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Research

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Brawls Bring Buzz: Male Size Influences Competition and Courtship in *Diadasia rinconis* (Hymenoptera: Apidae)

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Abstract

Sexual selection on male body size in species with a female-biased sexual size dimorphism is common yet often poorly understood. In particular, in the majority of bee species, the relative contribution of intrasexual competition and female choice to patterns of male body size is unknown. In this field study, we examined two possible components of male mating success with respect to body size in the solitary bee *Diadasia rinconis* Cockerell (Hymenoptera: Apidae): 1) ability to procure a mate and 2) the duration of copulation. We found that larger males were better able to procure mates and copulated for shorter periods of time. Although consistent with sperm competition theory, differences in copulation duration were slight; possibly, the shorter copulations of larger males instead reflect in copulo female choice. Consistent with this notion, males engaged in complex courtship while mounted, characterized for the first time in any bee in such detail via audio recordings and high-speed, high-definition video. The number of pulses in male courtship behavior was also positively associated with copulation duration and may have stimulated females to continue copulating, thereby potentially allowing smaller males to transfer a full ejaculate. Females were shown to be potentially polyandrous and although we did not observe precopulatory rejection in the field, captive females frequently rejected copulation attempts by captive males. Our work indicates that intrasexual competition selects for increased body size in a solitary bee.

Key words: female-biased sexual size dimorphism, large male advantage, vibrational signal, copulatory courtship, sexual selection

Sexual size dimorphism is common among animals (Maklakov et al. 2004, Himuru and Fujisaki 2014). When males are the larger sex, strong sexual selection is thought to be the cause (Zamudio 1998, Maklakov et al. 2004, Kelly et al. 2008). Two mechanisms of sexual selection frequently explain increases in male size: intrasexual (male-male) competition and female choice (Simmons 1995, Maklakov et al. 2004, Wong-Muñoz et al. 2013). Larger body size often confers an advantage in male-male competition, thereby improving access to mates; likewise, females may choose to mate and/or sire more offspring with larger males. Yet even when males are the smaller sex, as is the case in many spider, insect, lizard, and fish taxa (Zamudio 1998, Kelly et al. 2008, Himuru and Fujisaki 2014), sexual selection on male body size can be strong. In female-biased sexual size dimorphisms (i.e., females larger than males), sexual selection can drive increases or decreases in male size (Fairbairn and Preziosi 1994, Maklakov et al. 2004, Herberstein et al. 2017). Female-biased sexual dimorphism is typical among bee species (Alcock 1996), and although greater than 85% of bee species are solitary (Batra 1984), their mating behavior is rarely observed (Paxton 2005). Does male body size in such species play a role in male-male competition and female mate choice? We addressed this question using *Diadasia rinconis* Cockerell (Hymenoptera: Apidae) (hereafter '*Diadasia*'), a solitary bee species exhibiting female-biased sexual dimorphism.

Diadasia is a member of a taxonomically diverse group of solitary ground nesting bee species (that includes the bee families, Andrenidae, Apidae, Colletidae, and Halictidae) that is characterized by dense nest aggregations and mass male emergence that precedes periodic female emergence. In these species, operational sex ratio is thus heavily male-biased and associated with intense intrasexual competition. Males patrol the nesting aggregation in large numbers and form 'mating balls' in which multiple males grapple with each other and attempt to couple with the female (reviewed in Eickwort and Ginsberg (1980), Neff and Simpson (1992), Neff et al. (2003), and Shimamoto et al. (2006)). In these species, competition might influence male mating success in several ways with respect to body size. Prowess in these intense intrasexual physical interactions could conceivably be greater for larger males (Neff and Simpson (1992), but see Shimamoto et al. (2006)). Large body size could improve a male's ability to acquire the female within the mating ball and, having acquired the female, to resist takeovers from other males inside or outside of the ball.

Body size might affect mating success in other ways too. When male competitive ability depends on body size, and when males at least partly control the duration of a copulation, sperm competition theory predicts that the duration of each copulation should also depend on male body size (Parker et al. 1999, Wong-Muñoz et al. 2013, Herberstein et al. 2017). Although longer copulations may improve paternity (e.g., Snow and Andrade 2004, Mazzi et al. 2009), they also trade off against searching for and mating with another female. If larger males are more successful in securing matings, they would be expected to engage in shorter copulations (Parker et al. 1999, Teuschl et al. 2010, Herberstein et al. 2017), compared with smaller males.

