



Male postcopulatory reproductive success in the beetle, *Diaprepes abbreviatus*

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For most species that have been studied, considerable variation exists in male postcopulatory reproductive success, including female sperm use, reproductive investment and remating patterns. We investigated postcopulatory sexual selection in the beetle *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) by examining the correlation of two measures of a male's success (proportion of eggs fertilized (P_2) and number of eggs that a female laid after mating with him) with male body size, copulatory behaviour and copula duration. We allowed each female to mate with an irradiated sterile male and a normal male. Relative duration of intromission (i.e. the difference in the intromission durations of the two males) and relative stroking rate, but not the absolute duration and stroking rate, were positively correlated with P_2 . The number of eggs that a female laid was negatively related to the thrusting rate of her mate. Irradiated males mated for a shorter duration and thrusting at a lower rate than normal males. Irradiated males also fertilized a lower proportion of eggs than normal males, and females were more likely to remate after mating with an irradiated male than after mating with a normal male. These findings suggest that behavioural traits may influence male postcopulatory reproductive success. Furthermore, since variation in male stroking rate and intromission duration are associated with variation in male fertilization success, these traits may have evolved through postcopulatory sexual selection.

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Competition among males for reproductive success does not end with the onset of copulation. Rather, in many species, there are processes that occur within the female during and after copulation (postcopulatory) that influence the quantity and quality of offspring sired by particular males (Parker 1970; Eberhard 1996). In most species in which male postcopulatory reproductive success (MPCRS) has been measured, substantial unexplained variation occurs both in the likelihood that a particular male

will fertilize his mate's eggs (Birkhead & Møller 1998) and in the reproductive investment by the female after mating with a particular male (e.g. differential allocation; Arnqvist & Danielsson 1999a; Sheldon 2000). In this study, we investigated the correlation of MPCRS with male body size, copulatory behaviour and copula duration in the beetle *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae).

In insects, the best-documented pattern of MPCRS is that, on average, the first or last male to mate fertilizes the majority of eggs (Simmons 2001a). However, in many cases, mating order actually explains very little variation in sperm use patterns, with male fertilization success ranging from 0 to 100% for both first and last males to mate (Lewis & Austad 1990; Simmons 2001a). Investigations into other correlates of sperm use patterns have resulted in important insights regarding the strength and direction

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of sexual selection on male traits (Danielsson 2001) and the mechanisms underlying sperm transfer, storage and use (Simmons & Parker 1992; Otronen 1997a, b; Andrés & Cordero 2000; Schäfer & Uhl 2002; Tallamy et al. 2002; Bloch Qazi 2003). Yet, for most species of insects, the study of MPCRS has not gone beyond the effect of mating order on sperm use patterns. There is a need for a more comprehensive understanding of the causes of variation in MPCRS both to determine whether the currently known patterns can be generalized and to gain a better understanding of mechanisms of postcopulatory sexual selection, circumstances under which it occurs, and how it affects the evolution of traits.

Like many insect species, *D. abbreviatus* is characterized by (1) promiscuous mating, (2) female storage of sperm from multiple males and (3) substantial variation in MPCRS (Harari et al. 2003). *Diaprepes abbreviatus* provides an appropriate system in which to investigate correlates of MPCRS because body size, copula duration and male copulatory behaviour vary greatly within populations (Sirot 2004). In this study, we investigated influences on MPCRS by studying the correlation of two measures of a male's success (the proportion of eggs that he fertilized and the number of eggs that a female laid after mating with him) with male body size, copulatory behaviour and copula duration. Based on the results of previous studies of postcopulatory sexual selection, we made the following predictions.

(1) Male body size should be positively correlated with MPCRS. This prediction is based on previous studies that have shown that a female's sons and daughters will benefit from larger size through increased competitive ability and increased fecundity (Harari et al. 1999). Male size also may be positively related to MPCRS if larger males contribute more to egg production than smaller males. Although male contribution to female fecundity has not been demonstrated in *D. abbreviatus*, male-derived molecules are found in the ovaries and eggs of mated females (Sirot et al. 2006).

(2) Duration of intromission (Table 1) should be positively related to MPCRS since it may (1) be related to the number of sperm that a male transfers or to the number of rival sperm that a male removes (Simmons 2001a) and/or (2) indicate male competitive ability and endurance.

(3) Male stroking rate (Table 1) during copulation should be positively related to MPCRS, as it may be an indicator of male endurance (Watson & Lighton 1994; Kotiaho et al. 2001; Tallamy et al. 2002). In other insect species, the rate of similar behavioural patterns is positively related to male fertilization success (Edvardsson & Arnqvist 2000; Tallamy et al. 2002; Bloch Qazi 2003).

(4) The number or rate of genitalic thrusts (Table 1) by males should be positively related to male fertilization success because thrusting could (1) be a method of sperm transfer or sperm positioning within the female reproductive tract (Eberhard 1993) or (2) stimulate the female to (a) release stored sperm from previous matings (Córdoba-Aguilar 1999) or (b) relax muscles that prevent full penetration by and expansion of the male genitalia (Eberhard 1993; Eberhard & Kariko 1996; Tallamy et al. 2002).

