Cost of reproduction in Callosobruchus maculatus: effects of mating on male longevity and the effect of male mating status on female longevity

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Received 19 April 2005; received in revised form 27 June 2005; accepted 28 June 2005

Abstract

One of the most studied life-history trade-offs is that resulting from the cost of reproduction: a trade-off arises when reproduction diverts limited resources from other life-history traits. We examine the cost of reproduction in male, and the effect of male mating status on female Callosobruchus maculatus seed beetles. Cost of reproduction for male C. maculatus was manifested as reduced longevity. There was also a positive relationship between male body size and male longevity. Females mated to males that had already copulated twice did not live as long as females mated to males that had copulated once or not at all. The third copulation of males also lasted longer than the two previous ones. We conclude that even though the cost of reproduction for males has been studied much less than that in females, there is growing evidence that male reproductive effort is more complex than has traditionally been thought.

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Keywords: Bruchidae; Copulation; Cost of mating; Longevity; Seed beetle; Trade-off

1. Introduction

Evolution of life-histories is moulded by trade-offs between traits that have an influence on fitness (Williams, 1966; Reznick, 1985; Roff, 1992; Tatar et al., 1993; Messina and Slade, 1999; Koivula et al., 2003). One of the most studied trade-off situations is that resulting from the cost of reproduction, in which a trade-off arises when investment to reproduction reduces longevity, growth, future fecundity or for example immune function (Williams, 1966; Partridge and Harvey, 1985; Fowler and Partridge, 1989; Roff, 1992; Siva-Jothy et al., 1998; McKean and Nunney, 2001; Roff and Siva-Jothy, 2002; Kotiaho and Simmons, 2003; Messina and Fry, 2003). Most often trade-offs arise because organisms have a limited pool of resources (Fowler and Partridge, 1989; Roff, 1992); virtually everything in the life-history of an organism consumes resources and a decision to allocate resources into one life-history trait, e.g. into reproduction, simultaneously precludes the opportunity to allocate those resources into other life-history traits.

Reproduction incurs costs for both females and males, and for females, costs of reproduction are often documented (e.g. Fowler and Partridge, 1989; Tatar et al., 1993; Westendorp and Kirkwood, 1998; Messina and Slade, 1999). Overall, the cost of reproduction for females can be composed of several factors including tolerating harassment and courtship by males, copulation, harmful effects of male seminal products, producing eggs, pregnancy, giving birth, parental care or, as is most likely, some combination of these factors (e.g. Roff, 1992; Kotiaho and Simmons, 2003). For example, the greatest reproductive effort for the dung beetle

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Onthophagus taurus females is parental care (Hunt et al, 2002). Cost of reproduction may also be mediated from other mechanisms that are not directly related to individuals own reproductive effort. For example, if female and male interests over mating do not coincide, there may develop a conflict over mating increasing the cost of reproduction (Chapman et al., 1995; Clutton-Brock and Parker, 1995; Crudgington and Siva-Jothy, 2000; Dobson et al., 2000; Johnstone and Keller, 2000; Simmons, 2001; Royle et al., 2002). Despite the fact that reproduction consumes resources, in some cases reproductive activities may also provide resource benefits. For example, females may receive nutrients as nuptial gifts, and these nutrients can be used in producing the progeny (e.g. Thornhill, 1976; Wedell, 1993; Reinhold, 1999). In optimal situation, there is a balance between benefits and costs of reproduction that maximizes the fitness of the reproducing individual (Reznick, 1985).

The cost of reproduction for males on the other hand, has been studied much less than that in females. Historically, it was assumed that sire invests in offspring only by copulating and donating cheap sperm with its genes (see Simmons, 2001). However, there is evidence from D. melanogaster fruit flies and Hydrolycosa rubrofasciata wolf spiders that, for example, courtship alone is capable of reducing the longevity of males (Cordts and Partridge, 1996; Mappes et al., 1996; Kotiaho, 2000, see also Kotiaho, 2001). Indeed, now it is recognized that investing resources into offspring may be an important strategy also for males (Wedell and Karlsson, 2003). There is also growing evidence that male reproductive effort is more complex than has traditionally been thought. For instance, males may invest in mate choice, preferring as mates those females that signal their receptive reproductive state (LeBas and Marshall, 2000). Besides sperm production and copulation, also contests between males, courtship and parental care are components of male reproduction, all potentially causing a cost of reproduction for males (Partridge and Farquhar, 1981; Van Voorhies, 1992; Clutton-Brock and Langley, 1997; Siva-Jothy et al., 1998; Kotiaho, 2001; Kotiaho and Simmons, 2003). For example, when the cost of reproduction is considered, it has not always been clear that cost of sexual signalling i.e. cost of courtship forms a part of the cost of reproduction.

