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ORIGINAL ARTICLE

A good day to die: bridging the gap between costs and benefits of parental care

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Abstract

In species that provide parental care, offspring survival is often completely dependent on protection and resources afforded by the parents. Therefore, parents gain no fitness unless they raise offspring to a critical point of independence. In these species, selection should shape parental life history to increase their chances of surviving to this critical point. We test this hypothesis using females of two species of burying beetles, *Nicrophorus orbicollis* and *N. marginatus*. Burying beetles breed on small, vertebrate carcasses, and reproduction can be divided into two stages: carcass preparation and larva provisioning. Females were allowed to reproduce repeatedly until they died, and the stage in which each female died was recorded. Most females died while waiting for another carcass for their next reproductive bout or during carcass preparation, which indicates that females may have a physiological mechanism that allows them to delay death until their final brood is independent of parental care.

Significance statement

In this paper, we show that female burying beetles of two species do not die at random times during reproduction, but instead die after they have finished caring for their offspring. This is the first time that non-random death has been shown in burying beetles. We show that females that die after offspring care is complete have a higher fitness than females that die at random because offspring in many species of burying beetles depend on parental care for food provisioning and protection. We also propose a hormonal mechanism through which females might extend their lives to allow them to finish caring for their offspring.

Introduction

Gradual senescence is typically seen in iteroparous organisms (Cole 1954) while catastrophic senescence is typically seen in semelparous organisms (Rose 1991; Partridge and Barton 1993; Charlesworth 1994;). In semelparous organisms, it is critical to reach the end of the reproductive cycle because death before the end of the cycle results in no fitness for that individual. Death before the end of the terminal reproductive cycle of iteroparous organisms carries a lower fitness cost because those organisms have multiple chances to reproduce. However, iteroparous organisms should behave like semelparous organisms in their terminal reproductive bout (Coleman et al. 1985) because they have no residual reproductive value.

In organisms with parental care, offspring survival often depends entirely on resources and protection provided by the parents (reviewed in Clutton-Brock 1991). If both parents desert or die before the brood becomes independent, reproduction fails, and the parents suffer reduced fitness (reviewed in Klug and Bonsall 2014). When parental care is necessary for offspring survival, a mismatch occurs between the timing of costs and benefits of reproduction. Costs of reproduction accumulate on an ongoing basis, but the fitness payoff is not received until offspring reach independence. For this reason,

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each reproductive bout is similar to that of semelparous organisms because a critical point in reproduction must be reached to gain fitness from the reproductive bout.

Burying beetles (Coleoptera: Silphidae) are an excellent model system for evaluating end-of-life behaviors and strategies resulting from parental care. They provide biparental care to their offspring by burying and preserving a small vertebrate carcass, which serves as the sole food source for parents and offspring until the larvae leave the carcass to pupate (Scott and Traniello 1990). Parents regulate brood size to match carcass size through filial cannibalism (Scott and Traniello 1990; Trumbo 2006), which results in a positive correlation between offspring number and carcass size (Scott and Traniello 1990; Creighton 2005). Larvae of several species do not survive without their parents the first couple of days on the carcass, and older larvae have reduced growth rates in the absence of their parents (Trumbo 1992; Eggert et al. 1998). Burying beetles are capable of breeding multiple times, although reproduction is costly (Creighton et al. 2009; Cotter et al. 2010).

Burying beetle reproduction can be divided into three distinct stages: preparing a carcass, provisioning larvae, and locating a new carcass. In burying beetles, parental care behaviors are partitioned so that males provide substantial care during the carcass preparation stage (Scott 1998), but females are the primary parents during larval provisioning (Müller et al. 1998). Therefore, the larval provisioning stage of reproduction should be the most stressful for females. In this study, we assessed the timing of death relative to the stage of the reproductive cycle in females of two species of burying beetles, *Nicrophorus orbicollis* and *N. marginatus*. We allowed females to reproduce throughout their lives, and noted the stage of reproduction during which each female died.