Taken together, our study provides evidence consistent with postcopulatory sexual selection acting on male behavioural traits in *D. abbreviatus* and provides insights into the mechanisms underlying variation in MPCRS.

METHODS

Study Subjects

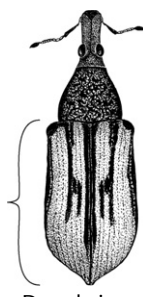
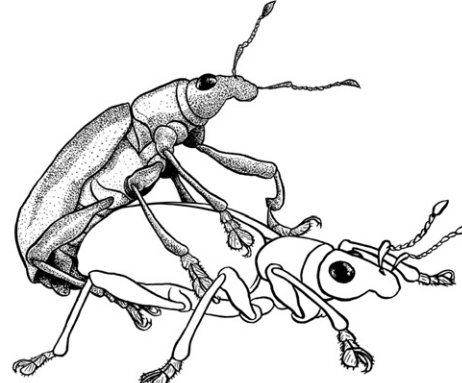
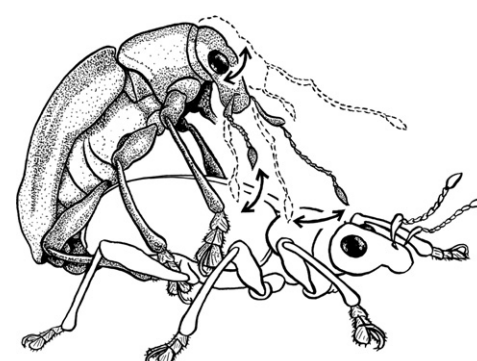
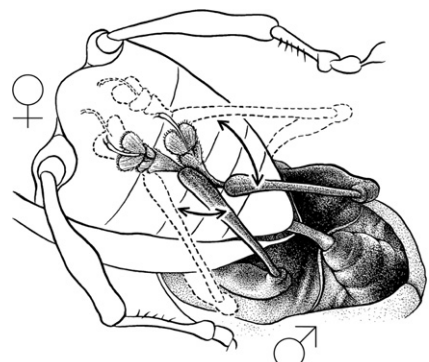
Diaprepes abbreviatus is a weevil found in Florida, Texas and California, U.S.A. (accidentally introduced into these areas), and much of the Caribbean (native to this region), where it feeds on a variety of plants including citrus. Females used in our experiment were reared in 30-ml plastic cups containing an artificial insect diet (product no. F1675, Bio-Serv, Inc., Frenchtown, New Jersey, U.S.A.) mixed with water, agar and preservatives (one female per cup; 26°C, >95% RH; Lapointe & Shapiro 1999; Lapointe 2000). After adult emergence, we maintained females in all-female cages (45 × 45 × 45 cm; wire mesh; 30 females per cage) and provisioned them with new citrus leaves and oviposition substrate every 3–4 days. Females were 2–4 weeks postemergence at the time of the experiment and had not mated. Females had a narrow range of body size (coefficient of variation = 5%), which minimized the effect of female size on the outcome of the experiment. Males were collected in the wild from a plant nursery in Homestead, Florida (Dade County) 2–5 days before the beginning of the experiment and were maintained in all-male cages under the same conditions as females. Wild males were used because their body sizes are more variable than laboratory-reared males. The length of the right elytron (Table 1) of males used in our study ranged between 7.4 and 11.9 mm ($\bar{X} \pm SE = 9.5 \pm 0.1$; $N = 150$). We used each male for only one mating in the experiment.

Experimental Design

We used males sterilized by gamma radiation to measure paternity (Boorman & Parker 1976). We allowed virgin laboratory-reared females to mate with two males on consecutive days; one of her mates was sterilized. We assigned females to either the IN (Irradiated male first, Normal male second) or NI (Normal male first, Irradiated male second) treatment. This allowed us to determine the paternity of eggs laid after the two matings. We then tested for correlations of the two measures of MPCRS (proportion of eggs fertilized by the second male to mate or P_2 and the number of eggs females laid after mating) with body size, copulatory behaviour and copula duration (Table 1). The experiment was conducted in June and July 2003 at the U.S. Horticultural Research Laboratory (USHRL), Ft Pierce, Florida (FL).

