 2007; Parker et al. 2015; Benowitz and Moore 2016). Part of the difficulty is that the behavior of burying beetles seems to be remarkably flexible, and one can often find biparental, uniparental females and even uniparental males in a single population (Müller et al. 1998; Parker et al. 2015). This flexibility extends to circumstances where in *N. pustulatus*, offspring of some families actually perform better without care than with care, although the majority of families still benefit most by parenting (Rauter and Moore 2002). How, then, should we characterize care in burying beetles? To what extent is it uniform across the genus and within species?

Here we conducted behavioral observations under standard conditions to describe patterns of parenting by both uniparental males and females in the two most commonly studied species, *N. orbicollis* and *N. vespilloides*. Although these species are highly diverged within the genus (Sikes and Venables 2013), the effects of their parental care are very similar (Benowitz et al. 2015). Our goal was to more accurately describe parenting behavior involving direct provisioning of regurgitated food by parents to their offspring. We therefore observed more than 60 families of each sex and species, and described the variation in direct parental provisioning of resources. We also characterized the extent that individuals were consistent in their behavior throughout a day. Given burying beetles provide direct care for their offspring for only a few days, this is a significant amount of the total care that they provide. We found that *N. orbicollis* and *N. vespilloides* had very different distributions of direct care, but were equally individually consistent. We suggest this reflects differences in the strength and perhaps form of selection acting on the care in these two species, and that characterization of nuanced variations in behavior between species deserves empirical attention.

Materials and methods

We used stock colonies of *N. orbicollis* and *N. vespilloides*, originating from Georgia, USA and Cornwall, UK, respectively, and maintained in our laboratory at the University of Georgia as previously described (Benowitz et al. 2015). We randomly mated twenty non-sibling *N. orbicollis* in plastic boxes ($17.2 \times 12.7 \times 6.4$ cm; Pioneer Plastics, Dixon KY)

filled with 2cm of dirt with a mouse carcass ranging in size from 22-25 grams. Two days after *N. orbicollis* were paired, we then paired *N. vespilloides* in the same manner, so as to align their reproductive schedules (Benowitz et al. 2015). Four days after the pairing of *N. orbicollis* (one day before expected hatching), we removed the male or the female from all containers to ensure uniparental female or male care. The following day, we checked all pairs for hatched larvae. Observations began the day following hatching (day 6 for *N. orbicollis*, day 4 for *N. vespilloides*). We observed bouts of parental care in 61 females and 78 males in *N. orbicollis*, and 64 females and 84 males in *N. vespilloides*. We only observed pairs that had eggs before 5:00 pm on this day to ensure that during observations larvae were between 16 and 24 hours old, around the time of peak parental care in *Nicrophorus* (Smiseth et al. 2003). We observed both species at the same stage on the same day to ensure that environmental conditions were randomized identically for both species. We moved breeding boxes into a dark room lit with only red light illuminating the areas of observation. After moving the boxes, we removed the lids from each box and then left them undisturbed for 30 minutes so that mothers had a chance to recover from the disturbance of the box lids being removed.

We recorded parental behavior in the presence of one-day old larvae eight times throughout the day under red light, one observation per hour. A single observation took 10 minutes, and consisted of 10 separate scan samples at one-minute intervals (Martin and Bateson 2007). At each scan, we recorded whether or not the mother was feeding larvae (i.e., mouth-to-mouth contact suggesting regurgitation of food; Smiseth and Moore 2002). Thus, for each individual, we recorded eight behavioral scores, each ranging from 0-10. We then summed these to create a total score for each individual with a potential range of 0-80. We also used this information to count the number of transitions into feeding, defined as an observation of a parent not feeding immediately followed by an observation of the parent feeding. For example, a behavioral score of 4 indicates four instances of care, but this could be one continuous bout of care lasting four minutes or 4 distinct bouts with 4 transitions spread over the observation period. As for total care, each individual had eight behavioral scores, but each score for transitions into care ranged from 0-5, meaning total scores ranged from 0-40. These two behavioral measures provide information on intensity and consistency of care. After observations, we removed parents from the breeding box, and left broods to develop without a parent present. We then recorded the number of larvae present at or soon before dispersal.

