



# Size-assortative mating, male choice and female choice in the curculionid beetle *Diaprepes abbreviatus*

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In the beetle *Diaprepes abbreviatus* (L.) females are larger on average than males, as indicated by elytra length. Size-assortative matings were observed in wild populations in Florida and in laboratory mating experiments. We tested three mechanisms for this size-assortative mating: (1) mate availability; (2) mating constraints; and (3) mate choice. We found that mate choice influenced size-assortative mating by: (1) large and small males preferring to mate with large females; (2) large males successfully competing for large females, leaving small males to mate with small females; and (3) females accepting large males as mates more readily than small males. Males increased their reproductive success by mating with larger, more fecund females. They transferred protein to females during mating.

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Nonrandom mating has attracted the attention of evolutionary biologists mainly because of its role in sexual selection and because it can influence the genetic variation in a population (Wade & Arnold 1980; Parker & Partridge 1998); it may thus increase the rate of evolutionary change and even promote speciation in some circumstances (Udovic 1980). Behavioural biologists have recently addressed the causes of nonrandom mating and, in particular, size-assortative mating (Crespi 1989a, b; Brown 1993; Enders 1995; Cooper & Vitt 1997; Santos-Filho & Pisaneschi 1997). Clarifying the mechanisms by which assortative mating is achieved may lead to a better understanding of the evolutionary forces underlying sexual selection.

Size-assortative mating has been reported throughout the animal kingdom (Crespi 1989a). The intensity of the phenomenon depends on the population density and the operational sex ratio, and may vary widely at different times and in different populations (Arak 1983; Bernstein & Bernstein 1998; Boell & Linsenmair 1998). However, the mechanisms are not fully understood and the causes are often not clear (Alcock & Hardley 1987; Enders 1995).

Crespi (1989a) suggested three hypotheses to explain size-assortative mating: (1) mate availability, that is, the body sizes of both males and females are strongly correlated in time or space; (2) mating constraints, that is,

physical or other difficulties derived from differences in the body sizes of males and females, prevent random mating; and (3) mate choice, that is, males, females or both sexes prefer bigger mates, and large males succeed in competition for large females, leaving small males to mate with small females. The mate availability hypothesis may explain size-assortative mating if individuals of similar sizes aggregate in patches (Johannesson et al. 1995), or if male and female body sizes covary in time (Miyashita 1994). Mating constraints may result in size-assortative mating if copulation between mismatched pairs is physically difficult either because of a lack of coincidence in anatomical parts, or because of differing physical abilities (Robertson 1990; Brown 1993), as in species where males carry females and may fail to carry large females (Crespi 1989a). On the other hand, size-assortative matings may result if size-matched pairs achieve intromission more easily (Brown 1993), or can fly in tandem more efficiently (Otronen 1993). The mate choice hypothesis may explain size-assortative mating if males, females, or both sexes select large mates (Gwynne 1981; Rutowski 1982; Sigurjonsdottir & Snorrason 1995; Rowe & Arnqvist 1996), and, in competition for large mates, large individuals are more likely to win (Johnson 1982; Sigurjonsdottir & Snorrason 1995; Rowe & Arnqvist 1996). Males may benefit from selecting large females as mates if these are more fecund (Rhainds et al. 1995; Marco et al. 1998; Savalli & Fox 1998) while females may gain from selecting large males if these are more fertile (Cohn 1990; Bukowski & Christenson 1997; Howard et al. 1998; McLain 1998; Uhl 1998) or provide larger nuptial gifts (LaMunyon 1994).

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Adult sugarcane rootstalk borer weevils, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), form aggregations on new growth of citrus and ornamental trees on which they feed and mate (Jones & Schroeder 1984). Ridley (1983) suggested considerable intrasexual variation in body size and prolonged postcopulatory guarding by males as prerequisite conditions for size-assortative mating. Our preliminary observations indicated intrasexual and intersexual variations in body size in *D. abbreviatus*, and male postcopulatory guarding that may last more than 12 h. Postcopulatory guarding may be important for the establishment of size-assortative mating because it opens the arena for male–male competition after mating and enables large males to win and mate with large females.

Our aims in this study were to document and quantify size-assortative mating in *D. abbreviatus* and to elucidate the mechanisms by which it occurs. Using field and laboratory tests, we evaluated each of Crespi's three hypotheses regarding the mechanisms that lead to size-assortative mating. The results indicate that male and female mate choice are both significant components of the size-assortative mating observed in this species.

