Longevity cost of reproduction for males but no longevity cost of mating or courtship for females in the male-dimorphic dung beetle *Onthophagus binodis*

Janne S. Kotiaho a,*, Leigh W. Simmons a

a Evolutionary Biology Research Group, Zoology Building, School of Animal Biology (M092), University of Western Australia, Nedlands, WA 6009, Australia

Received 27 September 2002; accepted 8 May 2003

Abstract

Life history theory predicts a trade-off between current and future reproduction. Despite a wealth of research on the cost of reproduction for females, there have been very few studies that have looked at the cost of reproduction for males. Longevity is closely related to the opportunity for future reproduction, and thus decreased longevity in response to current reproductive effort has been used as a measure of the cost of reproduction. Here we examine the cost of reproduction for males and females in the dung beetle *Onthophagus binodis*. Like many onthophagines, *O. binodis* exhibit dimorphic male morphology; major males develop a large pronotal horn while minor males remain hornless. Alternative morphologies are associated with alternative reproductive tactics. Thus, we ask whether major and minor males pay different costs of reproduction. We found that in contrast to previous work on Diptera, mating is not costly in terms of reduced longevity for female dung beetles. Despite a longevity cost of reproduction for males, we found no evidence for differential longevity costs associated with alternative reproductive tactics.

© 2003 Elsevier Ltd. All rights reserved.

Keywords: Alternative reproductive tactics; Cost of courtship; Cost of mating; Cost of reproduction; *Onthophagus* dung beetles

1. Introduction

Life history theory predicts a trade-off between traits that influence individual fitness, such as current and future reproduction (Williams, 1966; Partridge and Harvey, 1985, 1988; Reznick, 1985; Bell and Koufopanou, 1986; Roff, 1992; Stearns, 1992). Longevity is closely related to the opportunity for future reproduction, and thus decreased longevity in response to current reproductive effort has been used as a measure of the cost of reproduction. There is now good evidence that reproduction reduces female longevity and is thus costly for females (Maynard Smith, 1956, 1958; Partridge et al., 1986, 1987; de Souza Santos and Begon, 1987; Gustafsson and Sutherland, 1988; Fowler and Partridge, 1989; Gustafsson and Pärt, 1990; Sivinski, 1993; Clutton-Brock and Langley, 1997; Mangan, 1997; Chapman et al., 1998; Westendorp and Kirkwood, 1998; Hunt et al., 2002). However, the cost of reproduction for females may accumulate at four different stages: courtship and/or harassment by males, copulation, egg production, and maternal care. With the notable exception of work on *Drosophila melanogaster*, relatively few studies have attempted to evaluate the separate contributions of these different components to the overall cost of reproduction. There is limited evidence that male courtship can be costly for females. In *D. melanogaster* and the tsetse fly *Glossina morsitans*, females exposed to courting males but not allowed to mate suffered reduced longevity (Partridge and Fowler, 1990; Clutton-Brock and Langley, 1997). In contrast, costs of copulation for females, and the proximate causes for these costs, have been demonstrated in a number of species. In the seed beetle *Callosobruchus maculatus* and the bed bug *Cimex lectularius*, reduction in female longevity is caused by physical injuries inflicted by the male’s aedeagus during copulation (Crudgington and Siva-Jothy, 2000; Stutt and...
Siva-Jothy, 2001). In *D. melanogaster* there is a reduction in female longevity due to copulation, but in this case the cost arises due to the harmful effects of male seminal fluid products (Fowler and Partridge, 1989; Chapman et al., 1995). In the Mediterranean fruit fly *Ceratitis capitata* there is also a cost of copulation that is independent of the cost of egg production (Chapman et al., 1998). Nevertheless, there are many studies in which a cost of copulation has not been found (see Table 1 in Chapman et al., 1998), and indeed, in many species seminal fluid products received during copulation can increase female lifespan (for a review see Simmons, 2001).

Evidence for a cost of egg production comes mainly from *D. melanogaster*, where egg production has been shown to decrease female longevity independently of other costs (Maynard Smith, 1956, 1958; Partridge et al., 1987). Similarly, there is an independent cost of egg production in *C. capitata* (Chapman et al., 1998). Finally, where it occurs, maternal care may also incur costs (Lindén and Möller, 1989; Clutton-Brock, 1991; Visser and Lessells, 2001). In the dung beetle, *Onthophagus taurus*, for example, females provide their offspring with provisions for larval growth and development. Females that provision broods alone have a reduced longevity compared with females that receive assistance from males at this stage of reproduction (Hunt et al., 2002).