Selection on male body size in these species could also be driven by female choice (Blanckenhorn et al. 2000, Maklakov et al. 2004). Female choice can be exercised prior to copulation, by rejecting mating or copulation attempts of inferior males (Thornhill and Alcock 1983, Blanckenhorn et al. 2000). Female choice may also be cryptic, in that choice occurs during or even after copulation (Eberhard 2017, Firman et al. 2017). Polyandry may favor cryptic female choice, which reduces the costs associated with multiple mating (Welke and Schneider 2009). When female choice occurs during copulation and when females have at least some control over the duration of copulation, a longer copulation could reflect the female allowing the higher quality male to transfer more sperm (Eberhard 1996). To assess male quality, females may rely on complex male courtship signaling (Firman 2017, Eberhard 2017). Such displays are often costly to the male and may thus serve as honest indicators of male fitness (e.g., Hoefler 2008, Suzaki et al. 2013).

A variety of bee species produce powerful and presumably energetically expensive vibrations immediately before or during copulation (e.g., Rozen 1977, Larsen et al. 1986, Alcock and Buchmann 1985, Wcislo et. al. 1992); females of at least one species select among males partly on the basis of the duration of precopulatory bouts of vibrations (see Conrad et al. (2010) and Conrad and Ayasse (2015)), although patterns of copulation duration were not analyzed. Male *Diadasia* also produce bouts of vibrations during copulation (Alcock and Buchmann 1985, Neff and Simpson 1992). However, this copulation behavior and its possible association with male quality and copulation duration have yet to be characterized. If male size is associated with male quality, they might signal their higher quality by making relatively more or longer bouts of vibrations during copulation than expected based on the duration of copulation alone.

In this field study, we aimed to show how *D. rinconis* male body size related to mate procurement and retention, as well as the occurrence and duration of copulation. We further sought to characterize male vibratory copulation behavior and to determine how acoustic characteristics of copulation behavior were related to body size and copulation duration. To achieve these goals, we made audio and high-speed, high-definition video recordings of mating behavior at a *Diadasia* nesting aggregation, conducted random sweep samples of male *Diadasia* bees at a nesting aggregation and measured body size, collected mating pairs and measured the body size of individuals in the pair, and tested whether males and females mated multiply.

Methods

Field Sites

We studied two nesting site aggregations of the cactus bee *D. rinconis* Ckll. The 2016 nesting aggregation was located at the base of Sentinel Peak ('A Mountain') near downtown Tucson, Arizona (32° 13.021′ N; 110° 59.552′ W, elevation 716 m). We first visited the site on 28 April 2016 and studied it intensively over a 2-d period (17 and 19 May 2016). The 2017 site was located near Picture Rocks, Arizona (32° 19.397′ N; –111° 10.332′ W, elevation 740 m). We studied the site on 8 and 11 May 2017. Both sites were studied at the peak intensity of observed virgin female bee emergence and mating, from approximately 8 to 1300 h each day: activity was very limited before and after these hours (S.L.B., A.L.R., and D.R.P., personal observation). Only mating ball size was studied in both years; we assessed whether males mated multiply only in 2017.

Observations of Searching, Mating Balls, and Courtship

Observers watched for mating balls (a group of males grappling with each other and a single female) and waited for courting pairs to run a short distance away from the ball. To study mating balls, the leg, wing, and antennal movements of copulating (genital coupling) pairs, and interruptions by other males, movies were made using a GoPro Hero4 digital video camera fitted with a 3.8× macro close-up lens (GoPro, Inc.). Bees were video-recorded at ground level from a distance of up to ~12.5 cm with the GoPro camera mounted on a GoPro stand, at 120 or 250 frames per second. We could videotape mating balls as soon as a small number of individuals were grappling and before the mating ball had reached its maximum size. To estimate maximum size, we counted the small number of bees initially present in each video and tracked arrivals and departures. Unevenness in bee sample sizes for different components in a mating sequence are due to differences in observability among videos (e.g., hindleg movements were frequently obscured).

To study whether males in mating pairs differed in size from searching males, random samples of searching males were collected via aerial net (N = 83 bees). A collector walked rapidly across the emergence site making 1-m wide sweeps about 10 cm above the ground surface. Some courting pairs were also captured and isolated in vials for later size measurement (N = 52 pairs).

To study how body size related to the occurrence and duration of copulation and sounds made during mating, other courting pairs (N = 40 pairs) were captured and gently confined within 9-oz clear Dixie cups fitted with a soft fabric mesh base affixed with a rubber band. The containers were then placed directly atop the foam wind screen of a stereo digital recorder (a Zoom H2 or H4 pro, Zoom North America, NY). Recordings of sounds made during mating were collected at a sampling rate of 44,100 Hz; thus, the maximum frequency that could be detected is 22,050 Hz. To record sounds without interference from bird sounds and traffic noise, other pairs (N = 27 pairs) were captured and placed 2–5 cm from a Zoom H4 pro inside a Coleman 48-Quart Cooler (bottom lined with foam), the lid of which was then closed. Pairs were allowed to mate repeatedly. Some members of pairs were lost during handling, resulting in unequal male-female sample sizes reported in the results.