## METHODS

### Beetle Collection and Handling

We collected weevils in a citrus grove near Apopka, Orange County, Florida, U.S.A. on 3 days in 1995 (in May, June and August) and from ornamental trees in an orchard near Homestead, Dade County, Florida, on three days in 1996 (in May, August and September). For each sample, aggregated weevils were shaken from a branch into a cylindrical box (20 × 30 cm) with leaves from the same tree as a food source. All weevils were sorted by sex in the laboratory (Harari & Landolt 1997) and were segregated into different cages. On each sampling date, we measured with callipers the right elytron length of 75 males and 75 females (or of all weevils if fewer than 75 males and females were collected), to the nearest 0.01 mm ( $N=364$  males and 364 females). We divided the measured males and females into three body size groups, approximately in thirds for each sex (range, mm: males: small:  $<8.40 \pm 0.01$ ;  $8.40 \pm 0.01 \leq \text{medium} \leq 9.40 \pm 0.01$ ; large:  $>9.40 \pm 0.01$ ; females: small:  $<9.90 \pm 0.01$ ;  $9.90 \pm 0.01 \leq \text{medium} \leq 11.00 \pm 0.01$ ; large:  $>11.00 \pm 0.01$ ). We divided the rest of the individuals into the same groups by estimating their body size by sight only ( $N=937$ ). Up to 50 weevils of each sex and size category were maintained in Plexiglas frame cages covered on five sides with 1-mm-mesh plastic screening (30 × 30 × 30 cm). Green beans, *Phaseolus vulgaris* (L.), were supplied as food. During May–October, cages with males were kept in a large (2.4 m wide, 2.4 m tall) Saran-screened field cage and those containing females were kept in a similar field cage 50 m away.

For some experiments, we needed virgin weevils. These were reared in the laboratory at the USDA-ARS in Orlando, Florida. The laboratory stock was derived, and

occasionally renewed, from citrus groves in Orange County, Florida.

### Sex Ratio

The operational sex ratio may influence size-assortative mating. In a male-biased sex ratio, some males will not find a mate and competition for mates may be intense. Large males may then mate with females of all sizes and small males may not mate at all. In a female-biased sex ratio, all males will mate with all females. In an unbiased sex ratio, size-assortative mating may occur. Using the field-collected weevils, we tested the null hypothesis that the sex ratio in the populations sampled did not differ from 1:1 (chi-square test with Yates' correction for continuity; Sokal & Rohlf 1981).

### Size-assortative Mating

#### Field populations

To determine whether size-assortative matings occur in different populations of *D. abbreviatus*, we collected mating weevils from unidentified ornamental trees in Homestead, Florida, on 3 days in 1996 (May, August and September). Each mating pair was gathered by hand from a branch and placed in a vial (2.5 cm in diameter and 5 cm high), with one pair in each vial with two leaves from the host tree. Weevils were sexed in the laboratory (Harari & Landolt 1997) and the right elytron length of the copulating males and females was measured with callipers to the nearest 0.01 mm ( $N=201$  pairs). We tested for the correlation between the body sizes (elytron length) of females and males in copula, for each sampling date. The significance of the difference in slopes was tested with GLM (Systat 1997), and the significance of the overall assortative mating was tested with an ANCOVA with date as a factor, female size as the dependent variable and male size as a covariate (Systat 1997).

#### Laboratory experiments

To test for size-assortative mating in controlled conditions, we randomly selected 10 males from a cage (30 × 30 × 30 cm) in which 30 small, 30 large and 30 medium-sized males were placed 24 h before the experiment. We randomly selected 10 females in the same way. The selected males and females were placed in cages (30 × 30 × 30 cm) with green beans as a food source, and were allowed to mate ( $N=6$  replicates). To ensure that the copulation was stable, we marked each pair with a different colour dot 10 min after copulation and observed the pairs again after 6 h. The elytron lengths of the female and male of each pair were measured with callipers to the nearest 0.01 mm. We tested for the correlation between the body sizes (elytron length) of females and males in copula, for each cage. The significance of the difference in slopes was tested with GLM (Systat 1997), and the significance of the overall assortative mating was tested with an ANCOVA with cages as a factor, female size as the dependent variable and male size as a covariate (Systat 1997).

## Mate Availability Hypothesis

To test the hypothesis that assortative mating is a consequence of the availability of mates, we compared the sizes (measured as elytron length) of males and females from different sampling dates in two locations (as described above). The body size distributions of males and females on different dates were compared with the Kolmogorov–Smirnov two-sample test (Systat 1997), and the mean body sizes of males and females on different dates and from different locations were compared with an ANOVA (Systat 1997), with body size as the dependent variable and date as a factor.

## Mating Constraints Hypothesis

To test the hypothesis that assortative mating is the result of mating constraints, we gave extremely large males (11.5–11.9 mm) extremely small females (7.5–7.9 mm) to mate with, and we gave extremely small males (7.5–7.9 mm) extremely large females (13.8–14.2 mm). Males and females of mean sizes (males: 8.6–9.0 mm,  $\bar{X} \pm \text{SE} = 8.8 \pm 1.1$ ; females: 10.1–10.5 mm,  $10.4 \pm 1.2$ ) were used as a control. We set up cages (30 × 30 × 30 cm) with five small females and five large males, five large females and five small males, and five males and five females of average size ( $N=3$  cages of each type). Each cage contained green beans as food. Weevils were allowed to interact for 4 h, after which we noted the number of pairs. Establishment of pairs of different sizes would indicate a lack of mating constraints, whereas mating attempts ending with no copulations may be a result of mating constraints.