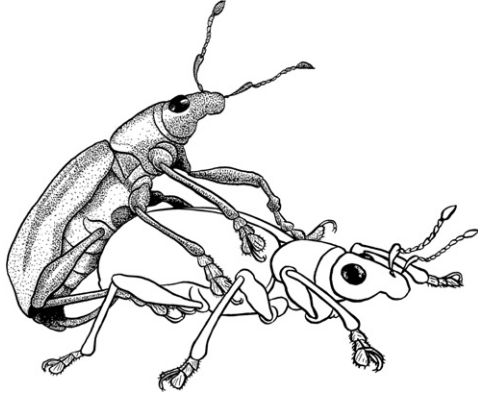
The day before the experiment began (Rugman-Jones & Eady 2001), we exposed a subset of males to 10 kR of irradiation (dosed intermittently: 1 min of irradiation at 1020 R/min and 4 min without irradiation; Cesium-137 source: Florida Department of Agriculture and Consumer Services, Gainesville, FL). Matings were conducted in an

Table 1. Morphological and behavioural measurements of *Diaprepes abbreviatus*

Trait	Description	Measurement	Diagram
Body size	Length of male elytron	Natural log of right elytron length (accurate to 0.01 mm)	 <p>Dorsal view</p>
Intromission duration	Total time that the male's aedeagus (i.e. penis) was inside female	Time between first observation of intromission and first observation in which male was no longer intromitted (rounded to nearest 30 min)*	
Stroking	There are two types of stroking. (1) With antennae: male lowers head, touches female's thorax or elytra one to several times with antennae, lifts antennae and raises head, in bouts during mounting and copulation	Total number of stroking bouts performed with legs or antennae divided by total time that the male was in physical contact with female	
	(2) With legs: male wipes hind tarsi alternately on female's abdominal sternites in bouts during copulation		 <p>Ventral view of abdominal area</p>

(continued on next page)

Table 1. (continued)

Trait	Description	Measurement	Diagram
Thrusting	Thrust begins with approximately three-fourths of the male's aedeagus (i.e. penis) intromitted into the female's genital opening. Male then pulls his body posteriorly away from female until only approximately one-eighth to one-half of his aedeagus is inside the female. Male then rapidly moves his body anteriorly, pushing his aedeagus fully into female	Number of thrusts or number of thrusts per unit time that the male was in physical contact with female	

*In cases in which the male remained mounted and later resumed intromission, we included all of the time periods during which he was intromitted in our measurements of intromission duration.

environmental chamber (27°C; 50% RH). For the females' first mating, we placed two to four males and females together in a plastic round cage (diameter: 10 cm; height: 8 cm) with fresh citrus leaves. For the females second mating, we placed each female together with two to four males in the same type of cage as for the first mating. The males in each cage were either all irradiated or all normal (i.e. untreated) and varied in body size. The number of males placed with females in the two treatments (i.e. NI and IN) did not differ consistently. As pairs began to mate, we isolated them by ushering them onto a piece of mesh screen and placing them in their own cage. This was to prevent take-overs by other males in the cage. We used multiple males and multiple females in the experimental design because matings are more likely to occur and occur more quickly in this context than when a single male and female are placed together. Although this design may allow for precopulatory sexual selection, this is unlikely to affect the outcome of our study since we eliminated the major known source of precopulatory sexual selection (take-overs by large males; Harari et al. 1999) and only looked at correlates of MPCRS in the pairs that mated. We monitored pairs every 30 min to determine duration of copulation. We observed a subset of the pairs (both matings for 10 IN females and 13 NI females) continuously and recorded all male and female behaviour on a hand-held Pision data recorder using the Noldus Observer 3.0 software program (Noldus Information Technology, Wageningen, The Netherlands). Observations began between 0900 and 1000 hours Eastern Standard Time (EST) and ended between 1900 and 2330 hours EST. Pairs still mating at the end of the observation period (five pairs) were excluded from the analyses because we did not have complete data on their intromission duration or behaviour. Pairs that mated for less than 10 min (three pairs) were also excluded from the analyses because

matings lasting less than 10 min result in few or no fertilizations (L.K.S., unpublished data).

Determining Paternity and Egg Number

To collect eggs, we placed females individually in cages with oviposition substrate (strips of wax paper; Wolcott 1933) each evening after mating and for two more nights after the second mating. Each morning, we removed the wax paper strips and placed them in capped plastic vials coated with a mist of distilled water and incubated them at 26°C. We determined whether eggs were (1) fertilized and developing, (2) fertilized but not developing, or (3) unfertilized after 5–10 days based on shape and colour (Harari et al. 2003). Developing eggs were assigned to the normal male, whereas eggs that were fertilized but not developing were assigned to the irradiated male.

The sterile male technique may incorrectly assign paternity if eggs that are fertilized by irradiated males sometimes develop fully and eggs that are fertilized by normal males sometimes fail to develop. To correct our counts of eggs fertilized by normal and irradiated males (following Boorman & Parker 1976), we used the percentage of normal developing eggs laid over nights 2–4 by females mated only to an irradiated male (0% in our study; $N = 8$ females) and the percentage laid by females mated only to a normal male that failed to develop (6%; $N = 12$ females). We also estimated 'nonsperm representation' (NSR; García-González 2004), or failure of males to fertilize eggs, in singly mated females ($N = 9$ mated to irradiated males and 14 mated to normal males) to estimate the proportion of NSR matings. NSR could result from sexual selection processes (i.e. sperm competition or cryptic female choice), but it could also result from other mechanisms such as naturally occurring male sterility

(García-González 2004) or egg–sperm incompatibility (Zeh & Zeh 1997, 2003; Simmons 2001b). Only two of these matings (both with normal males) resulted in NSR. However, in 23 of the 75 experimental matings (NI and IN), one or both males failed to fertilize eggs ($N = 11$ with no fertilizations by irradiated male; $N = 6$ with no fertilizations by normal male; $N = 6$ with fertilizations by neither male). We conducted analyses with and without data from NSR matings included (Table 2).

