

# The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*

Martin Edvardsson<sup>a</sup> and David Canal<sup>b</sup>

<sup>a</sup>Animal Ecology/Department of Ecology and Evolution, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, SE 752 36 Uppsala, Sweden and <sup>b</sup>Departamento de Zoología y Antropología Física, Facultad de Ciencias Biológicas, Universidad Complutense de Madrid, José Antonio Novais 2, E-28040 Madrid, Spain

Control over copulation duration is a potentially important generator of sexual conflict that has received little empirical attention. The copulatory behavior of the bruchid beetle *Callosobruchus maculatus* may reflect a sexual conflict over copulation duration. Males have spines on their intromittent organs that puncture the female reproductive tract, and females kick their mates during copulation. If females are prevented from kicking, copulations last longer and the injuries females sustain are more severe. Males supposedly use the spines as anchors to prolong copulation duration, and females kick to terminate copulations. We manipulated copulation duration experimentally and quantified its effects on male and female fitness components to test whether or not there is a conflict over copulation duration in *C. maculatus*. Females did not suffer from long copulations but instead experienced increased lifetime fecundity. Ejaculate size increased with copulation duration, and females apparently derive material benefits from the ejaculates. Males that mated first and had long copulations were relatively unsuccessful when competing with sperm from other males. However, there was a trend for female remating propensity to decrease with long copulation durations, and first males may therefore also benefit from long copulations. The copulation duration of the second male to mate did not have a significant effect on sperm precedence. We conclude that even though it seems likely that the male spines have evolved to act as an anchor during copulation, there seems to be little conflict over copulation duration per se in *C. maculatus*. **Key words:** *Callosobruchus maculatus*, copulation duration, ejaculate, harmful male traits, mating costs, nuptial gifts, sexual conflict, sperm competition. [*Behav Ecol* 17:430–434 (2006)]

Conflicting evolutionary interests of individual males and females appear to be a common and important driving force behind the evolution of traits involved in reproduction (Chapman et al., 2003; Parker, 1979; Partridge and Hurst, 1998). The underlying reason for these sexual conflicts is that the optimal outcome of an interaction between potential mates often differs between males and females. As a consequence, male traits that manipulate female reproductive processes can evolve even if they are harmful to females. This may lead to sexually antagonistic coevolution with males evolving traits to manipulate females and females evolving resistance to male traits (Holland and Rice, 1998; Parker, 1979). Traits that have been suggested, and in some cases shown, to be involved in sexual conflicts include mating rate, egg production, and female remating propensity (Chapman et al., 2003; Parker, 1979; Partridge and Hurst, 1998; Rice, 2000). One example that has received little empirical attention is copulation duration.

Copulation duration can have important effects on male and female fitness. For females, it may be important that copulations last long enough for them to receive sufficient amounts of sperm. In addition, even though the empirical evidence supporting substantial benefits to females from nuptial gifts is still inconclusive (Arnqvist and Nilsson, 2000; Vahed, 1998), long copulations may be beneficial to females when males transfer nutrients or feed females during mating (Thornhill, 1976; Vahed, 1998). The costs of mating, such as

time waste, energetic costs, increased predation risk and injuries caused by male behavior, and the substances they transfer, may also increase with copulation duration (Daly, 1978; Simmons, 2001; Watson et al., 1998). Therefore, the payoff from continuing to copulate should diminish rapidly once females have received sufficient numbers of sperm. The situation is somewhat different for males. Faced with female influence over the fate of their sperm and with potential competition from sperm of other males, copulating males typically do much more than just transfer enough sperm to fertilize the eggs of their mates (Eberhard, 1996). Sperm numbers often exceed what females need to fertilize their eggs or even the capacity of their sperm storage organs (Eady, 1995; Eberhard, 1996; Simmons, 2001). Males also transfer substances that affect female remating propensity and rate of offspring production, stimulate females physically, and manipulate sperm from previous matings (see Eberhard, 1996; Simmons, 2001, for reviews). The optimal mating time for males will not only depend on how much time is required for these behaviors but also on the availability of mates and risk of sperm competition (Parker, 1974). Furthermore, some males prolong copulations as a form of mate guarding to keep other males from mating (Parker, 1970; Simmons, 2001). In addition, stimuli provided by copulating males or their ejaculates often trigger a decrease in female receptivity to courting males (Eberhard, 1996). It has been suggested that some males may prolong copulations so that enough time passes for these stimuli to take effect (Parker, 1970). Although this could be of great importance to the evolution of copulatory behavior in many species, this suggestion has received little empirical attention.