## Mate Choice Hypothesis

### *Male mate choice*

To observe male mate choice we placed either a large or small single male together with 10 females (five small and five large) in a screen cage (30 × 30 × 30 cm) outdoors in shaded conditions between 1000 and 1700 hours. Each male was followed for 10 min or until contact with a female was observed and attempts to copulate were clearly seen. We separated the mounting male from the female by gently pulling him away from the female's back, and put each weevil in a separate cage. All other females were then taken back to their cages, and a random draw of five large females and five small females was repeated before the next assay. Pairs that did not mate after 10 min were excluded from the test. We tested 20–35 large and 20–30 small males each day on 7 days (individual males were used only once). Chi-square tests with Yates' correction for continuity (Sokal & Rohlf 1981) were used to test the null hypothesis that males choose large or small females randomly.

### *Male–male competition*

To test the outcome of male–male competition we placed five large females with five large males or five small males in a cage (30 × 30 × 30 cm) outdoors in

shaded conditions. To distinguish individual mating pairs and to verify that the members of a pair remained together, we marked each pair with Testor gloss enamel paint 10 min after copulation, with the same colour dot for males and females within a pair and different colour dots for different pairs. One hour after copulation was established (allowing the mating male to inseminate the female; personal observation), we introduced 10 small males into the cage where large males were copulating and 10 large males into the cage where small males were copulating. We observed the mating pairs for 1 h and noted the rate of mate replacement (when a male was replaced by another of the same size) and the rate of take-overs of females by introduced males (of a different size to the original male;  $N=10$  for cages of small copulating males (50 pairs) and  $N=10$  for cages of large copulating males (50 pairs)). We compared the replacement and take-over rates when males of different sizes initiated copulations ( $t$  test, after arcsine square-root transformation on the dependent variables; Sokal & Rohlf 1981).

### *Female mate choice*

To distinguish between passive female choice (accepting the winner in a male–male competition) and active female choice, we judged a female to have accepted a male if she opened the genitalia aperture to facilitate male insertion of the aedeagus. To observe female mate choice, we placed a single large female with five small males or five large males in a screened cage (30 × 30 × 30 cm) outdoors in shaded conditions between 1000 and 1700 hours. Each female was followed for 15 min or until contact with a male was made and insertion of the male's aedeagus into the female genitalia aperture was clearly observed. We noted the time elapsed from mounting until insertion of the aedeagus. We then put the pair into a different cage. The remaining males were taken back to their cages and a random draw of five small males or five large males was repeated before the next assay. Females that were not mounted by males within 15 min were removed and were excluded from further calculation. We conducted this assay 20 times with females placed in cages of small males and 20 times with females placed in cages of large males on each of 5 consecutive days ( $N=100$  females with large males and 100 females with small males). We used a  $t$  test to compare the mean time between mounting and observed intromission of small and large males (SAS Institute 1985).

### *Male reproductive success in relation to female size*

To test whether female size has an effect on her reproductive potential, we used virgin females (laboratory reared) to avoid any effect of males on female fecundity. Virgin females lay unfertilized eggs in a similar daily pattern to mated females. We placed 1-month-old ( $\pm 6$  days) virgin females of each size category (large, medium and small) in three cages, 25 females of one size category in a cage, with green beans as a food source. Double parafilm sheets (3 cm wide and 10 cm long) attached to the inside wall of the cage with adhesive tape served as an

oviposition substrate. Since females oviposit during the scotophase, we removed the sheets the next morning. We repeated this experiment on 4 successive days ( $N=4$ , a total of 12 cages). All eggs in egg batches were counted after 48 h with a stereoscopic microscope. A two-way ANOVA was used to test for the effect of female size on number of eggs, and Tukey HSD multiple comparisons were used to compare the total number of eggs deposited by females of different size categories (Systat 1997).