Data Analysis

To test for correlates of PCRS, we used both relative and absolute measures of male traits and postcopulatory success. We used the difference between the values of the predictor variables of the two males because the proportion of eggs fertilized by a male is likely to be related to his traits relative to the particular male that he is competing against. However, absolute measures of the second male's traits may be important to consider for traits involved directly in removal of sperm (Waage 1979) or stimulation of sperm ejection (Córdoba-Aguilar 1999) since the total amount of stimulation may affect these processes. In addition, previous studies of correlates of male fertilization success have used absolute values of predictor variables (e.g. Arnqvist & Danielsson 1999b; Edvardsson & Arnqvist 2000; Bloch Qazi 2003). Therefore, we conducted separate analyses using absolute values of predictor variables for the second male and using the difference between the values of predictor variables for second and first males. We analysed the proportion of eggs fertilized by the second male to mate (P_2) on nights 2–4 after mating. We used generalized linear models (GLMs) with a logit link and correction for overdispersion (using the 'quasibinomial' model in R; Williams 1982) to analyse the relationship between P_2 and (1) size, duration of

intromission, stroking rate and number of thrusts of normal and irradiated males, (2) mating order and (3) irradiation treatment. The data from 11 females were excluded from the analyses of P_2 because these females either laid no eggs (six females) or laid an unusually low number of eggs (five females) over the 3 nights following the second mating.

To examine the correlates of the number of eggs that a female laid after mating, we used the eggs laid on the first night after mating so that results were not confounded by traits of the second male. We used a GLM with a log link and a correction for overdispersion (using the 'quasipoisson' model in R) to analyse the relationship between the number of eggs that a female laid and five predictors: male size, duration of intromission, male stroking rate, male thrusting rate and irradiation treatment.

Since we recorded stroking and thrusting only for a subset of matings (Table 2), we analysed the relationship between these traits and MPCRS in separate models from those that included intromission duration and male size. Variables were eliminated from the models using backward iteration until only variables with P values ≤ 0.15 remained. To assess the predictive power of each GLM, we used the simple correlation coefficient, r , between the observed measure of MPCRS and the model's predicted values (Zheng & Agresti 2000). All GLMs were conducted using the program R (version 2.2.1; Venables & Ripley 2002).

RESULTS

Mating Behaviour

Of the 89 females that mated on the first day of the experiment, 84% mated with a second male on the next day. Females were more likely to remate if they had mated with an irradiated male than if they had mated with a normal male: 36 of 49 females assigned to the NI treatment and 39 of 40 females assigned to the IN treatment mated with a second male (chi-square test: $\chi^2_1 = 9.59$, $P = 0.002$).

Intromission duration ranged from 30 min to 12 h ($\bar{X} \pm \text{SE} = 6.5 \pm 0.1$ h; $N = 150$) and was longer for normal males than for irradiated males (ANOVA: $F_{1,146} = 13.39$, $P < 0.001$; Fig. 1a). However, intromission duration was unrelated to mating order ($F_{1,146} = 0.03$, $P = 0.87$; Fig. 1a). Male stroking rate ranged between 0 and 56 stroking bouts/h (5.5 ± 1.5 bouts/h; $N = 46$). The second male to mate stroked at a higher rate than the first male (Wilcoxon matched-pairs signed-ranks test: $T = 72$, $N = 23$, $P = 0.05$; Fig. 1b); this pattern resulted primarily from higher stroking rates by irradiated males when they were the second to mate (8.5 ± 4.2 bouts/h; $N = 13$) than when they were the first (1.4 ± 0.5 bouts/h; $N = 10$). Stroking rate was not related to irradiation treatment ($T = 138$, $N = 23$, $P = 1.00$; Fig. 1b). Male thrusting rate ranged between 0 and 33.6 thrusts/h (11.2 ± 1.3 thrusts/h; $N = 46$). Male thrusting rate was higher for normal males than for irradiated males ($T = 71$, $N = 23$, $P = 0.04$), but was unrelated to mating order ($T = 129$, $N = 23$, $P = 0.78$; Fig. 1c).

Table 2. Sample size summary for analyses of male postcopulatory success

	Number of females used for analysis	
	Number of eggs laid	P_2
Complete data set		
NI treatment*	36 (31)†	29 (22)‡
IN treatment	39 (36)	35 (29)
Total	75 (67)	64 (51)
Subset with behavioural observations		
NI treatment	13 (11)	10 (10)
IN treatment	10 (7)	9 (6)
Total	23 (18)	19 (16)

*NI: female mated with a normal male first and an irradiated male second; IN: female mated with an irradiated male first and a normal male second.