Methods

Sources of burying beetles

N. orbicollis was captured in central Wisconsin during summers from 2009 to 2011, and *N. marginatus* was captured in central Utah during the summer of 2013. Wild-caught pairs were placed on 30 g mouse carcasses and allowed to breed to generate the laboratory population. All mouse carcasses used in this study were purchased frozen from Layne Laboratories (Arroyo Grande, CA, USA). The date of eclosion was recorded for all laboratory-bred beetles, and all beetles used in these experiments were F_1 or F_2 offspring of wild-caught beetles. Experimental beetles were placed individually in small plastic containers ($15.6 \times 11.6 \times 6.7$ cm) with ad libitum raw chicken liver and maintained on a 14:10-h light:dark cycle. Experiments on *N. orbicollis* were run at Purdue University Calumet from 2009 to 2012. Experiments on *N. marginatus* were run at Brigham Young University from 2013 to 2014.

Experimental design

N. orbicollis

The purposes of this experiment were to determine whether the timing of death in female *N. orbicollis* was random relative to the stage of the reproductive cycle, and to determine whether carcass size or carcass manipulations affected timing of death. These data were collected as part of a larger experiment addressing the costs of reproduction in burying beetles (Smith et al. 2015; Smith et al. in prep). The experimental design consisted of four treatments: 20-g control, 30-g control, $30 \rightarrow 20$-g experimental, and $20 \rightarrow 30$-g experimental. In each of the control treatments, the original carcass was left with the parent for the duration of the trial. In the $30 \rightarrow 20$-g experimental treatment, the 30 g carcass was replaced with a pre-prepared 20 g carcass within 12 h of the arrival of larvae on the carcass to cause overproduction of offspring. In the $20 \rightarrow 30$-g experimental treatment, the 20 g carcass was replaced with a pre-prepared 30 g carcass within 12 h of the arrival of larvae on the carcass to cause underproduction of offspring. Although carcasses were switched in the experimental treatments, carcasses were not switched in the control treatments because previous research found that switching a carcass with one that was pre-prepared of the same size had no effect on reproduction (JCC unpublished data). In addition, each brood was handled daily, regardless of treatment to count larvae and check brood progress. Twelve replicates were completed for each of the four treatments for a total of 48 replicates. We did not use blinded methods in this study because we switched carcasses in some treatments, which required that we know the treatment for each replicate. Also, we observed the timing of death for each beetle, which is not subject to bias or influence.

We randomly paired a genetically unrelated virgin male and female aged 28–35 days old to begin each trial. The pair was placed in a small brood container ($16.5 \times 15 \times 9$ cm) filled with 6 cm of moist soil and either a 20 g (± 1.0 g) or a 30 g (± 1.0 g) mouse carcass. The containers were kept in an environmental chamber at 21 °C on a 14:10-h light:dark cycle. We checked broods daily, and after larvae arrived on the carcass, the lid of the small container was removed, the carcass was switched if the trial was in an experimental treatment, and the container was placed in an abandonment chamber ($37.5 \times 25.5 \times 14.5$ cm) flush with an elevated styrofoam platform. Two cups (diameter: 8 cm, height: 9.5 cm) were placed in diagonal corners of the larger abandonment chamber, also flush with the elevated ledge (see Smith et al. 2014). The cups in each corner of the container were checked daily to see whether an adult had abandoned the brood. If a parent was found in a cup, its mass and the date were recorded, and it was placed back in the small container with the brood. If the parent abandoned the brood for a second time, it was removed if it

was the male parent, or removed and placed on ad libitum chicken liver for 2 days if it was the female parent. After a 2-day break, the female was given a new genetically unrelated virgin male aged 28–35 days and a new carcass. If the female parent remained with the brood when the larvae dispersed into the soil, she was placed on ad libitum chicken liver for 2 days and then set up to reproduce again. This cycle continued for each female until death.

N. marginatus

The purpose of this experiment analysis was to determine whether the timing of death in female *N. marginatus* was random relative to the stage of the reproductive cycle. These data were collected as part of a larger experiment. All females of this species reproduced biparentally on 30 g carcasses. The experimental protocol was identical to that of *N. orbicollis* females, using the same abandonment chambers, to allow comparison between the two species. Fifty-four individual trials of *N. marginatus* females were completed in this experiment.