#### Female reproductive success in relation to male size

**Sperm load.** Female reproductive success may also be affected by the size of her mate. Small males may provide females with less sperm than larger males and deplete their sperm reservoir faster than larger ones. To test this hypothesis, we used virgin males (laboratory reared) to avoid any effect of their mating history on sperm storage. We placed 15 large females with 20 large males in one cage and 15 large females with 20 small males in another. Both cages were supplied with green beans as a food source. When all females were mated (after 30 min), we removed all surplus males to prevent male–male competition. Postcopulatory guarding of females lasted until sunset when the males climbed off the females' backs. Each female was then taken to a separate cage and provided with double parafilm sheets as an oviposition substrate and beans for food. We removed the sheets the next morning, placed them in an incubator and attached new sheets to the cage. After 5 days of incubation at room temperature, fertilized eggs could be distinguished by the dark head capsules of the embryos. We counted the fertilized eggs every day until no more were observed (because of either sperm depletion or loss of sperm viability in the females' spermathecae). Only large females were used in this experiment so that the full potential of eggs would be expressed and males of different sizes would maximize their fertilization ability. We used *t* tests to compare the mean numbers of eggs sired by large and small males after the first night of ovipositing and after 30 days of oviposition (SAS Institute 1985).

**Male contribution to females during mating.** If males provide nutrients to the female as a nuptial gift, large males may provide more material. To test whether females benefit materially by mating with large males, we conducted an experiment to determine whether male-specific factors are transferred into female haemolymph (following Monsma et al. 1990). Male proteins were labelled with  $^{35}\text{S}$ -methionine by injection into the male haemocoel. Males were allowed to mate 1 day prior to the labelling experiments so that the de novo synthesized protein would be labelled with  $^{35}\text{S}$ -methionine prior to their next mating. Males were anaesthetized on ice and injected with 6–8  $\mu\text{Ci}$  of  $^{35}\text{S}$ -methionine (>1000 Ci/mmol) in Ringer's solution through the posterior part of the dorsal–ventral intersegmental membrane. Injected males were isolated for a recovery labelling period of 24 h ( $N=57$ ), and then each was placed with a single female and monitored. At different times during mating (0.5–6 h), we separated females from the males and placed

**Table 1.** GLM analysis of size-assortative matings of *D. abbreviatus* in a field population

| Source                   | df  | F       | P      |
|--------------------------|-----|---------|--------|
| Male elytron length      | 1   | 105.989 | <0.001 |
| Date                     | 2   | 0.865   | 0.423  |
| Male elytron length*Date | 2   | 0.788   | 0.456  |
| Error                    | 195 |         |        |

Dependent variable: elytron length of female;  $N=201$ .

them in ether for 5 min. Females were then decapitated with a razor blade and 5–20  $\mu\text{l}$  of haemolymph were drawn with a 100- $\mu\text{l}$  capillary pipette. The haemolymph was mixed with 40  $\mu\text{l}$  water and was then added to a scintillation cocktail for liquid scintillation counting. Haemolymph taken from 10 females that were mated to nonlabelled males served as a control. The method of haemolymph collection precluded contamination from sperm, female reproductive organs and genitalia. Radioactivity (counts per min, cpm) from control and experimental samples was normalized to the amount of haemolymph taken from each female. The cpm value of unlabelled female haemolymph controls ( $\bar{X} \pm \text{SE} = 37.0 \pm 1.4$ ) after scintillation counting was subtracted from the total cpm value of each experimental sample. We compared the counts of the labelled haemolymph after different recovery-labelling times and different mating durations with that of the control after log transformation of the observed data (Dunnett's *t* test; SAS Institute 1985).

Statistical tests are two tailed.

## RESULTS

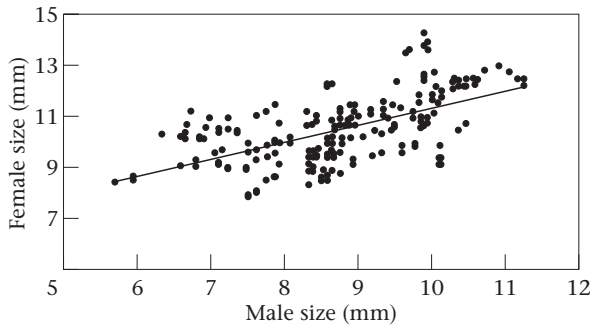
### Sex Ratio

The sex ratio was significantly male biased ( $\chi^2_1=5.93$ ,  $N=203$ ,  $P<0.025$ ) on only one sampling date (Apopka, August 1995). On the other five sampling dates at the two locations the sex ratio did not differ significantly from 1:1. The sex ratio on all six sampling dates did not differ significantly from 1:1 after Bonferroni correction for six comparisons ( $P>0.05/6$ ; pooled data:  $\chi^2_1=0.52$ ,  $N=2888$ ,  $P>0.9$ ).

### Size-assortative Mating

#### Field populations

Size-assortative mating was characteristic of pairs under field conditions ( $N=201$  pairs). Male and female size were positively correlated on all dates sampled ( $r_{74}=0.666$ ,  $P<0.0001$ ;  $r_{63}=0.490$ ,  $P<0.0001$ ;  $r_{58}=0.600$ ,  $P<0.0001$ ). The slopes of the assortative mating with sampling dates were not significantly different (Table 1), and we therefore combined all the body size data from field samples. For the combined data, the body size correlation was  $r_{199}=0.606$  (Fig. 1); there was a significant overall assortative mating effect (males as covariate;  $F_{1,197}=109.024$ ,



**Figure 1.** The correlation between elytron lengths of mating male and female *D. abbreviatus* in a field population (Homestead, Florida).