†Numbers in parentheses include only females with which both males fertilized eggs (see text for details). Analyses were conducted with and without these females included.

‡Data from 11 females (7 NI and 4 IN) were excluded from the analyses of P_2 because these females laid few or no eggs following the second mating.

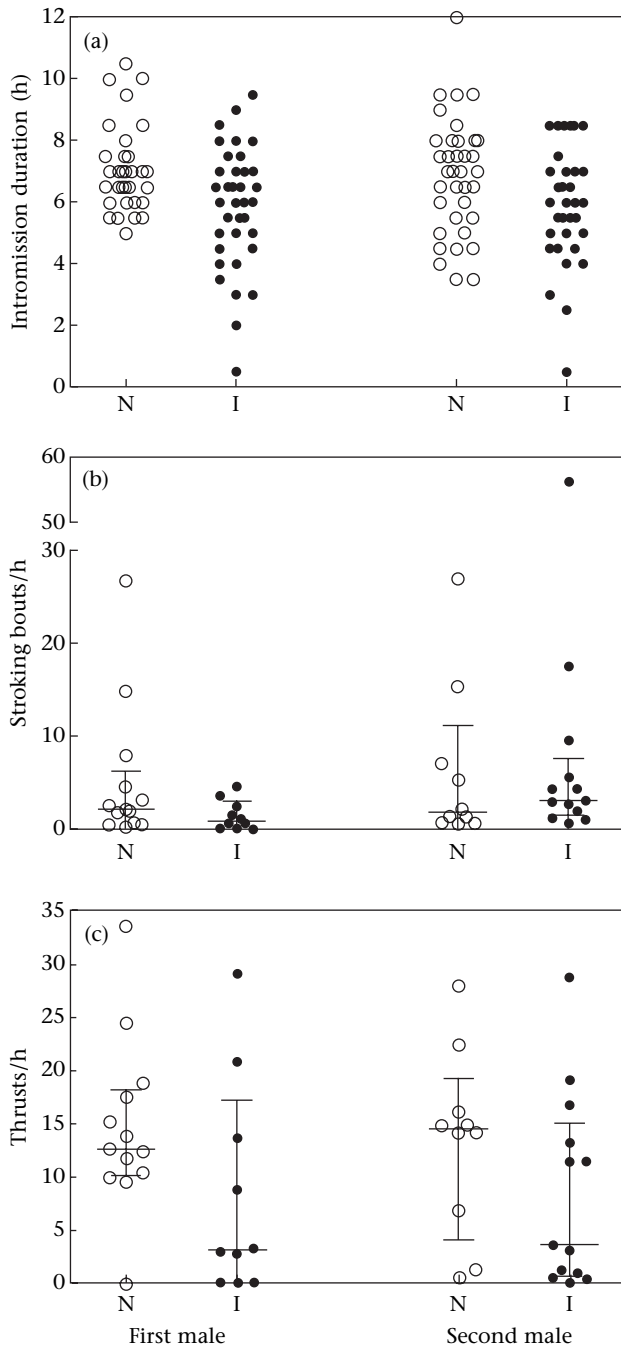


Figure 1. (a) Relation between intrusion duration and mating order for irradiated (I) and normal (N) males ($\bar{X} \pm SE$ duration: normal first: 7.1 ± 1.4 h; irradiated first: 5.9 ± 1.9 h; normal second: 6.9 ± 1.8 h; irradiated second: 6.0 ± 1.9 h). (b) Relation between stroking rate and mating order for males in each treatment. Bars depict medians and interquartile ranges. (c) Relation between thrusting rate and mating order for males in each treatment. Bars depict medians and interquartile ranges.

Proportion of Eggs Fertilized by Second Male to Mate (P_2)

The proportion of eggs fertilized by the second male to mate (P_2) ranged between 0 and 1 for both normal (0.76 ± 0.06 ; $N = 35$) and irradiated males (0.42 ± 0.07 ;

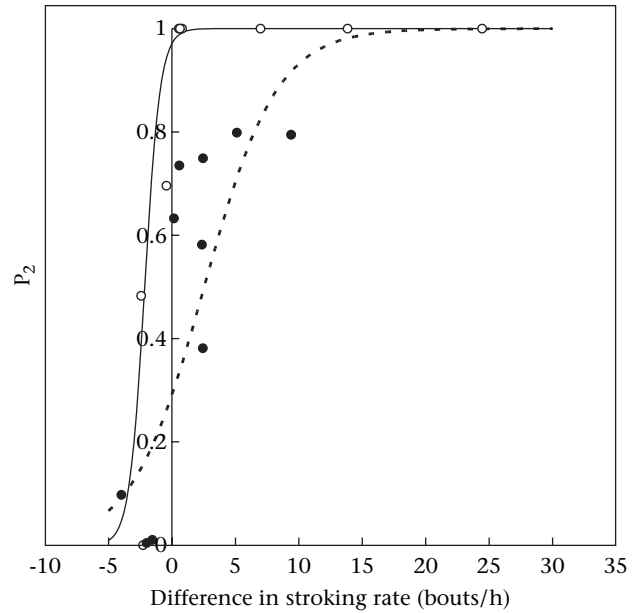


Figure 2. Proportion of eggs fertilized by the second male to mate (P_2) as a function of relative stroking rate and irradiation treatment. \circ : normal males; \bullet : irradiated males. Lines are fits of generalized linear models (solid line: normal males; dotted line: irradiated males).