Statistical analyses

Reproduction was divided into three stages for our analysis: (1) carcass preparation, (2) larval provisioning, and (3) rest period. The number of days in each stage of reproduction differed between species. In *N. orbicollis*, stage 1 was an average of 5 days in length, stage 2 was an average of 6 days in length, and stage 3 was 3 days in length. In *N. marginatus*, stage 1 was an average of 4 days in length, stage 2 was an average of 5 days in length, and stage 3 was 3 days in length.

To determine whether carcass size or presence of larvae affect the timing of death in female *N. orbicollis*, we used a generalized linear model (Proc GENMOD; SAS version 9.4, SAS Institute Inc. Cary NC). In the model, the response variable was the stage during which death occurred (1, 2, or 3), and the predictor variable was the carcass treatment (four treatment combinations). We found no significant differences among the carcass treatments (F value = 0.835; $p = 0.365$), so all the treatments for *N. orbicollis* were combined for the timing of death analysis. To determine whether death occurs randomly relative to reproductive stage in female *N. orbicollis* and *N. marginatus*, we used X^2 goodness-of-fit tests and compared the number of females that died in each stage. Expected values were set to account for the different number of days in each stage of reproduction for each species.

Results

Females of both *N. orbicollis* ($X^2 = 44.8258$; $p < 0.0001$) and *N. marginatus* ($X^2 = 35.1169$; $p < 0.0001$) did not die at

random times during the reproductive cycle (Fig. 1). In *N. orbicollis*, 31 % of females died during the carcass preparation stage, 58 % died during the break period, but only 10 % of females died during the larval provisioning stage of reproduction. In *N. marginatus*, 61 % of females died during the carcass preparation stage, 37 % died during the break period, but only 2 % of females died during the larval provisioning stage of reproduction.

Discussion

Females of both *N. orbicollis* and *N. marginatus* did not die at random times during reproduction (Fig. 1). In burying beetles, parental care in the form of protection (Fetherston et al. 1990; Scott 1990) and offspring provisioning (Eggert et al. 1998) is necessary for offspring survival, which creates a mismatch between the timing of the costs and benefits of reproduction in burying beetles. Parents must care for offspring and raise them to independence before maximum fitness benefits from the current brood are obtained (i.e., additional offspring). Females of both species were less likely to die during the larval provisioning stage of reproduction than during the carcass preparation stage or the brief rest period between bouts.

Females are the primary parents during larval provisioning (Fetherston et al. 1990; Rauter and Moore 1999; Rauter and Moore 2004), and this stage is more costly for them than the carcass preparation stage (Trumbo and Rauter 2014; Smith et al. in prep). Female burying beetles typically gain mass during carcass preparation, then lose mass rapidly during larval provisioning (Steiger et al. 2012; Trumbo and Rauter 2014), which indicates that females gain mass in anticipation of the costly stage of reproduction—larval provisioning. Females seem to be investing all of their remaining energy
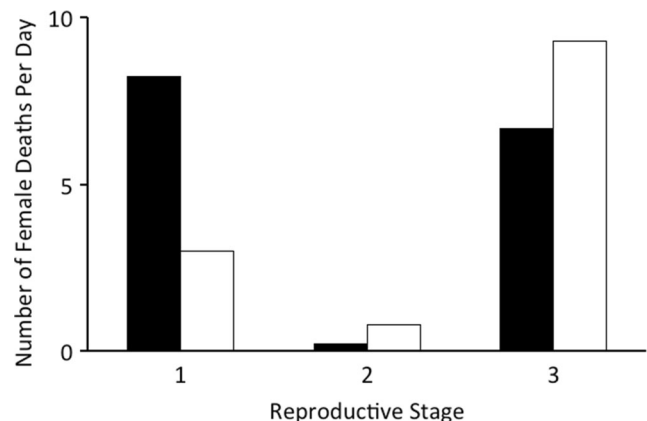

Fig. 1 Number of female deaths per day in each stage of reproduction. *Nicrophorus orbicollis* (*n* = 48) is represented by *white bars*, and *N. marginatus* (*n* = 54) is represented by *black bars*. If females die at random, the expected values for *N. orbicollis* are stage 1 = 17, stage 2 = 21, and stage 3 = 10, and the expected values for *N. marginatus* are stage 1 = 18, stage 2 = 23, and stage 3 = 13

into caring for offspring, and then die either while they are waiting for another carcass or during preparation of the next carcass. If females died during carcass preparation, they typically had not produced eggs for that reproductive bout (ANS personal observation), so they did not leave offspring to be cared for by their partner alone. Also, there was no difference in the timing of death for female *N. orbicollis* in any of the carcass size or stress treatments, which indicates that reproductive conditions or past reproductive history has no effect on the timing of death.