We used Callosobruchus maculatus seed beetles in our experiments. In this species adults do not need nutrition or water to reproduce but collect all necessary energy reserves during their larval stage (e.g. Fox and Tatar, 1994). Thus, at emergence these beetles have a fixed amount of energy to allocate into different activities during their adult life. This makes the species ideal for manipulative studies of trade-offs. Because the beetles do not eat as adults, the energy reserves that are used for reproduction can not be compensated, and thus energy loss should be seen as a trade-off with some other fitness component.

For C. maculatus females, reproduction is costly in terms of reduced longevity (e.g. Fox, 1993; Messina and Slade, 1999). The cost is composed of copulation and egg production but it has also been suggested that mating causes damages that may contribute to the cost of reproduction (Crudgington and Siva-Jothy, 2000). Here we report two experiments examining the cost of reproduction for male C. maculatus. Our aim was to determine whether reproduction is trading off with male longevity and to examine whether male mating status has an effect on longevity of its partner.

2. Materials and methods

2.1. C. maculatus

C. maculatus is a post-harvest pest of legumes in tropical regions. This species uses Vigna-beans, e.g. black-eye beans (V. unguiculata, also known as cowpea) and mung beans (V. radiata) as nutrition during their larval stage. Females lay eggs singly on beans, and 4–5 days later, first instar larvae burrow themselves inside the bean, where they develop into adults in about 25–40 days (Fox, 1993; Fox and Tatar, 1994; Savalli and Fox, 1998). However, development time is strongly dependent on environmental factors, such as temperature. Both sexes can copulate soon after emerging and both sexes can copulate several times during their life time.

The population used in our experiment was derived from a laboratory culture that was originally established from animals collected from Brazil. This population has been maintained on black-eye beans (V. unguiculata) in the laboratory since 1982.

Here we report results from two experiments. For both experiments, we collected beans with a single egg from our laboratory population. By using beans with only one egg, we avoided larval competition inside the bean (Colegrave, 1993; Horng, 1997). Each single-egg bean was labelled and placed individually into a pierced 1.5 ml Eppendorf tube. When the beetle emerged, the bean was removed from the tube. We used pronotum width as a measure of body size. During the experiment, beetles were kept in 20 °C in the laboratory, where light conditions were 15L: 9D.

2.2. Experiment 1

In the first experiment, we determined the effect of reproduction on the longevity of the males. We also examined the effect of male mating status, i.e. the effect of number of earlier matings (0, 1 or 2), on the fecundity and longevity of the males’ partner, and on the duration of copulation.
Emergence of individuals was observed daily. We allocated 50 males into a mating group and 89 males into a control group. In total there were also 150 females in the experiment. Mating group males were mated once in every three successive days, altogether three times. Males were 3–6 days old at the first mating. Mating group males were mated by placing them together with a 1–5 day old virgin female into a pierced Eppendorf tube, one pair into each tube. The duration of copulation was recorded. After copulation males were returned into their original tubes, and females were placed into 0.1l containers (+4.5cm, height 6.5cm) with 100 black-eye beans on which to lay eggs. Each female was mated only once. Control group males were handled as mating group males, but they were not introduced to females. Adult longevity of each beetle from emergence to death was observed daily. After death, pronotum width of the mating group males was measured to the nearest 0.01mm using a dissecting microscope.