**Table 2.** Correlation of elytron lengths of male and female *D. abbreviatus* in copula in six cages in the laboratory

| Cage | <i>r</i> | <i>df</i> | <i>P</i> |
|------|----------|-----------|----------|
| 1    | 0.739    | 8         | 0.015    |
| 2    | 0.822    | 8         | 0.013    |
| 3    | 0.643    | 8         | 0.046    |
| 4    | 0.649    | 8         | 0.043    |
| 5    | 0.696    | 6         | 0.026    |
| 6    | 0.778    | 8         | 0.007    |

**Table 3.** GLM analysis of size-assortative matings of *D. abbreviatus* in the laboratory

| Source                   | <i>df</i> | <i>F</i> | <i>P</i> |
|--------------------------|-----------|----------|----------|
| Male elytron length      | 1         | 46.007   | <0.001   |
| Cage                     | 5         | 1.550    | 0.193    |
| Male elytron length*Cage | 5         | 1.261    | 0.297    |
| Error                    | 46        |          |          |

Dependent variable: elytron length of female; *N*=58.

*P*<0.001), and no significant effect of sampling date ( $F_{2,197}=0.939$ , *P*=0.393).

**Laboratory experiments**

Size-assortative mating was seen under laboratory conditions. In one cage only (cage 5), two pairs were separated during the observation time and did not mate again. These pairs were excluded from further analysis, leaving eight pairs in that cage (total *N*=58 pairs). Male and female size were positively correlated in each cage (Table 2).

The slopes of the assortative mating in all cages were not significantly different, when allowing for the effect of different cages. The effect of the replicated cages was not significant, whereas the effect of male length on female length was highly significant (Table 3). This means that we cannot assume that the slopes of different cages share the same intercept. This may be a consequence of different size ranges of both males and females in each cage. For the combined size data from all cages  $r_{56}=0.632$ , *P*<0.001.

**Table 4.** GLM analysis of size-assortative matings of *D. abbreviatus*, field and laboratory data combined

| Source                     | <i>df</i> | <i>F</i> | <i>P</i> |
|----------------------------|-----------|----------|----------|
| Male elytron length        | 1         | 45.339   | <0.001   |
| Cage and dates             | 8         | 0.715    | 0.679    |
| Male length*Cage and dates | 8         | 0.620    | 0.761    |
| Error                      | 241       |          |          |

Dependent variable: elytron length of female; *N*=259.

To test whether size-assortative mating of weevils in the laboratory was different from that of the weevils in the orchards, we compared the slopes of female length on male length of all samples in the orchards and in the laboratory. These were not significantly different (Table 4). For the combined size data,  $r_{257}=0.604$ . There was a significant overall assortative mating effect (males as covariate;  $F_{1,249}=145.824$ , *P*<0.001) and no significant effect of sampling date or cage ( $F_{8,249}=1.361$ , *N*=259 pairs, *P*=0.214). This may indicate that the sizes of mating weevils in the laboratory were in the range of sizes of mating weevils in the orchards.

**Mate Availability Hypothesis**

There was no evidence that the size distributions of males and females varied during the season. Females (mean elytron length ± SD=10.354 ± 1.156 mm, *N*=364) were significantly larger than males (8.777 ± 1.065 mm, *N*=364) on all dates sampled (date:  $F_{1,725}=1.906$ , *P*=0.168; length:  $F_{1,725}=368.099$ , *P*<0.001).

Male body size distribution (elytron length) did not differ significantly between sampling dates (Kolmogorov–Smirnov’s two-tailed probabilities for all tests: *P*=0.054–0.999); nor did the mean body size on different dates and from different locations ( $F_{5,358}=1.493$ , *P*=0.191). Female body size distribution (elytron length) also did not differ significantly between sampling dates (Kolmogorov–Smirnov’s two-tailed probabilities for all tests: *P*=0.131–0.990), nor did the mean body size on different dates and from different locations ( $F_{5,358}=1.837$ , *P*=0.105). Hence, both males and females of all size ranges were available on all dates and from all locations sampled.