Despite this obvious wealth of research on the cost of reproduction for females, there have been very few studies that have looked at the cost of reproduction for males. The major components of reproduction for males include sperm production, male contest competition, courtship, copulation, and more rarely paternal care. Early research on the nematode *Caenorhabditis elegans* concluded that sperm production decreased lifespan in males, based on the observation that mutant spermless males had longer lifespans than normal males (Van Voorhies, 1992). However, subsequent work by Gems and Riddle (1996) failed to support this finding. Although mating, independent of egg production, decreased lifespan of hermaphrodites, males were unaffected by mating suggesting that the longer lifespan of mutant males in Van Voorhies study arose from pleiotropic effects of the mutation that affects sperm production.

Reproduction has been shown to reduce lifespan for male *D. melanogaster* (Partridge and Farguhar, 1981; Partridge and Andrews, 1985; Prowse and Partridge, 1997) with the main cost arising from courtship (Cordts and Partridge, 1996). A significant cost of courtship has similarly been found for male *G. morsitans* (Clutton-Brock and Langley, 1997) and the drumming spider *Hygrolycosa rubrofasciata* (Kotiaho et al., 1998; Kotiaho, 2000). The paucity of empirical evidence for costs associated with mate attraction and/or courtship is surprising, given that most theories of sexual selection via female choice are based on the requirement that sexual advertisement should be costly so that it trades off against other components of fitness (Andersson, 1994; Johnstone, 1995). The distinction between expenditure and costs in the sexual selection literature has often been neglected, and on many occasions the evidence that sexual advertisement causes evolutionarily significant costs is at best circumstantial (Kotiaho, 2001). Male contest competition is also a significant aspect of sexual selection, and can have deleterious consequences for male lifespan (reviewed in Gaskin et al., 2002). Finally, some studies of biparentally caring birds have revealed reductions in male survival associated with paternal care while others have not (for review see Lindén and Möller, 1989).

Here we examine the cost of reproduction for males and females in the dung beetle *Onthophagus binodis*. Like many onthophagine, *O. binodis* exhibit dimorphic male morphology; major males develop a large pronotal horn while minor males remain hornless (Cook, 1987; Simmons et al., 1999). Dimorphic male morphology is associated with alternative mating tactics. As with other onthophagine (Lee and Peng, 1981; Sowig, 1996; Emlen, 1997; Hunt and Simmons, 1998; Moczek and Emlen, 2000) major male *O. binodis* compete for access to females and assist them in the production of brood masses. In contrast, minor males sneak copulations with females guarded by major males (Cook, 1988a, 1990). Moreover, there is an asymmetry in sperm competition risk. Minor males are always subject to sperm competition from major males because of their sneaking tactic. Major males are subject to a lower risk of sperm competition, depending on the probability of sneak matings occurring. In accord with sperm competition theory (Parker, 1990) minor males have a greater expenditure on the ejaculate, investing more of their body resources into testes and producing larger ejaculates (Simmons et al., 1999). Thus, because the two male morphs exhibit very different modes of reproduction, we ask whether they differ in the cost of reproduction incurred.

2. Materials and methods

In this study we used laboratory reared F1 offspring from parents collected from a field population in Walpole, south Western Australia, in January 1998. Adults were maintained in large mixed sex population cultures for a period of 10 days before females were established individually in breeding chambers (PVC piping, 30 cm in length and 9 cm in diameter, three quarters filled with moist sand topped with 250 ml of cow dung) to construct brood masses. A single egg is laid into each brood mass which constitutes the resources available for the growth and development of the larva to adulthood. Breeding chambers were sieved after 10 days and batches of c. 50
brood masses were buried in moist sand in 10 l plastic containers held under constant laboratory conditions of $28 \pm 2$ °C and 14:10 h light:dark photoperiod. After 20 days, the containers were checked daily for emerging beetles and sexes were separated at emergence.

From these virgin F1 offspring we established six populations of beetles, two with both sexes (mating treatment), two with females only (female control treatment), and two with males only (male control treatment). Two populations with both sexes consisted of 28 minor and 22 major males and 50 females, two populations with males only consisted of 28 minor and 22 major males and two populations with females only consisted of 50 females. The ratio of minor to major males was chosen to reflect that of natural populations, with morphs recognised on the basis of pronotum width; males larger than 5.80 mm were categorised as major males (Simmons et al., 1999). The ages of the beetles within a single population were matched such that the variance in age was between 3 and 5 days. Mean (±SE) pronotum width of major males, minor males and females in our experiment were 6.18 ± 0.02, 5.35 ± 0.03 and 5.75 ± 0.04 mm, respectively and all were significantly different from each other ($F_{2296} = 178.39, P < 0.001$; Tukey MD > 40.0, $P < 0.001$ for all comparisons).