In all, it can be expected that males will often benefit from extending copulations beyond what is optimal for females (Simmons, 2001). The resulting conflict may lead to selection

Address correspondence to M. Edvardsson, who is now at the Centre for Conservation and Ecology, School of Biosciences, University of Exeter in Cornwall, Tremough Campus, Penryn, TR10 9EZ, UK. E-mail: martin.edvardsson@ebc.uu.se.

Received 7 April 2005; revised 9 December 2005; accepted 12 January 2006.

on female ability to terminate copulations and on male ability to prolong them (Arnqvist, 1997). This could, in turn, lead to an arms race of sexually antagonistic coevolution with males evolving to become better at prolonging copulations and females becoming better at terminating them.

Copulating females of the beetle *Callosobruchus maculatus* (Coleoptera, Bruchidae) kick their mates vigorously during the last third of copulation (Eady, 1991a; Qi and Burkholder, 1982; Tufton, 1993). Males of this species have spines on their intromittent organs. These spines unfurl during mating, puncture the female reproductive tract, and leave wounds (Crudgington and Siva-Jothy, 2000). If females are prevented from kicking their mates, copulations are prolonged, and the wounds caused by the spines are more extensive (Crudgington, 2001). The female kicking behavior has therefore been interpreted as a way for females to minimize the injuries they sustain during mating by keeping copulations short (Crudgington, 2001). It is unclear why males inflict these injuries, but it has been suggested that males may evolve harmful traits to induce favorable responses, such as delayed remating or increased rate of oviposition, in females (Johnstone and Keller, 2000; Lessells, 1999; Michiels, 1998). However, previous studies have failed to find such effects in *C. maculatus* (Edvardsson and Tregenza, 2005; Morrow et al., 2003). Another possibility is that the male spines are used as an anchor to enable males to remain in copula and not be displaced by other males or dislodged by the kicking females. Copulating males are attached firmly to their mates by their genitalia. It is therefore possible that the female kicking behavior has coevolved with the male spines because of a conflict over copulation duration.

In the present study, we assessed whether or not there is a sexual conflict over copulation duration in *C. maculatus*. We did this by manipulating copulation duration experimentally and quantifying its effects on size of the transferred ejaculate, female lifetime offspring production, female remating propensity, and sperm precedence. These variables are central to the reproductive success of males and females.

## METHODS

*C. maculatus* is a widely distributed pest on legumes. Females attach single eggs to the surface of beans, and the larvae develop inside (Southgate, 1979). If females have access to oviposition substrate, oviposition starts soon after the first mating and occurs continuously thereafter. Females are polyandrous, often mating with several males, but typically have a refractory period after mating when they are not receptive to courting males (Shu et al., 1996). Females that have mated with more than one male produce offspring of mixed paternity with the last male to mate generally fertilizing the majority of the eggs laid subsequently (Eady and Tubman, 1996). Copulating males may therefore compete with sperm from both previous and subsequent matings.

We used the "South India" strain in this study. Our cultures were maintained on black-eyed beans *Vigna unguiculata* and kept at 28°C. Experimental matings were carried out at 21 ± 1°C under diffuse room lighting. All beetles were virgin and 2–4 days posteclosion at the time of their first experimental mating.

Copulating males are attached firmly to females by their genitalia and are virtually impossible to dislodge. However, severing the abdomen of a copulating male with a pair of microscissors will cause the male genitalia to deflate, and it is then not difficult to pull them out of the female. We used this technique to manipulate copulation duration. In all experiments described below, except for the immediate remating experiment, copulating pairs were allowed to mate for 6, 8, or 10 min in petri dishes with a 30-mm diam. Females

usually start to kick after about 6 min at this temperature, and matings normally last for about 8 min (personal observation). To be able to extend matings beyond their normal length, we ablated the hind legs of all females, except the ones used in the immediate remating experiment, thus rendering females unable to kick their mates. Copulations are then prolonged, and the injuries females sustain are more severe (Crudgington, 2001; Edvardsson and Tregenza, 2005). We immobilized females on ice and used a pair of microscissors to cut their hind legs at the midpoint of the tibia. All females, not only the ones assigned to the 10-min treatment, had their hind legs ablated the day before their first mating.