**Mating Constraints Hypothesis**

Our findings partly support the mating constraints hypothesis. Almost all males were involved in copulation regardless of the size of their mates. Whereas all small males initiated mating and remained in copula with large females until sunset, however, some large males never initiated mating with small females, and some ceased guarding after only a few hours. Half an hour after being placed in a cage, a mean ± SE of 86.67 ± 11.55% (13/15) of the large males placed with small females were engaged in copulations but 4 h after they established matings only 60.0 ± 20.0% (9/15) were still engaged in mating pairs. These pairs lasted until sunset when all males climbed off

the females' backs. Of the small males caged with large females, all 15 were engaged in mating pairs after 30 min and remained in this position until sunset when they climbed off the females' backs. In the control, all 15 beetles were engaged in copulation 30 min after being placed in the cage. All males in copula, large, small and control, showed the characteristic postcopulatory guarding behaviour.

## Mate Choice Hypothesis

### Male mate choice

Our results support the hypothesis of assortative mating as a result of male choice. Both large and small males chose large females as mates over small females ( $\bar{X} \pm \text{SE} = 75.0 \pm 2.3$  versus  $25.0 \pm 2.3\%$  for large males and  $75.7 \pm 2.5$  versus  $24.3 \pm 2.5\%$  for small males; pooled data for large males:  $\chi^2_1 = 11.38$ ,  $N = 200$ ,  $P < 0.005$ ; small males:  $\chi^2_1 = 10.30$ ,  $N = 161$ ,  $P < 0.005$ ).

### Male-male competition

Our results indicate that large males can defeat smaller males when in competition over a female, whereas small males cannot win, and males of the same size sometimes win. The replacement rate was low for both large males ( $\bar{X} \pm \text{SE} = 0.12 \pm 0.14$ ; six males out of 50) and small males ( $0.04 \pm 0.08$ ; two males out of 50;  $t$  test:  $t_{18} = 1.501$ ,  $P = 0.151$ ). The take-over rate by large males when small males initiated copulations was high ( $\bar{X} \pm \text{SE} = 0.96 \pm 0.08$ ; 48 males out of 50), but no small males displaced large males ( $t$  test:  $t_{18} = 30.874$ ,  $P < 0.001$ ).

### Female mate choice

Our results support female choice of large mates. The time between a male mounting a female and inserting the aedeagus into the female genitalia was significantly shorter for large males ( $\bar{X} \pm \text{SE} = 1.46 \pm 0.13$  min) than for small males ( $4.10 \pm 0.43$  min;  $F_{1,96} = 34.4$ ,  $P < 0.0001$ ; there was no significant interaction between replicates and male sizes:  $F_{1,96} = 0.32$ ,  $P = 0.573$ ). When mounted by large males, females 'assisted' the males by widening the opening leading to the genitalia aperture. They did not do so when small males attempted mating.

### Male reproductive success in relation to female size

Our results suggest that larger males gained a reproductive advantage by mating with large females. Females deposited a total of 20 427 eggs in 537 egg batches. Large females deposited significantly more eggs in 12 h than either medium-sized or small females; but medium-sized females did not deposit significantly more eggs than small females (Table 5; Tukey HSD test:  $MSE_9 = 42\ 809.17$ ,  $P < 0.001$ ,  $P < 0.001$  and  $P = 0.062$ , respectively;  $\bar{X} \pm \text{SD} = 2507.50 \pm 215.438$ ,  $1493.75 \pm 250.037$  and  $1105.50 \pm 139.629$ , respectively).

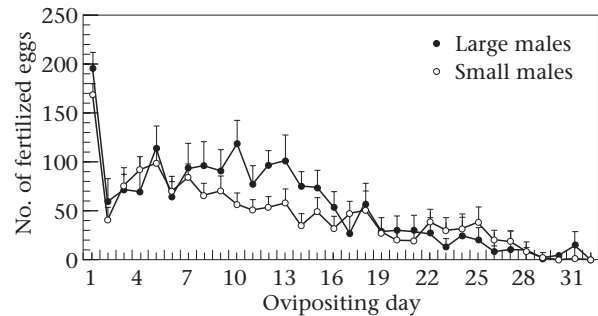
### Female reproductive success in relation to male size

**Sperm load.** Our results cannot explain female preference for large males as a result of differences in sperm quantity

**Table 5.** Two-way ANOVA of the total number of eggs oviposited by large, medium and small virgin female *D. abbreviatus*

| Source      | df | F      | P      |
|-------------|----|--------|--------|
| Cage        | 1  | 0.009  | 0.929  |
| Female size | 2  | 43.568 | <0.001 |
| Error       | 8  |        |        |

Dependent variable: total number of eggs; female size as factor; and different cages as covariate;  $N = 12$ .



**Figure 2.** Cumulative number of eggs+SE sired by large and small male *D. abbreviatus* ( $N = 15$  large males and 15 small males, 1232 egg clutches).

inseminated by males of different sizes. In total, the 30 females deposited 1232 egg clutches over 30 days (when depletion or sperm mortality occurred). There was no significant difference between the numbers of fertilized eggs deposited per female that were sired by large or small males, either on the night after mating or over 30 days ( $\bar{X} \pm \text{SE}$  eggs per female on the first night of oviposition sired by large males:  $195.33 \pm 16.77$ ; small males:  $168.31 \pm 12.46$ ;  $t_{29} = 0.327$ , NS; cumulative number of eggs after 30 days of oviposition sired by large males:  $2023.35 \pm 112.72$ ; small males:  $1867.51 \pm 142.37$ ;  $t_{1229} = 0.257$ , NS; Fig. 2).