We examined the distribution of parental care in each species and sex using histograms of total parental care, ranging from 0-80. We tested for homogeneity of variance between species and sexes using Levene's test. Next, we calculated repeatabilities of parental care for each species and sex using the intraclass correlation coefficient (Boake 1989; Falconer and Mackay 1996). We then performed pairwise tests using Fisher's *r*-to-*z* transformation to compare repeatabilities between sexes and species (Cohen et al. 2003). We performed two-way ANOVA to examine the effects of species and sex on the number of transitions into feeding made. Lastly, we used Pearson's correlations to analyze whether the amount of parental care provided was related to the number of surviving larvae. Statistical analyses were performed in JMP 11 (SAS Institute, Cary NC, 2013).

In *N. vespilloides*, several individuals provided no parental care during any of the observation periods, yet still produced surviving larvae. Thus, we cannot say whether they simply abandoned the carcass or whether they repeatedly declined to feed their offspring. Thus, to avoid potentially inflating repeatability values for *N. vespilloides* artificially, we chose to remove these individuals from our analyses of repeatability. However, they are represented in the histograms.

Results

Visual inspection of the data suggests that feeding behavior across the total of eight ten-minute observations followed a roughly normal distribution pattern in both uniparental male and female *N. orbicollis* (Fig. 1a,b). The distribution of provisioning in *N. vespilloides*, on the other hand was more uniform (Fig. 1c,d), with no obvious peak and greater variance ($F_{1,273} = 61.193$, $p < 0.001$). This suggests that parental care might reflect more consistent individual differences in *N. vespilloides*, as values of feeding behavior were less likely to fall in the middle of the distribution. Consistency of parental care behavior in *N. vespilloides* was confirmed by estimates of repeatability, which were substantial (female $n = 57$, $r = 0.36$; male $n = 79$, $r = 0.37$), and moderately higher than estimates for *N. orbicollis* (female $n = 61$, $r = 0.25$; male $n = 78$, $r = 0.18$). There were no statistically significant differences between the sexes in either *N. vespilloides* ($z = 0.078$, $p = 0.938$) or *N. orbicollis* ($z = 0.425$, $p = 0.670$) and so sex was no longer taken into consideration. The overall repeatability of *N. vespilloides* was 0.37 and for *N. orbicollis* was 0.24. However, this difference was not statistically significant ($z = 1.158$, $p = 0.123$). There were more transitions into feeding behavior by *N. orbicollis* than in *N. vespilloides* ($F_{1,271} = 47.573$, $p < 0.001$; Fig. 2). Only species influenced the number of transitions; there was no statistically significant effect of sex ($F_{1,271} = 0.023$, $p = 0.878$) or the interaction between species and sex ($F_{1,270} = 3.413$, $p = 0.066$). Finally, we found no statistically significant correlation between number of larvae produced and amount of feeding provided in either *N. vespilloides* ($n = 128$, $r = 0.045$, $p = 0.614$) or *N. orbicollis* ($n = 139$, $r = 0.057$, $p = 0.505$).

Discussion

Parental care is unusual but taxonomically widespread in insects (Tallamy and Wood 1986, Costa 2006). It is especially well-developed in burying beetles (Milne and Milne 1976; Eggert and Müller 1997; Scott 1998). However, defining parental care is difficult and the term is perhaps better as a descriptor or category of behavior (Royle et al. 2012) than as a quantifiable phenotype. Therefore, although all burying beetle species provide parental care, how similar is the expression of behavior that contributes to parental care across different species of *Nicrophorus*? Can we assume that the differences between species are limited to immediate environmental effects? Or might there be subtle evolutionary forces influencing the production of behavior reliably differently in different species? Here, we provide a detailed examination of parental behavior in two burying beetle species, *N. orbicollis* and *N. vespilloides*, with the goal of determining the generalizability of a shared behavioral trait, direct provisioning of food to offspring.