2.3. Experiment 2

In the second experiment, we examined the effect of reproduction on the longevity of the males, and the effect of male mating status on the longevity of his partner. The second experiment was conducted with a larger sample size to confirm the results observed in the first experiment. In addition, to verify the effect of male mating status on the longevity of the males’ partner, we now prevented females from laying eggs. Emergence of individuals was observed daily. In this experiment we had 537 males as a mating group and 658 males as a control group. We also had 1611 females. Mating group males were mated three times with a 1–5 day old virgin female on three successive days, once with each female. Males were mated for the first time 4–6 days after their emergence. Mating procedures followed the ones described for experiment 1, except that the duration of copulation was not recorded. Each female was mated only once, but now they were prevented from laying eggs. C. maculatus females lay some eggs even if there are no natural oviposition sites (beans) available. However, excluding beans substantially reduces the egg laying (Wilson and Hill, 1989; own observation). Adult longevity of each beetle from emergence to death was observed daily. After death, pronotum width of all individuals was measured to the nearest 0.01 mm using a dissecting microscope.

Statistical analyses were conducted with SPSS statistical software, version 12.0.1. Effect sizes estimate the magnitude of a relationship between two factors and have been calculated according to Rosenthal (1991). Population parameters have been presented in Table 1.

3. Results

3.1. Experiment 1

Males in the control group lived longer than males in the mating group (Cox regression, Wald = 17.38; d.f. = 1; P<0.001, Fig. 1a). There was no effect of body size on male longevity (linear regression: \( t_{46} = 0.73; N = 48; P = 0.471 \); effect size, Pearson correlation coefficient: \( r = 0.08, \) Fig. 2a).

In this first experiment we determined also the effects of male mating status and male identity on the duration of copulation. Male mating status had an effect on the duration of copulation (Table 2), such that the third copulation of the male lasted longer than the first or second, but there was no difference between the first and the second copulation (Table 3). Also male identity tended to have an effect on the duration of copulation (Table 2).

Next we analysed the effect of male mating status and identity on the longevity and egg production of his mate. Male mating status had an effect on the longevity of his mate such that the female that was the third mate of the male lived a shorter time than the first or the second mate, but there was no difference between longevities of the first two mates (Tables 3 and 4). Male identity had also an effect on the longevity of his mate (Table 4), but the duration of copulation had no effect (Table 4). However, there tended to be an interaction between male mating status and the duration of copulation (Table 4). In a separate analysis we determined the effect

<table>
<thead>
<tr>
<th>Group</th>
<th>Longevity (d)</th>
<th>Pronotum width (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  s.d.</td>
<td>Median  n</td>
</tr>
<tr>
<td>Exp. 1 mated males</td>
<td>22.64 5.03 22 50</td>
<td>1.35 0.056 1.36 50</td>
</tr>
<tr>
<td>Exp. 1 control males</td>
<td>26.87 5.98 26 89</td>
<td>1.35 0.062 1.36 536</td>
</tr>
<tr>
<td>Exp. 1 females</td>
<td>17.30 2.93 17 132</td>
<td>1.34 0.060 1.34 658</td>
</tr>
<tr>
<td>Exp. 2 mated males</td>
<td>24.00 4.62 24 502</td>
<td>1.41 0.066 1.42 1147</td>
</tr>
<tr>
<td>Exp. 2 control males</td>
<td>28.72 5.19 29 623</td>
<td></td>
</tr>
<tr>
<td>Exp. 2 females</td>
<td>29.85 6.49 30 1101</td>
<td></td>
</tr>
</tbody>
</table>
of female fecundity on female longevity but found no effect (linear regression, \( t_{72} = -1.03; \ N = 74; \ P = 0.309 \)).

Male mating status (ANOVA, \( F_{2,30} = 0.34; \ P = 0.717 \)) or male identity (\( F_{26,30} = 0.87; \ P = 0.636 \)) had no effect on their mate’s egg production. However,
between male body size and treatment on male longevity (Wald = 0.14, d.f. = 1; P = 0.47), but body size had a positive effect on longevity (Wald = 225.65, d.f. = 1, P<0.001; effect size: r = 0.17; Fig. 2c and d). Also females that were larger lived longer (Cox regression, Wald = 25.50; d.f. = 1; P<0.001; effect size: r = 0.20; Fig. 2b). However, even though it was significant, the effect of body size on longevity of males was moderate at the most (males of experiments 1 and 2: combined effect size: r = 0.16).