$N = 29$) and was significantly higher than the proportion fertilized by the first male to mate (GLM: $t_{63} = -2.0$, $P = 0.05$, $r = 0.23$). P_2 was higher for normal than for irradiated males ($t_{63} = -3.08$, $P = 0.003$, $r = 0.42$; Figs 2, 3). P_2 was positively associated with both relative stroking rate ($t_{18} = 3.16$, $P = 0.006$, $r = 0.80$ for the model including

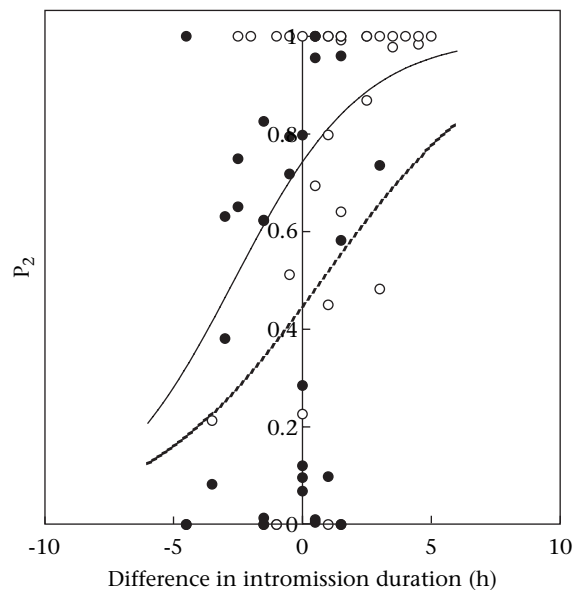


Figure 3. Proportion of eggs fertilized by the second male to mate (P_2) as a function of relative intrusion duration and irradiation treatment. \circ : normal males; \bullet : irradiated males. Lines are fits of generalized linear model (solid line: normal males; dotted line: irradiated males).

relative stroking rate and irradiation treatment; Fig. 2) and relative intromission duration ($t_{63} = 2.71$, $P = 0.009$, $r = 0.46$ for the model including relative intromission duration and irradiation treatment; Fig. 3), but the relationship with relative intromission duration was no longer significant when NSR matings were excluded ($t_{50} = 1.79$, $P = 0.08$). There was also a nonsignificant tendency for P_2 and relative male size to be positively related (with NSR: $t_{63} = 1.95$, $P = 0.06$; without NSR: $t_{50} = 1.74$, $P = 0.09$). P_2 was not significantly related to any of the absolute measures of the predictor variables.

Number of Eggs Laid

The total number of fertilized eggs that a previously unmated female laid on the first night after mating ranged between 0 and 668 eggs (199 ± 19 ; $N = 75$). There was a negative relationship between this measure and male thrusting rate when NSR matings were included ($t_{22} = -2.09$, $P = 0.05$; Fig. 4), but this relationship was no longer significant when NSR matings were excluded ($t_{17} = -2.00$, $P = 0.06$). Thrusting rate was significantly higher in matings involving females that subsequently laid no eggs on the night after mating than in those involving females that laid eggs (Mann–Whitney U test: $U = 19.5$, $N_1 = 8$, $N_2 = 15$, $P = 0.01$). Stroking rate showed a similar pattern, although the difference was not significant ($U = 36$, $N_1 = 8$, $N_2 = 15$, $P = 0.13$). Number of eggs laid was not related to intromission duration, male irradiation treatment, or male size.

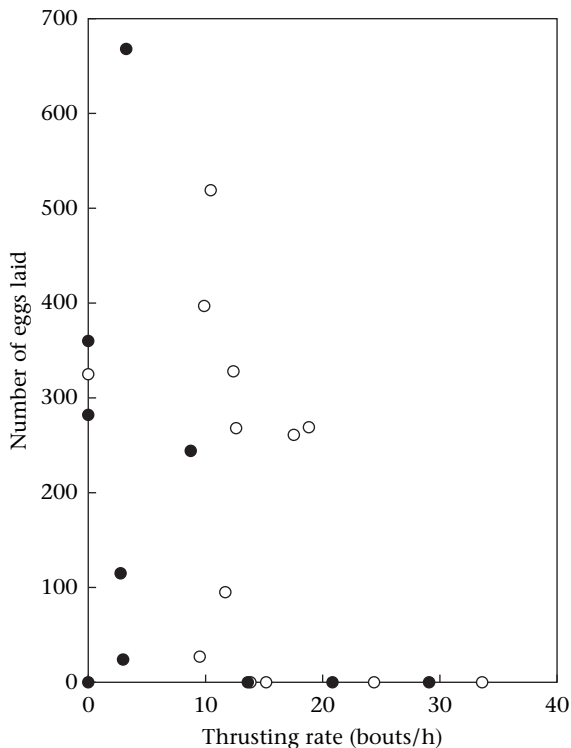


Figure 4. Number of eggs laid by females during the first night after mating as a function of male thrusting rate. ○: normal males; ●: irradiated males.