**Male contribution to females during mating.** Our results support the assumption that males transfer proteins to the females during mating. A significant amount of labelled  $^{35}\text{S}$ -methionine was detected in the female's haemolymph after a 24-h recovery labelling time, at times ranging from 0.5 to 6 h after initiation of copulation ( $F_{2,65} = 6.47$ ,  $P = 0.003$ ; range 0–880 cpm). No significant difference was observed in the amount of labelled materials in female haemolymph after different copulation durations ( $F_{13,55} = 0.99$ ,  $P = 0.475$ ). Thus a factor was transferred from the male to the female during mating. Unfortunately the techniques we used did not allow us to detect significant differences of labelled  $^{35}\text{S}$ -methionine in female haemolymph after she mated with either a large or a small male.

## DISCUSSION

Assortative mating by size (defined here by elytron length) was characteristic of *D. abbreviatus* mating pairs,

with a correlation coefficient of 0.6 which is similar to correlations found in natural populations of other organisms (Fairbairn 1988). There was no difference between the body sizes of males and females on different sampling dates and in different locations. Therefore, the mate availability hypothesis, as demonstrated for the orb-web spider *Nephila clavata* (Araneae: Araneidae) (Miyashita 1994), can be ruled out as an explanation for assortative mating in *D. abbreviatus*.

Our results partly rule out the mating constraints hypothesis as suggested for the caddisfly, *Athripsodes cinereus* (Trichoptera: Leptoceridae) (Peterson 1995). In our study, small males mated large females and showed a typical mate-guarding behaviour. However, this was not the case when large males encountered extremely small females. Even though the majority of the large males mated with small females (86.67%), some of them (40.0%) stopped mating and guarding the females a few hours before sunset. Guarding time might have been reduced because small females are low-quality mates or because of physical mismatching, which was obvious to the observer. In the wild large males mated with small females may not guard them for long, instead searching for additional matings and competing with smaller males for their mates. Such combats end with the larger male winning and eventually mating with the large female.

Assortative mating in *D. abbreviatus* is best explained as a result of active mate choice by both females and males, which is maintained by male–male competition. A combination of male and female choice for large mates and male–male competition has been suggested as having a significant role in size-assortative matings of water striders, *Gerris lateralis*, *G. buenoi* and *G. lacustris* (Rowe & Arnqvist 1996).

### Male Choice of Mates

Males actively chose females. Both large and small males preferred large females, when a choice of either large or small females was given, in the absence of intramale competition. The relative size of females may be important for the reproductive success of *D. abbreviatus* males, since larger females were more fecund. In the majority of invertebrates, female fecundity is positively correlated with size (Clutton-Brock 1988). Male choice for larger, more fecund females has been described by Gwynne (1981) for the Mormon cricket, *Anabrus simplex*, by Rutowski (1982) for the checkered white butterfly, *Pieris protodice*, and for the yellow dung fly, *Scatophaga stercoraria* (Sigurjonsdottir & Snorrason 1995) and other species (Rhainds et al. 1995; Uhl 1998). Males are expected to be choosy if mating is costly, such as providing the female with nutrients (Johnstone et al. 1996; Kvarnemo & Simmons 1998), and if by doing so they increase their reproductive success (Andersson 1994). Since it is costly to transfer nutrients to females during mating (Boggs & Gilbert 1979; Gwynne 1981), and to spend time and energy in mating and prolonged postcopulatory guarding (Polak & Brown 1995), male *D. abbreviatus* should choose their mates to maximize their reproductive success.

Male–male competition is an important force generating assortative matings (Arak 1983; Rowe & Arnqvist 1996). In competition for large females, large males win in take-overs and mate with large females, leaving small males with the option of mating less contested small females (Johnson 1982; Sigurjonsdottir & Snorrason 1995). Male *D. abbreviatus* are attached to their mates after intromission in prolonged postcopulatory guarding. This behaviour provides an opportunity for large males to displace small males in take-over attempts, and mate with large females. Females may help large males remain attached to their backs, and push away smaller males, but we did not see such behaviour.

### Female Choice of Mates

Female choice for a larger male adds to the intensity of size-assortative matings (Johnson 1982, 1983). When competition between males of different sizes was prevented, female *D. abbreviatus* showed mate choice and delayed copulation with small males. Females opened the genitalia aperture when mounted by large males, facilitating aedeagus insertion, but kept it closed when mounted by small males. Active female choice should be distinguished from passive female choice. In the latter, a female need not prefer any male. She can either mate with a successfully competing male or be attracted by a more intense cue released by a particular male. In the former case, however, females are able to reject certain males and should favour others (Parker 1983; Andersson 1994), thus contributing to size-assortative mating.