Each population was housed in a 15 l bucket, two thirds filled with sand and topped with 1 l of cow dung per 50 individuals. Beetle density was thus set at 1 beetle/20 ml of dung. Although the numbers of beetles per bucket differed between our treatments, the population densities used in our experiments were reflective of natural populations; up to 1000 beetles have been recorded arriving at bated pitfall traps within 24 h (Ridsdill Smith and Hall, 1984; Ridsdill Smith and Matthiessen, 1988) and population counts from natural dung pads in the field have been reported to range from 13 to 149 (mean 65 ± 35, $n = 25$; Cook, 1988b) and can be much higher at the peak of the breeding season (personal observation). More importantly, Ridsdill Smith et al. (1982) examined the effect of population density on adult survival across populations of 2, 6, 8, 10, 20, 30, 60, and 100 beetles housed, as in our experiments, in 15l containers provided with 1 l of cow dung. They found no significant influence of beetle density on longevity. If anything, high density populations tended to have a higher survival than low density populations (populations of 60 had 28% at 12 weeks vs. 46% for populations of 100, see Table 1 in Ridsdill Smith et al., 1982) so that any potential reductions in survival in our mating treatment compared to our controls are likely to be conservative estimates.

Every week during the experiment, dung and sand were changed and the survival of majors, minors and females recorded. The amount of dung available was not sufficient to allow beetles to construct brood masses. Thus, the experimental design tests for cost of reproduction for males pooling costs of sperm production, courtship, contest competition and mating. For females the experiment tests for the pooled costs of male courtship and mating.

### 3. Results

First we analysed whether there was any heterogeneity in survival patterns between the populations that had received the same treatments. Between the two replicates of populations that received the mating treatment, there was no significant heterogeneity in male or female survival patterns (survival rates estimated with Kaplan-Meyer method and their significance tested with Log rank test; Log rank = 2.29, df = 1, $P = 0.130$ and Log rank = 2.66, df = 1, $P = 0.103$, respectively). Similarly there was no significant heterogeneity in survival patterns between the two replicates of control populations for males or females (Log rank = 0.39, df = 1, $P = 0.533$ and Log rank = 0.55, df = 1, $P = 0.457$, respectively). Because of the homogeneity between the replicates of each treatment, replicates were pooled for further analysis.

For males there was a significant difference in survival patterns between the treatment and the control (Log rank = 8.08, df = 1, $P = 0.005$) (Table 1). This difference indicates that reproduction carries a cost for males in terms of reduced longevity (Fig. 1a). However, for females there was no significant difference in survival patterns between the treatment and the control (Log rank = 0.08, df = 1, $P = 0.777$). This indicates that male courtship and mating does not carry a longevity cost for females (Fig. 1b).

We found no evidence that male survival patterns were dependent on male morph. There was no significant interaction term between male morph and treatment on male survival (Cox regression, Wald = 0.50, df = 1, $P = 0.480$), and there was no significant main effect of morph on male survival (Log rank = 0.09, df = 1, $P = 0.765$).

#### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males in treatment</td>
<td>54.8</td>
<td>2.5</td>
</tr>
<tr>
<td>Males in control</td>
<td>62.6</td>
<td>2.7</td>
</tr>
<tr>
<td>Females in treatment</td>
<td>55.2</td>
<td>2.7</td>
</tr>
<tr>
<td>Females in control</td>
<td>54.7</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Fig. 1. Survival of the (a) males, (b) females and (c) the two morphs of the males. In (a) and (b) solid line represents survival of the populations that were housed in mixed sex groups and dashed line populations that were housed in single sex groups and in (c) solid line represents survival of minor males and dashed line survival of the major males.

4. Discussion

In contrast to work on the flies, *Drosophila* and *Ceratitis* (Fowler and Partridge, 1989; Chapman et al., 1995, 1998), our experiments have revealed that female dung beetles, *Onthophagus binodis*, do not suffer a cost of reproduction from courtship and/or mating. In general, existing data on the effect of mating on female lifespan fail to reveal any consistent pattern. Chapman et al. (1998) reviewed the literature for Diptera and found that in 10 species mating decreased lifespan and in 9 species it had no effect on lifespan. They point out that carefully controlled large scale or replicated experiments are required to show that mating can reduce survival. Our study provides such a test, and fails to show a cost of mating. Moreover, across a broader range of insect orders, mating, and the receipt of seminal fluids, can have significant positive influences on female lifespan, because nutrients contained in ejaculates can be utilised by females for egg production and contribute to somatic maintenance (see review in Simmons, 2001). The cost of mating seen in *Drosophila* females, and perhaps some other Diptera, may arise from the mechanism of sperm competition where toxic seminal peptides facilitate the displacement and/or incapacitation of sperm stored from previous males (Chapman et al., 1995; Wolfner et al., 1997; Price et al., 1999; Civetta and Clark, 2000).