## Rate of oviposition—lifetime offspring production

Females were mated to virgin males and allowed to copulate for 6, 8, or 10 min as described above. Immediately after matings, we transferred the females to petri dishes containing approximately 25 beans. To be able to monitor how oviposition rate changed over the first 24 h after mating, we moved females to new petri dishes with the same number of beans after 3, 6, 9, 12, and 24 h. The females remained in the last (24 h) petri dish until they died. The petri dishes containing ovipositing females and beans were kept at 21 ± 1°C. We counted the number of eggs each female laid by inspecting the beans visually under a dissecting microscope.

## Sperm competition—sperm precedence and remating propensity after 48 h

We conducted two sperm competition experiments to assess any effects of copulation duration on male fertilization success when mating as either the first or second male. In both experiments, virgin females were mated to two virgin males with 48 h separating the two matings. We used the sterile male technique to determine paternity (Boorman and Parker, 1976; Parker, 1970). One of the two males had been sterilized prior to mating by irradiation with the dosage of 70 Gy using a cesium-137 source at the division of Biomedical Radiation Sciences, Uppsala University. Sterilized males were used in experimental matings 1–3 h after the completion of irradiation. The sterile male technique has been shown to be a reliable way of assigning paternity in *C. maculatus* (Eady, 1991b). Sperm from a sterilized male can still fertilize eggs, but these eggs will not hatch. We measured the proportion of viable eggs laid by each female to assess the proportion of eggs fertilized by the first male (P1) and the second male (P2) to mate. Viable eggs can be readily distinguished from nonviable eggs because they fill up with shavings from the bean and turn a milky white as the larvae burrow into the bean. The hatched larvae also leave tunnels in the beans that are easy to see when eggs are removed.

In the P1 experiment, females were first mated to a normal male for 6, 8, or 10 min and then, 48 h later, to a sterile male for 8 min. Females were kept in petri dishes containing approximately 25 beans in between matings. After the second mating, females were transferred to a new petri dish containing approximately 25 beans and were then allowed to oviposit until they died. Pairs were given 30 min to mate. Paternity was assigned as described above.

Not all females remated after 48 h in the P1 experiment. We used remating propensity in the three treatments as a test of the effects of copulation duration on female refractoriness.

The P2 experiment was conducted in the same way as the P1 experiment except that here the females were first mated to a sterile male for 8 min and then, 48 h later, to a normal male for 6, 8, or 10 min. We checked the viability of all eggs

laid between the first and second mating to assess the efficiency of the irradiation for each individual sterilized male. A very low proportion of these eggs were viable (proportion of viable eggs  $\pm$  standard error of mean [SEM]:  $0.024 \pm 0.008$ ).

### Immediate remating propensity

In this experiment, we assessed the effects of copulation duration on female propensity to remate immediately after the first copulation. Unlike in the other experiments described above, females did not have their hind legs ablated prior to their first mating. Virgin females were mated to virgin males as described below. After their first copulation, four females were placed with eight virgin males in a petri dish with a diameter of 30 mm. This was done to assure that females would be detected and approached by males as soon as possible. The number of females that remated within 10 min was recorded. Before their first mating, we assigned virgin females randomly to three experimental groups. In the first group (6 min), copulations were interrupted after 6 min, and females were placed with new males directly. Females of the second group (6 + 4 min) also had their first copulations interrupted after 6 min but were not placed with new males until 4 min after the first copulation had been interrupted. In the third group (indefinite), copulations were not interrupted, and the females were placed with new males immediately after the first copulation had ended.

### Weight of the transferred ejaculate

Again, we assigned virgin females to the three copulation durations (6, 8, and 10 min) and mated them to virgin males as described above. Females were weighed immediately before and immediately after mating using a Cahn 28 microbalance with an accuracy of  $<10 \mu\text{g}$ . The weight they gained during copulation was taken as a measure of the weight of the transferred ejaculate. This is a reliable way of measuring the ejaculate size in *C. maculatus* because male weight loss and female weight gain are highly correlated (Edvardsson and Tregenza, 2005).

We used GLIM to estimate generalized linear models (GLMs) to analyze variation in the proportion of offspring fathered by focal males in the sperm competition experiments. To compensate for overdispersion (McCullagh and Nelder, 1989), we employed the method of Williams (1982). All other statistical analyses were carried out using SYSTAT 10.