Females can actively encourage male–male competition and then mate with the winner (Watson 1990). This tactic may be used by female *D. abbreviatus*. Females mounted by a small male delayed copulation. By doing so they may have allowed time for a larger male to be attracted to the pair (Harari & Landolt 1997) and to displace the smaller male. Females may then mate with the larger male.

Females may increase the number and quality of their offspring by actively choosing high-quality males (Trivers 1972; Phelan & Baker 1986; LaMunyon 1994). In our experiments, however, female *D. abbreviatus* did not produce more offspring by mating with larger males. Females mated with larger males did not lay more fertile eggs, and the sperm from only one mating of both large and small males were enough to fertilize eggs up to 30 days after mating. Furthermore, females in the wild mate more than once and in the laboratory they mated and laid eggs every other day (on average) for more than 30 days. Therefore, there was probably no selective pressure on large males to maximize sperm quantity.

Success in male–male competition is strongly related to male size (Simmons 1986) as we have shown for male *D. abbreviatus*. Therefore, females could use large size as an overall indicator of male fitness in mate choice decisions to improve offspring quality, and later mating success (Trivers 1972). Large males have better reproductive success by winning fights and mating with large females. Thereby, females mating with large males may produce large sons and thus may gain better reproductive

success through their sons' progeny (the sexy son effect: Fisher 1930; Arnold 1983).

Females may also benefit from male-derived nutrients (Rutowski et al. 1987; Oberhauser 1988, 1989, 1998; Savalli & Fox 1998). Male proteins were clearly detected in the haemolymph of female *D. abbreviatus* after mating. The nature of the substances transferred and the relation between male size and protein quantity are not yet known. There is some evidence that larger males transfer larger nuptial gifts to females during mating, although the majority of these examples come from spermatophore-producing insects (Wiklund et al. 1991; Rutowski 1997; but see Wedell 1997). Thus, female *D. abbreviatus* may benefit by choosing larger males as mates if these males transfer more protein to females during mating.

Reynolds & Gross (1990) suggested that females may select males for mating, despite gaining few direct benefits from being choosy, because the cost of searching is low (the so-called 'lek paradox'). This may be in agreement with the low cost of *D. abbreviatus* females searching for high-quality males when aggregated on the host plant foliage (Jones & Schroeder 1984).

### Sex Ratio and Size-assortative Mating

Size-assortative mating strongly depends on the population operational sex ratio, the population density and the size distribution (Arak 1983). Male-biased sex ratios in wild populations might result in females being mated regardless of their sizes, and mated males being larger than unmated males, as a consequence of male-male competition. Female-biased sex ratios may lead to females mating with males regardless of male sizes. A male's fighting ability should not affect his ability to mate. However, mated females would be larger than unmated females because of male choice for larger females (Lawrence 1986). Assortative mating may be most intense when the operational sex ratio is equal to or close to 1 (as it was in our laboratory experiments). In this case every individual, male and female, will find a mate but large males will win in male-male competition for large females. Therefore, assortative mating in the laboratory with an equal sex ratio is expected to be more intense than in the orchard where sex ratios may differ in different aggregation patches, and may be equal, female or male biased. In our experiment, the intensity of size-assortative mating was similar in the laboratory and in the field ( $r=0.632$  and  $0.606$ , respectively). This could be the consequence of the small sample sizes in the cages ( $N=5$  pairs) and random changes in the size range of both males and females in different cages. This may lead to a significant difference between the slopes of female length on male length, and, thus, a decrease in the value of the correlation coefficient of the matings held in laboratory cages. When all samples of mating weevils in the field and in the laboratory were combined, however, the slopes of female length on male length of all samples were not significant, indicating that the sizes of mating weevils in the laboratory were within the range of those in the field.

### Conclusions

Ridley (1983) showed that long copulation periods, male-male competition and high fecundity of large females are all necessary and sufficient for the evolution of size-assortative mating. We suggest mate choice of both males and females for large individuals as the mechanism of size-assortative mating in *D. abbreviatus*. The evolutionary explanation for this behavioural mechanism may be as suggested by Ridley (1983), postcopulatory mate guarding over several hours, which forms the arena for male-male competition. Large males are the winners in such combats and they mate with large females. Males prefer large females as mates because large females are more fecund than small ones. In our study we did not find an ultimate explanation for female preference for large males but there may be a positive relationship between a male's size and the amount of protein in the nuptial gift (Rutowski et al. 1987), or between a male's size and the size of his sons (Fisher 1930).

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