DISCUSSION

Understanding variation in male postcopulatory reproductive success is a major goal for studies of sexual selection (Eberhard 1996; Birkhead & Møller 1998; Simmons 2001a). Most previous studies have investigated only one aspect of MPCRS: the proportion of offspring sired or the number or size of eggs or offspring produced (but see Arnqvist & Danielsson 1999a; Nilsson et al. 2003). By incorporating a variety of traits (i.e. morphological and behavioural, absolute and relative measures) and two measures of MPCRS into our study of *D. abbreviatus*, we made several discoveries that have implications for future research.

First, although both mating order and intromission duration were related to the proportion of eggs that a male fertilized, these variables explained little of the variation in sperm use patterns. In contrast, the rate of male antennal and leg stroking explained much more of the variation in the proportion of eggs fertilized by the second male to mate. Second, the proportion of eggs fertilized by the second male was related to his relative and not his absolute measures of both intromission duration and stroking rate. Third, the number of eggs that a female laid the night after mating was negatively related to the thrusting rate of her mate. We also found that irradiated males mated for a shorter duration, thrust at a lower rate and fertilized a lower proportion of eggs than normal males, and that females were more likely to remate after mating with an irradiated male than after mating with a normal male.

Mating Order and Duration of Intromission

As has been found for several other species of insects (Simmons 2001a), the proportion of eggs fertilized by the second male to mate was greater than the proportion fertilized by the first male and was positively related to his intromission duration relative to that of the male that he was competing against. However, like other insects (Simmons 2001a), these two variables explained little of the variation in sperm use patterns. Harari et al. (2003) noted that intromission duration of *D. abbreviatus* is generally longer than necessary for insemination and inferred that the remainder of mating constituted mate guarding by the male. Our finding that P_2 increased with relative intromission duration suggests that mate guarding (in the sense of preventing the female from remating with other males) is not the only function of lengthy matings in this species. Take-overs and remating were not an issue in this study since pairs were separated from conspecifics.

The relationship between relative intromission duration and P_2 may be due to a time-dependent process related to sperm transfer or displacement. For example, the number of sperm that a male transfers, the number of rival sperm that he blocks from entering storage (in the case of the first male to mate) and/or the number of rival sperm that he displaces (in the case of second males to mate) may increase with time (Thornhill 1983; Dickinson 1986; Siva-Jothy 1987; Arnqvist & Danielsson 1999a;

Andrés & Cordero 2000). The relationship between relative intromission duration and P_2 may also result from female choice. Females may preferentially use sperm of males that endure long matings, either through selective storage or use, if, for example, intromission duration is an indicator of a male's ability to resist take-over attempts by other males, or endure food or dehydration stress (since males do not eat during mating).

Stroking and Thrusting

In comparison to intromission duration and mating order, male stroking rate was more closely related to sperm use patterns. P_2 was related to a male's relative stroking rate but unrelated to his own stroking rate. Stroking with legs or antennae is related to male mating or fertilization success in at least five species of beetles from five different families (Chrysomelidae: *Diabrotica undecimpunctata howardi*: Tallamy et al. 2002, 2003; Curculionidae: *D. abbreviatus*: this study; Scarabaeidae: *Onthophagus taurus*: Kotiaho et al. 2001; Melyridae: *Psilothrix viridicoeruleus*: Shuker et al. 2002; Tenebrionidae: *Tribolium castaneum*: Edvardsson & Arnqvist 2000; Bloch Qazi 2003). The mechanisms that underlie the relationship between male copulatory behaviour and sperm use patterns are still poorly understood for most species (Watson & Lighton 1994; Edvardsson & Arnqvist 2000; but see Tallamy et al. 2002); yet, there is evidence that this relationship is mediated by cryptic female choice in two species in which underlying mechanisms have been investigated (*D. undecimpunctata howardi*: Tallamy et al. 2002; *T. castaneum*: Edvardsson & Arnqvist 2000).