## RESULTS

### Rate of oviposition—lifetime offspring production

Copulation duration had a significant effect on lifetime offspring production. Females with long copulations laid more eggs than females with shorter copulations (mean  $\pm$  SEM—6 min:  $33.2 \pm 6.6$ , 8 min:  $35.8 \pm 6.1$ , 10 min:  $54.5 \pm 4.1$ ; one-way Kruskal-Wallis:  $H_{0.05,17,19,20} = 7.8$ ,  $p = .02$ ).

Initial rate of offspring production was not affected significantly by copulation duration. For instance, there was no significant difference in number of eggs laid over the first 12 h between the treatment groups (ANOVA:  $F_{2,49} = 0.6$ ,  $p > .5$ ). Hence, the effects seen appeared later in life.

### Sperm competition—sperm precedence and remating propensity after 48 h

In the P1 experiment, copulation duration of the first male had a significant effect on P1 in a GLM, using binomial errors of the number of eggs fertilized by the first of two males to

mate with the total number of eggs as the binomial denominator and a logit link function. Contrary to expectation, males with short copulation durations were more successful than males with long copulation durations (mean P1  $\pm$  SEM—6 min:  $0.38 \pm 0.07$ , 8 min:  $0.21 \pm 0.05$ , 10 min:  $0.16 \pm 0.04$ ; GLM:  $\chi^2_2 = 6.902$ ,  $p = .032$ ). There was no difference in numbers of eggs laid in between the two mating opportunities (48 h) between females of the three experimental groups (ANOVA:  $F_{2,254} = 1.2$ ,  $p > .3$ ), confirming the results of the first experiment.

Copulation duration of the second male did not have a significant effect on P2 in the P2 experiment in a GLM, using binomial errors of the number of eggs fertilized by the second male to mate with the total number of eggs as the binomial denominator and a logit link function. However, there was a trend for males with longer copulation durations to be more successful than males with short copulation durations (mean P2  $\pm$  SEM—6 min:  $0.51 \pm 0.01$ , 8 min:  $0.49 \pm 0.09$ , 10 min:  $0.62 \pm 0.07$ ;  $\chi^2_2 = 1.487$ ,  $p > .4$ ).

Copulation duration did not have a significant effect on female remating propensity 48 h after the first mating (Cochran's test of linear trend:  $\chi^2_1 = 0.68$ ,  $n = 255$ ,  $p = .41$ ). There was, however, a trend for females that had mated for short times to be more likely to remate than females that had copulated for longer times (proportion of females remating—6 min: 0.28, 8 min: 0.27, 10 min: 0.22). Females that remated after 48 h did not lay more eggs in between the two mating opportunities than females that did not remate ( $t_{(2),249} = 1.1$ ,  $p > .25$ ), suggesting that sperm depletion was not a reason for remating.

### Immediate remating propensity

Very few females remated immediately after their first mating, and there were no significant differences between the three treatments ( $\chi^2_2 = 2.9$ ,  $p > .2$ ,  $n = 126$ ). One out of 42 females that were allowed to copulate for 6 min and then offered a new male immediately remated; four out of 43 females that were allowed to copulate for 6 min and then offered a new male after an additional 4 min remated; and five out of 41 females that were allowed to copulate indefinitely and then offered a new male immediately remated.

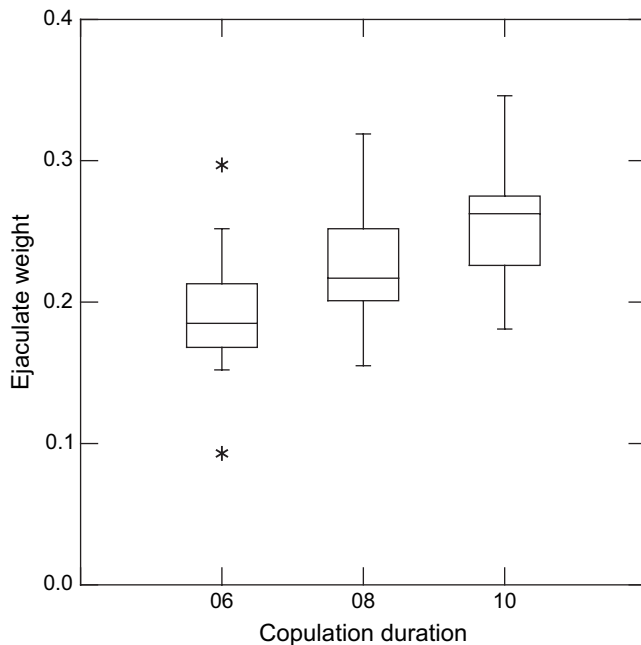
### Weight of the transferred ejaculate

Copulation duration had a significant effect on the weight of the transferred ejaculate (ANOVA:  $F_{2,52} = 11.4$ ,  $p < .001$ ). Longer copulations resulted in the transfer of heavier ejaculates (mean  $\pm$  SEM—6 min:  $0.193 \pm 0.010$  mg, 8 min:  $0.227 \pm 0.010$  mg, 10 min:  $0.260 \pm 0.010$  mg) (Figure 1).