We found striking differences in the distributions of feeding behavior between the two burying beetle species studied here. *N. orbicollis* behavior in both sexes followed a relatively normal distribution, with a large peak at an intermediate trait value. In contrast, *N. vespilloides* provisioning behavior followed a more uniform distribution, with more individuals populating the extremes of the possible phenotypic spectrum than in *N. orbicollis*. This difference may reflect alternate selective regimes acting on these two species. On one hand, *N. orbicollis* appears to be or have been experiencing strong stabilizing selection, which would reduce the variance of the population as we observe in our data (Brodie et al. 1995). *N. vespilloides*, on the other hand, may be or have been experiencing relaxed or disruptive selection, leading to an increased variance and occupation of phenotypic space (Brodie et al. 1995). Why might this difference in selection exist? One potentially important difference in the biology of these species is that parenting is obligate in *N. orbicollis* (Trumbo 1992), but less so for *N. vespilloides* (Eggert et al. 1998; Schrader et al. 2015ab). Thus, in *N. orbicollis*, the fitness cost of being a low caring parent may constrain the evolution of parenting, when parents are provisioning on their own. This explanation is not complete, however. It is less clear why *N. vespilloides* occupies the upper end of the parental care distribution. It is possible that high levels of care are beneficial, but come at some (unknown) cost. If so, then the increased variance in this species reflects life history tradeoffs involved with parenting (e.g. Tallamy and Denno 1982). Alternatively, this pattern could simply reflect differences in larval number between these species. *N. vespilloides*, even though a smaller species, produces more larvae than *N. orbicollis* and also displays more variance in this trait (K.M. Benowitz unpub. data). However, our results suggest that number of surviving larvae produced is not linked to feeding behavior in either species.

We next asked whether these population level differences reflected any changes in the way individuals produced this behavior. Based on the prevalence of extreme phenotypic values in *N. vespilloides*, we expected to see higher levels of individual repeatability in provisioning behavior. However, this prediction was not borne out, as we observed moderately high repeatability for behavior in both species across our eight periods of observation. However, we did find that despite providing less care overall, *N. vespilloides* individuals were more likely to remain feeding once it began. In other words, on the finest scale, *N. orbicollis* transition rapidly between provisioning larvae and other behaviors, whereas *N. vespilloides* perform a single task (feeding or otherwise) for longer periods of time. Thus, not only does population variance differ between species, but the temporal patterns of individual behavioral expression also differ. The fitness consequences of this species difference are likely to be minimal, given that the effects of parenting in these two species are very similar (Benowitz et al. 2015). This may instead reflect differences in larvae, which tend to beg more aggressively in *N. orbicollis* (Benowitz et al. 2015), and thus may be more likely to manipulate *N. orbicollis* parents into feeding while they were attempting to perform other tasks. Further work is needed to characterize the possible causes of this stark species difference.

Despite a broad similarity of behavior amongst all burying beetles, our results suggest that we cannot generalize our conclusions from a single species to all of *Nicrophorus*. In fact, even the core *Nicrophorus* trait of offspring provisioning displays striking differences

between species when subtle aspects of this phenotype are considered. This type of between species variation opens the door to potentially fascinating phylogenetic and comparative studies within the *Nicrophorus* clade that could untangle the evolutionary factors affecting the specific nature of parental care in this genus.

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Fig. 1.

The distribution of total parental care in (a) *N. orbicollis* females, (b) *N. orbicollis* males, (c) *N. vespilloides* females, and (d) *N. vespilloides* males.

Fig. 2.
The number of transitions into parental care performed. Black bars represent females; grey bars represent males.