If the relationship between male relative stroking rate and fertilization success in *D. abbreviatus* is mediated by cryptic female choice, then our results provide evidence that female *D. abbreviatus* generally favour sperm from the mate that strokes the fastest but they do not distinguish between mates if the difference between stroking rates is small (Fig. 3). To our knowledge, cryptic female choice based on relative trait values of current and past mates has been suggested only for a few species in any taxonomic group (moths: LaMunyon & Eisner 1993; fish: Pitcher et al. 2003; Macías-García & Saborío 2004; Pilastro et al. 2004). Strong evidence for cryptic female choice based on relative male trait values comes from a study of guppies in which the number of sperm transferred depended on female perception of male coloration relative to a competitor (Pilastro et al. 2004). In *D. abbreviatus*, it is also possible that the proportion of eggs fertilized is not related to stroking itself but rather to another trait associated with stroking. For example, stroking rate could be related to the amount of sperm or seminal fluid proteins transferred (Matthews et al. 1997), or stroking could be a response to a female behaviour affecting sperm use or a method by which males transmit pheromones that influence sperm use patterns. Male-derived substances that affect female reproductive behaviours have been reported in other arthropods (e.g. a pheromone induces female quiescence during courtship in spiders: Becker et al. 2005; accessory gland proteins affect numerous female

postcopulatory behaviours including sperm storage in insects: Gillot 2003). Of course, the way to more fully understand this relationship would be to manipulate stroking rate without altering other variables.

Our observations of male and female behaviour during mating suggest that stroking may be a method by which males can increase their postcopulatory reproductive success by influencing female behaviour. At some point during most (59%) of the matings that we observed, females shook their bodies vigorously from side to side in apparent attempts to dislodge the male and indeed this shaking was sometimes followed immediately by the male dismounting. Intense stroking by the mounted male almost invariably followed shaking behaviour by the female. In some cases, the female stopped shaking, but, in others, the female continued shaking in sporadic bouts for up to several hours until the male ultimately dismounted. One interpretation of stroking is that it may be a method by which the male maintains his position or further influences sperm use by the female. Clearly, the interaction between male and female behaviour during mating and the subsequent effects of this interaction on MPCRS deserves more attention (Edvardsson & Tregenza 2005; Peretti et al. 2006; Cuatianquiz & Cordero 2006; Baena & Eberhard 2007).

Contrary to our prediction, thrusting was not correlated with male fertilization success. Based on other studies of beetles, we thought it possible that thrusting in *D. abbreviatus* played a role in mediating MPCRS by positioning sperm, stimulating the female to release stored sperm, or loosening the muscles of the female's vaginal canal. In other beetles, contraction of these muscles prevents the male from fully intromitting (Eberhard 1993; Tallamy et al. 2002) or from inflating the internal membranous sac of the genitalia (Eberhard & Kariko 1996), both of which are likely to influence sperm transfer and positioning. If this is the case in *D. abbreviatus*, the number of thrusts that a male performs may indicate the amount of resistance that he encounters within the female reproductive tract. According to this scenario, males may perform as many thrusts as are necessary to induce the female to relax her muscles. Our finding that thrusting rate was negatively related to the number of eggs that a female laid after mating is consistent with this hypothesis: high thrust rate matings may be ones in which the female was not receptive (i.e. never relaxed her muscles). This possibility would be interesting to investigate in *D. abbreviatus* as well as other beetles since rhythmic thrusting is common across Coleoptera (Eberhard 1994) and selective relaxation of reproductive tract muscles may be a widespread mechanism by which females control sperm transfer.

Male Size

We found a tendency towards a negative relationship between male size and P_2 , although this relationship was not significant. In contrast, a previous study found that male size was significantly related to precopulatory reproductive success (Harari et al. 1999). Large males were able to disrupt matings involving small males and displace

them from the backs of females (Harari et al. 1999). Once mounted, large males were also able to intromit more quickly than small males (Harari et al. 1999). These processes, together with male preference for large females, resulted in positive size-assortative mating (Harari et al. 1999). Size-assortative mating may reduce the opportunity for postcopulatory sexual selection on male size since it reduces variation in the sizes of those males that are competing to fertilize each female's eggs.

Irradiation, Mating Behaviour and Fertilization Success

Consistent with previous studies of insects (Lux et al. 2002; Kraaijeveld & Chapman 2004; Weldon 2005; Harmer et al. 2006), we found that irradiation of males affected mating behaviour, the probability that females would remate and the proportion of eggs fertilized. Specifically, irradiated males mated for shorter duration, thrusted at a lower rate, and, when they were first to mate, tended to stroke at a lower rate than normal males. Furthermore, females were more likely to remate after mating with an irradiated male. Our experimental design did not allow us to determine whether this effect was due to the treatment (i.e. irradiated or normal) of the first male, the second male, or both. That is, females may be more likely to remate after mating with an irradiated male than after mating with a normal male and/or more likely to remate with a normal male than with an irradiated male. Finally, the mean proportion of eggs fertilized by the first male to mate (P_1) was only 0.24 for irradiated males (versus 0.58 for normal males) and the mean P_2 was 0.42 (versus 0.76 for normal males). These results show that, at the irradiation level that we used, irradiated male *D. abbreviatus* were at a disadvantage relative to normal males in terms of postcopulatory reproductive success. Although this pattern may be explained, entirely or in part, by the effect of irradiation on the quality or quantity of sperm transferred, future studies should investigate the possibility that the reduced fertilization success of irradiated males may also result from the negative effects of irradiation on stroking rate.

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