## DISCUSSION

Divergent optimal copulation duration is a likely source of conflict between males and females (Simmons, 2001). To a human observer, the dramatic female kicking behavior and the spiny male genitalia of *C. maculatus* appear to reflect such a conflict. However, we found no support for a conflict over copulation duration in this study.

Size of the transferred ejaculate increased with copulation duration in the present study even when copulations were extended beyond their normal length (Figure 1). This demonstrates that ejaculate transfer has not been completed when females start kicking. This finding undermines the assumptions of some previous studies where time until kicking has been used as a measure of copulation duration (Savalli and Fox, 1998). Females with relatively long copulation durations did not suffer from reduced lifetime fecundity in the present



**Figure 1**

Weight of the transferred ejaculate measured as female weight gain in milligrams for females that mated for 6, 8, and 10 min. Ejaculate weight increased with copulation duration. \* denotes outliers (outside the 1.5 interquartile range).

study. On the contrary, females that copulated for long times produced significantly more offspring than did females that copulated for short times. Apparently, the benefits females obtain from prolonged copulations outweigh any associated costs caused by the male spines or ejaculates. These benefits are not simply a matter of females receiving enough sperm to fertilize all their eggs. Eady (1995) has shown that males transfer many more sperm than females need even when they have a reduced ejaculate size because of previous copulations. Male *C. maculatus* transfer large ejaculates that, in virgin males, can weigh up to 10% of their body weight (Edvardsson and Tregenza, 2005; Savalli and Fox, 1998). There is evidence suggesting that females derive material benefits from the ejaculates (Fox, 1993; Ofuya, 1995; Savalli and Fox, 1999; but see Eady, 1995). If so, females should benefit from receiving larger ejaculates. Ejaculate size could also affect female offspring production through the amount of secondary substances that females receive. These seminal substances have been shown to stimulate female oviposition in many insects (see Eberhard, 1996; Simmons, 2001, for reviews). However, there was no significant effect of copulation duration on the rate of oviposition early in life in the present study. Our results suggest therefore that females indeed derive material benefits from the ejaculates and that these resources are important to lifetime fecundity, confirming the findings of Savalli and Fox (1999).

Previous studies have found that the female kicking behavior mitigates the costs of mating in *C. maculatus*. Females that were prevented from kicking during mating suffered from more severe injuries to their reproductive tracts (Crudgington and Siva-Jothy, 2000) and reduced lifetime fecundity (Edvardsson and Tregenza, 2005). The lack of a negative effect of copulation duration on female fecundity in the present study suggests that the most important function of the kicking behavior is not to keep copulations short but to prevent injuries by some other mechanism such as limiting how deeply the spines on the male genitalia penetrate the walls of the female

reproductive tract. However, while females were only allowed to mate with one or two males in the present study, female *C. maculatus* typically mate with several males during their lifetime. Hence, it is possible that it becomes important for females to keep copulations short when injuries accumulate over many matings. Although it seems unlikely, we cannot rule out that the way in which we interrupted copulations in the present study somehow decreases the severity of the injuries females sustain compared to when males dislodge after an uninterrupted copulation. However, this should not change the relative effects of long and short copulations.

The relationship between copulation duration and male reproductive success appears to be complex. When given the opportunity to copulate for 10 min, males transfer larger ejaculates than when they are only allowed to copulate for shorter times (Figure 1). Surprisingly, long copulations are associated with lowered paternity success for males mating as first males with virgin females. Why P1 decreases with increasing copulation duration of the first male is unclear and contrasts to the number of studies that have found the opposite pattern (see Simmons, 2001, for a review). However, this result confirms the results of a previous study that found a negative association between copulation duration of the first male and P1 in *C. maculatus* (Edvardsson and Tregenza, 2005). In that study, as in the present study, copulation duration of the first male appeared to be much more important than copulation duration of the second male. Large ejaculates could stimulate oviposition and hence also sperm use. This would decrease the number of sperm present at the time of the second mating when the first ejaculate was relatively large. However, there was no positive association between ejaculate size or copulation duration and rate of oviposition in the previous study or between copulation duration and rate of oviposition. One possibility is that long copulations somehow facilitate sperm displacement at subsequent matings perhaps through permanent stretching of the female reproductive tract or because it is easier to replace a large mass of stored sperm completely compared to a smaller amount of sperm.