In contrast, egg production and brood provisioning do appear to be costly for female dung beetles. In their study of *O. taurus*, Hunt et al. (2002) found that increased access to dung resulted in an increase in the number of brood masses produced by females, and in a decline in female longevity. Moreover, brood provisioning represented a cost of reproduction that was independent of egg production, because females assisted by major males had a reduced cost compared with females breeding alone. Egg production and maternal care appear to be general, widespread costs of reproduction for females (Maynard Smith, 1956, 1958; Partridge et al., 1987; Lindén and Møller, 1989; Clutton-Brock, 1991; Visser and Lessells, 2001).

In contrast to our result for females, we found that male dung beetles housed with females suffered a reduced longevity. This cost of reproduction for males may arise from costs associated with courtship, contest competition, mating, or sperm production. Work on both *Drosophila* and tsetse flies suggests that it is courtship that has the greatest adverse effect on lifespan (Cords and Partridge, 1996; Clutton-Brock and Langley, 1997). Courtship does appear to be energetically expensive for male dung beetles, including *O. binodis*. During courtship males tap the female’s back with their head and forelegs (Cook, 1990; Kotiaho, 2002), and the rate of tapping is dependent on a male’s nutritional status (Kotiaho, 2002). Moreover, courtship rate in onthophagines seems important in male mating success, exhibiting genotypic variation that is dependent on heritable male condition (Kotiaho et al., 2001; Kotiaho, 2002). If the major cost of exposure to females comes from the costs of courtship, models of sexual selection under condition dependence predict that the marginal cost of exposure to females revealed in our experiments should be lower for males of high phenotypic and genotypic condition, despite their higher rate of courtship, as found for drumming spiders (Kotiaho, 2000). In our experiments the
density of beetles in each treatment was set at 1 beetle per 20 ml of dung. Because there were 50 single sex or 100 mixed sex individuals per 15 l bucket, it could be argued that the density between the treatments differed so that an alternative explanation for the effect of the treatment on male longevity could be an adverse effect of higher population density. However, earlier studies examining the effects of density on longevity have found the opposite tendency, that in high densities beetles live longer than in low densities (Ridsdill Smith et al., 1982). Moreover, the argument that higher density in mixed sex populations caused the observed reduction in male longevity relies on the assumption that males are more susceptible to assumed detrimental effects of density than are females, or that females benefit more from higher densities than do males. We know of no evidence for sex differences in the effects of density on survival.

We found no difference in lifespan between alternative mating tactics; major and minor males suffered the same reduction in lifespan when exposed to females. Minor males tend to have a lower courtship rate than major males (Cook, 1990; Kotiaho, 2002). More significantly however, minor males have larger testes than major males, and transfer larger ejaculates to females (Simmons et al., 1999; Tomkins and Simmons, 2002). Given that minor males do not suffer reduced longevity compared with major males, these data might imply that ejaculate production does not represent a significant cost for males. Or alternatively, that minor males trade off increased ejaculate expenditure with reduced courtship rate so that longevity is unaffected. However, Prowse and Partidge (1997) have shown that for Drosophila, reproduction has a much more marked effect on fertility than survival. Male Drosophila which were exposed to females showed complete and irreversible sterility at a time when more than 80% of their cohort were still alive. Thus, for Drosophila, reduced fertility represents a much greater cost of reproduction than reduced survival. It may be that the greater expenditure on the ejaculate by minor male O. binodis is associated with a greater sterility cost of reproduction, so that it is currently premature to claim that alternative mating tactics are not associated with differential costs of reproduction. Certainly differences in mate acquisition tactics, sneaking vs. fighting and guarding, do not appear to be associated with differences in lifespan.

Acknowledgements

We thank Julie Wernham for help in the laboratory, the Cooper family in Walpole for access to their farm to collect beetles and the McKay family in Serpentine for access to their farm to collect dung. This research was supported by the Academy of Finland to JSK and by the Australian Research Council to LWS.

References