Although we do not know the mechanism underlying the non-random pattern of mortality we document here, juvenile hormone may play an important role. Juvenile hormone (JH) regulates metamorphosis and reproduction (Gilbert 1976), as well as self-maintenance (González-Tokman et al. 2013) in insects. It also up-regulates lysozyme-like activity, which is an important component of insect immune function (Rolff and Siva-Jothy 2002). In female *N. orbicollis*, JH level increases rapidly after finding a carcass, then declines (Trumbo et al. 1995), then increases again 3 days later (Scott et al. 2001). Metabolic activity and JH are both increased during larva provisioning in *N. pustulatus* females, and this parental behavior reduces longevity (Trumbo and Rauter 2014). Therefore, female burying beetles may delay death during larva provisioning by increasing JH and lysozyme activity to defend themselves against pathogens, but at the cost of an increased energetic demand that ultimately leads to their death shortly after this terminal investment strategy. The evidence for JH manipulation as a mechanism to extend life until after larvae become independent is circumstantial, and we realize further experimentation is required to determine the causal mechanism that allows females to delay death until care of the terminal brood is completed.

Evolution of this strategy

The evolution of the pattern of non-random survivorship we observed requires increased fitness of individuals that control the timing of death relative to those that die at random times. A complete reproductive bout (carcass preparation and larva provisioning, plus the 3-day rest period) takes an average of 14 days in *N. orbicollis*, and 12 days in *N. marginatus*. Hypothetical females that die at random times have a 21 and 25 % chance (for *N. orbicollis* and *N. marginatus*, respectively) of surviving the first two stages of reproduction and successfully completing the current reproductive bout. In contrast, females that delay death until after caring for their offspring have a 64 and 67 % chance (for *N. orbicollis* and *N. marginatus*, respectively) of surviving the first two stages of reproduction and thus successfully completing the current reproductive bout. The following calculations assume that all offspring would die without care from the mother. Larvae are able to feed directly from the carcass when they reach the third instar stage of development (Scott 1998), but offspring are still vulnerable to intruders without protection from a parent.

Obviously, the fitness difference between females that die at random and females that die after offspring are independent depends on the number of offspring in the last brood and on the number of offspring produced in previous reproductive bouts (Creighton et al. 2009). For example, in *N. orbicollis*, number of offspring in the brood declines from about 14 in the first bout to about 11 by the fourth bout (ANS personal observation). If death occurs during the first bout, females that die after offspring care is complete will produce an average of 8.96 offspring (14 offspring multiplied by 64 % probability of surviving), whereas females that die at random will produce an average of 2.94 offspring (14 offspring multiplied by 21 % chance of surviving). This represents a 205 % increase in fitness for females that die after offspring are independent. Using similar calculations, if death occurs during the fourth reproductive bout, *N. orbicollis* females that die after offspring are independent achieve an 11 % increase in fitness relative to females that die randomly during the fourth reproductive bout. Numbers for *N. marginatus* would be comparable. For both *N. orbicollis* and *N. marginatus*, and at any reproductive bout, delaying death until after offspring are independent generates a substantial increase in fitness, and thus, this trait should be rapidly incorporated within populations.

Offspring of both *N. orbicollis* (Trumbo 1992) and *N. marginatus* (ANS personal observation) require regurgitation from parents for survival. Therefore, selection for a mechanism to delay death should be strongly selected for. In *N. defodiens*, *N. tomentosus*, and *N. pustulatus*, parental care is less important for offspring survival, and therefore, we would expect selection for this mechanism to be weaker. Future studies using other species of burying beetles would indicate whether a mechanism for delayed death is widespread among *Nicrophorus* or if it is specific to species where parental care is essential for offspring development.

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