Even though transferring large ejaculates apparently does not make males more successful when competing with sperm from subsequent matings, they may still benefit in other ways. In addition to increasing female offspring production, large ejaculates may prolong the female refractory period. If females derive material benefits from ejaculates and remate when they need nutrients or water, it may pay males to provide them with a large and nutritious ejaculate to delay remating. Savalli and Fox (1999) found that female *C. maculatus* remated more readily when they had mated with nonvirgin males, and hence received a smaller ejaculate, than when they had mated with virgin males. They also appear to be more reluctant to remate when they have had access to nutrients (Savalli and Fox, 1999) or water (Edvardsson M, unpublished results). In the present study, females that mated for 10 min, and therefore on average received the largest ejaculates, had the lowest proportion of rematings after 48 h followed by females that had mated for 8 min and then females that had mated for 6 min. However, these differences were not statistically significant. The potential benefits of mating for a long time as a first male (delayed female remating) and the costs (reduced success when competing with sperm from subsequent matings) may create an interesting trade-off for males. If females remate when they need nutrients or water, the optimal male strategy in terms of ejaculate size and copulation duration may well depend on environmental factors such as temperature, humidity, and female access to food and water.

We found no effect of copulation duration on immediate female remating propensity. Very few females remated

immediately or 4 min after their first copulation. Apparently, 6 min of copulation is enough time for the female refractory period to commence. Hence, ensuring that females have become resistant to other males cannot be the reason why males prolong copulations beyond the time when females start kicking.

In conclusion, we found little evidence for a conflict over copulation duration in *C. maculatus* because females do not suffer from relatively long copulations but instead benefit from the large ejaculates resulting from them. The mate kicking behavior mitigates the injuries caused by the spiny male genitalia (Crudginton and Siva-Jothy, 2000; Edvardsson and Tregenza, 2005) but apparently not through a shortening of copulation duration. However, it is important to note that we cannot rule out the possibility that copulation duration has negative effects when injuries accumulate over more than two matings. Even though there are several possible functions of the spines, including, for example, forceful manipulation of the female reproductive tract to facilitate ejaculate transfer, acting as an anchor during copulation seems like a particularly likely function. Copulating males are attached firmly to their mates in spite of only using their genitalia to hold on to females. Spiny genitalia may have evolved because decreasing the risk of copulations being interrupted prematurely through dislodgment by other males outweighs the costs of reduced female fecundity associated with the injuries caused by the spines.

We thank the division of Biomedical Radiation Sciences, Uppsala University, for access to their cesium-137 source and especially Bo Stenerlöv and Karin Karlsson for practical help with the irradiation treatments. Thanks to Göran Arnqvist for valuable discussions and ideas on how to conduct this study. We would also like to thank Göran Arnqvist, Fleur Champion de Crespigny, and three anonymous reviewers for commenting on earlier versions of this paper.