Also in this second experiment we analysed the effect of male mating status and male identity on their mate’s longevity. Interestingly, now there was no effect of male mating status (ANOVA, F_{2,548} = 0.00; P = 0.998) or male identity (F_{344,548} = 1.08; P = 0.200) on his mate’s longevity. There was also no correlation between male body size and longevity of his mate (Pearson correlation: r = 0.01, N = 478; P = 0.883).

4. Discussion

A cost of reproduction for male C. maculatus was manifested as reduced longevity for three-times mated males when compared to non-mated males. We expect that ejaculate formation could be the cause of the cost. This is because males lose between 5–10% of their body mass in their first ejaculate (Fox et al., 1995; Savalli and Fox, 1999; Paukku, Kotiaho and Tomkins, unpublished data). Thus, depending on what the ejaculate is composed of, males lose either a large amount of their limited energy reserves or water.

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Table 2
Mixed model analysis of variance for duration of copulation (Experiment 1)

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mating status</td>
<td>1028722.24</td>
<td>2</td>
<td>514361.12</td>
<td>16.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Male identity</td>
<td>2205405.22</td>
<td>48</td>
<td>45945.94</td>
<td>1.47</td>
<td>0.064</td>
</tr>
<tr>
<td>Error</td>
<td>2504487.76</td>
<td>80</td>
<td>31396.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Male identity was entered as a random factor.

Table 3
Tukey test for the effects of male mating status on the duration of copulation and female longevity

<table>
<thead>
<tr>
<th>Male mating status</th>
<th>Male mating status</th>
<th>Duration of copulation</th>
<th>Female longevity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0–2) (J)</td>
<td>(0–2) (J)</td>
<td>Mean difference (I–J)</td>
<td>Mean difference (I–J)</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>−6.99</td>
<td>0.38</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>−210.00</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>−203.01</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4
Mixed model analysis of covariance for the longevity of male’s mate (Experiment 1)

<table>
<thead>
<tr>
<th></th>
<th>SS</th>
<th>d.f.</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copula duration</td>
<td>0.59</td>
<td>1</td>
<td>0.59</td>
<td>0.11</td>
<td>0.744</td>
</tr>
<tr>
<td>Male mating status</td>
<td>63.57</td>
<td>2</td>
<td>31.79</td>
<td>5.75</td>
<td>0.005</td>
</tr>
<tr>
<td>Male identity</td>
<td>515.52</td>
<td>48</td>
<td>10.74</td>
<td>1.94</td>
<td>0.005</td>
</tr>
<tr>
<td>Male mating status &amp; copula duration</td>
<td>31.18</td>
<td>2</td>
<td>15.59</td>
<td>2.82</td>
<td>0.066</td>
</tr>
<tr>
<td>Error</td>
<td>425.90</td>
<td>77</td>
<td>5.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Copula duration was entered as a covariate and male identity was entered as a random factor. Interaction between male identity and the duration of copulation was eliminated with values F_{48,26} = 0.84; P = 0.711.

there was a positive correlation between male body size and the number of eggs produced by mates of the male (Pearson correlation: r = 0.38; N = 41; P = 0.016) (Fig. 3) as well as between male body size and the duration of copulation (r = −0.38; N = 49; P = 0.007). However, there was no correlation between duration of copulation and egg production (r = 0.09; N = 74; P = 0.468) or male body size and longevity of his mate (r = −0.17; N = 45; P = 0.257).

3.2. Experiment 2

Males in the control group lived longer than males in the mating group (Cox regression, Wald = 29.36, d.f. = 1, P<0.001, Fig. 1b). There was no interaction between male body size and treatment on male longevity (Wald = 0.14, d.f. = 1; P = 0.47), but body size had a positive effect on longevity (Wald = 225.65, d.f. = 1, P<0.001; effect size: r = 0.17; Fig. 2c and d). Also females that were larger lived longer (Cox regression, Wald = 25.50; d.f. = 1; P<0.001; effect size: r = 0.20; Fig. 2b). However, even though it was significant, the effect of body size on longevity of males was moderate at the most (males of experiments 1 and 2: combined effect size: r = 0.16).

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even though the cost of reproduction as reduced longevity has not been previously reported for male {\textit{C. maculatus}}, cost of reproduction is often documented in female {\textit{C. maculatus}}. For example, Messina and Fry (2003) showed that egg laying reduced the longevity of {\textit{C. maculatus}} females. However, interestingly in our experiment female longevity was not strongly related to her fecundity. Therefore, it may be that the cost of reproduction is not so straightforward even for females. Besides a female and her decisions, a male and his mating history may have an effect on the longevity of the female; in our first experiment male identity and his mating history had an effect on female longevity.

There are also other factors that may affect female longevity. For example, in {\textit{C. maculatus}} multiple mating increases female longevity (Fox, 1993; Fox et al., 1995, but see Savalli and Fox, 1999;Crudgington and Siva-Jothy, 2000), but it seems that females benefit from multiple mating only when they are maintained without water and nutrition, as is the norm in {\textit{Callosobruchus}} experiments (Fox, 1993). Furthermore, availability of oviposition substrates (beans) decreases female longevity (Messina and Fry, 2003) while availability of nutrition (food) increases female longevity (Tatar and Carey, 1995). Thus, when studying the cost of reproduction it seems that very many factors must be taken into consideration before general conclusions about the exact nature of the costs can be made.

Our results may be used as an example of the above difficulty. Male mating history had an effect on the longevity of his mate such that the female that was the third mate of the male lived a shorter time than the first or the second mate, but there was no difference between longevities of the first two mates. In this experiment the male’s third copulation was also longer than the first copulation. Because males may damage females during copulation (Crudgington and Siva-Jothy, 2000), our results could be interpreted as evidence of the cost of reproduction in terms of increased damage depending on the duration of the copulation. However, further analyses suggested that, in fact, the duration of the copulation had no independent effect on the longevity of the females. Thus, it seems difficult to explain the reduced longevity of the female that was the third mate of the male as a cost of reproduction.

On the other hand, it has been suggested that in {\textit{C. maculatus}} the ejaculate is composed of nutritious liquid (Savalli and Fox, 1998, but see Wilson et al., 1999) and it is known that the first ejaculate of a virgin {\textit{C. maculatus}} male is larger than the ones produced subsequently (Savalli and Fox, 1999; Paukku, Kotiaho and Tomkins, unpublished data). Therefore, it could be that our result arises because females, that were the first or second mates of a male, received extra energy thus allowing them to live longer. However, we suspect that the longevity benefit of females receiving larger ejaculates could also arise simply because of the larger quantity of water the females receive. In our lab {\textit{C. maculatus}} lives in a dry environment and extra water from a larger ejaculate may be a large benefit for females. Therefore, an alternative explanation to the apparent cost of reproduction for the females who were mates in the third copulation of the male, could be that they received less benefits from the smaller ejaculate. This explanation is in accord with results from earlier studies in {\textit{C. maculatus}}, where females live longer if they receive multiple ejaculates (Fox, 1993; Fox et al., 1995; Savalli and Fox, 1999).

In this paper we reported results from two experiments investigating partially the same questions. In the first experiment females were allowed to lay eggs while in the second one they were prevented from laying eggs. In the first experiment, male mating status had an effect on the longevity of his mate: females that were the third mates of the males died sooner than females that were the first or second mates of the male. Interestingly, in the second experiment where females were prevented from laying eggs, male mating status had no effect on longevity of his mate. Thus, it seems that male mating status has an effect on the longevity of his mate only if the female is allowed to lay eggs. This discrepancy suggests that the difference in female longevity may not be simply due to a beneficial increase in energy reserves of the first two mates of a male, as suggested above, but rather, that obtaining additional energy may increase the tolerance of the cost of reproduction of the first two mates of the male in comparison to the third mate.

Acknowledgments

We thank Paul Eady for providing us the beetle culture. We are grateful to Joseph L. Tomkins for greatly improving the manuscript. We also thank Frank Messina and the Monday Coffee Club: Tarmo Ketola, Mikael Puurtinen, Ville Selonen, Tero Toivanen and Katja Tynkkynen for comments on the manuscript and Kari Lahtinen and Mari Vaittinen for help in the laboratory. This study was funded by the Academy of Finland.

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