## REFERENCES

- Arnqvist G, 1997. The evolution of water strider mating systems: causes and consequences of sexual conflicts. In: The evolution of mating systems in insects and arachnids (Choe JC, Crespi BJ, eds). Cambridge: Cambridge University Press; 146–163.
- Arnqvist G, Nilsson T, 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim Behav* 60:145–164.
- Boorman E, Parker GA, 1976. Sperm (ejaculate) competition in *Drosophila melanogaster*, and the reproductive value of females to males in relation to female age and mating status. *Ecol Entomol* 1:145–155.
- Chapman T, Arnqvist G, Bangham J, Rowe L, 2003. Sexual conflict. *Trends Ecol Evol* 18:41–47.
- Crudginton HS, 2001. Sexual conflict in the bean weevil, *Callosobruchus maculatus* (PhD dissertation). Sheffield: University of Sheffield.
- Crudginton HS, Siva-Jothy MT, 2000. Genital damage, kicking and early death. *Nature* 407:855–856.
- Daly M, 1978. The cost of mating. *Am Nat* 112:771–774.
- Eady PE, 1991a. Sperm competition in *Callosobruchus maculatus* (PhD dissertation). Sheffield: University of Sheffield.
- Eady PE, 1991b. Sperm competition in *Callosobruchus maculatus* (Coleoptera: Bruchidae): a comparison of two methods used to estimate paternity. *Ecol Entomol* 16:45–53.
- Eady PE, 1995. Why do male *Callosobruchus maculatus* beetles inseminate so many sperm? *Behav Ecol Sociobiol* 36:25–32.
- Eady PE, Tubman S, 1996. Last male sperm precedence does not break down when females mate with three males. *Ecol Entomol* 21:303–304.
- Eberhard WG, 1996. Female control: sexual selection by cryptic female choice. Princeton, New Jersey: Princeton University Press.
- Edvardsson M, Tregenza T, 2005. Why do male *Callosobruchus maculatus* harm their mates? *Behav Ecol* 16:788–793.
- Fox CW, 1993. Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Funct Ecol* 7:203–208.
- Holland B, Rice WR, 1998. Chase away sexual selection: antagonistic seduction versus resistance. *Evolution* 52:1–7.
- Johnstone RA, Keller L, 2000. How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *Am Nat* 156:368–377.
- Lessells CM, 1999. Sexual conflict in animals. In: Levels of selection in evolution (Keller L, ed). Princeton, New Jersey: Princeton University Press; 75–99.
- McCullagh P, Nelder PA, 1989. Generalized linear models. London: Chapman & Hall.
- Michiels NK, 1998. Mating conflicts and sperm competition in simultaneous hermaphrodites. In: Sperm competition and sexual selection (Birkhead TR, Møller AP, eds). London: Academic Press; 219–254.
- Morrow EH, Arnqvist G, Pitnick S, 2003. Adaptation versus pleiotropy: why do males harm their mates? *Behav Ecol* 14:802–806.
- Ofuya TI, 1995. Multiple mating and its consequences in males of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J Stored Prod Res* 31:71–75.
- Parker GA, 1970. Sperm competition and its evolutionary consequences in the insects. *Biol Rev* 45:525–567.
- Parker GA, 1974. Courtship persistence and female-guarding as male time investment strategies. *Behaviour* 48:157–184.
- Parker GA, 1979. Sexual selection and sexual conflict. In: Sexual selection and reproductive competition in insects (Blum MS, Blum NA, eds). New York: Academic Press; 123–166.
- Partridge L, Hurst LD, 1998. Sex and conflict. *Science* 281:2003–2008.
- Qi Y, Burkholder WE, 1982. Sex pheromone biology and behaviour of the cowpea weevil *Callosobruchus maculatus* (Coleoptera: Bruchidae). *J Chem Ecol* 8:527–534.
- Rice WR, 2000. Dangerous liaisons. *Proc Natl Acad Sci U S A* 97:12953–12955.
- Savalli UM, Fox CW, 1998. Genetic variation in paternal investment in a seed beetle. *Anim Behav* 56:953–961.
- Savalli UM, Fox CW, 1999. The effect of male mating history on paternal investment, fecundity and female remating in the seed beetle *Callosobruchus maculatus*. *Funct Ecol* 13:169–177.
- Shu S, Koepnick WL, Mbata GN, Cork A, Ramaswamy SN, 1996. Sex pheromone production in *Callosobruchus maculatus* (Coleoptera: Bruchidae): electroantennographic and behavioral responses. *J Stored Prod Res* 32:21–30.
- Simmons LW, 2001. Sperm competition and its evolutionary consequences in the insects. Princeton, New Jersey: Princeton University Press.
- Southgate BJ, 1979. Biology of the Bruchidae. *Annu Rev Entomol* 24:449–473.
- Thornhill R, 1976. Sexual selection and paternal investment in insects. *Am Nat* 110:153–163.
- Tufton T, 1993. The cost of reproduction in *Callosobruchus maculatus* (PhD dissertation). Sheffield: University of Sheffield.
- Vahed K, 1998. The function of nuptial feeding in insects: a review of empirical studies. *Biol Rev* 73:43–78.
- Watson PJ, Arnqvist G, Stallmann RR, 1998. Sexual conflict and the energetic costs of mating and mate choice in water striders. *Am Nat* 151:46–58.
- Williams DA, 1982. Extra-binomial variation in logistic linear models. *Appl Stat* 31:144–148.