Measuring Bee Size

Bees collected for size measurements ($N = 135$) were placed in small plastic vials, chilled on ice in a Coleman Cooler, and transported to the Papaj laboratory where they remained frozen until measured. Bees' heads were removed and photographed in frontal view at 1.2× using a digital camera with a 5.2-mega pixel resolution (CMOS Camera, Microscope Cameras), affixed to the ocular lens of a DCM500 stereoscope. Photomicrographs were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, http://imagej.nih.gov/ij/) and an ocular micrometer scale was used to calibrate all measurements. Bee head widths were measured at their widest point. The thorax was similarly photographed to measure the intertegular span (Supplementary Fig. 6).

Assessing Multiple Mating

To study whether females mated multiply, we collected focal courting pairs and allowed them to copulate a single time in individual vials. The male was removed and novel males (collected via aerial net) were then introduced one at a time to vials, allowed to copulate once and then removed. We repeated these steps up to five times for a given female ($N = 6$ females). In 2017, to determine whether males mated multiply, we collected focal courting pairs and allowed them to copulate a single time in individual vials. To collect presumably virgin females (females left the nest site nearly immediately after mating and multiple mating was rarely observed in the field: see Results), courting pairs were collected and the female separated in an aerial net. These virgin females were individually introduced to the focal male and the number of copulation attempts and copulations recorded. If copulation occurred, the female was removed and a novel virgin female immediately introduced. If copulation with a given virgin female did not occur 8 min after it was introduced into the vial, we removed the female and substituted a novel virgin female. This process was repeated for a total of four novel virgin females per male ($N = 8$ males).

Data Analyses

Timings of the various phases of male courtship (to nearest 0.003 s) were determined by stepping movies of copulating pairs frame-by-frame and noting time codes using Avidemux 2.6 software. All behavioral and audio recording data from the experiment were analyzed using R v.3.3.2 (R Development Core Team). Male bee sounds made during courtship were analyzed using a series of sound analysis packages within R (tuner, ggplot2, compiler, manipulate, shiny, seewave, and viridis).

Generation and Shape Distribution of Male Mating Sounds

To examine whether male bees generated primary pulse sounds while making hindwing flicks, we compared the tempo of both using a Wilcoxon-signed rank test using the wilcox.test() function in R. We report effect sizes for Wilcoxon tests using Rosenthal's r. To test whether primary pulse number for all first courtships was unimodal, we fit a Gaussian Finite Mixture Model (GFMM) via the Mclust() function in the mclust package (Fraley et al. 2018). We forced a one-component fit and performed a likelihood ratio test. We determined that two normal components optimized the Bayesian information criterion (BIC).

Sexual Size Dimorphism

To examine sexual size dimorphism, we compared the head width of sweep males to mating females using a t-test via the t.test() function in R and examined variation in size between the sexes using an F-test via the var.test() function in the mgcv package (Wood 2018). We report effect sizes for t-tests using Cohen's d.

Relationship Between Male Body Size and Mate Procurement

To examine whether mating males were of higher quality than patrolling males, we tested whether head width of males captured during mating differed relative to head width of males captured in a random sweep using a Wilcoxon test. We further examined whether male head width of both groups was normally distributed using a Shapiro test via the shapiro.test() function in the mgcv package. We tested whether there was evidence of assortative mating for size via the Pearson's product moment correlation coefficient, using the cor.test() function in R.

Relationship Between Body Size, Male Mating Sounds, and Copulation Duration

For pairs' first courtship, we tested for an association between the number of primary pulses and the duration of the vibration train (the full sequence of sounds), and between the number of primary pulses and secondary pulses via Pearson's coefficient. For the second test, we discarded 21 of 64 recordings in which we could not identify all secondary pulses.

We used linear models (LMs) to investigate the possible association between male or female head width and vibration train duration, primary pulse number, or secondary pulse number in the first copulation. We specified male and female head width as fixed factors and vibration train duration or primary pulse number as the response variable. To report the effect of each factor and their interaction, we used post hoc Type II SS ANOVAs using the Anova() function when there was an significant overall effect. We apply a conservative Bonferroni correction for these analyses (α value = 0.017). We report effect sizes for LMs using Cohen's f^2.

Relationship Between Remating, Male Mating Sounds, and Body Size

To examine whether males invested less in subsequent copulations with the same female, we tested whether primary pulse number in a pair's second mating was significantly different from primary pulse number in its first mating, using a paired t-test. To investigate whether males or females that made second copulations were of higher quality than individuals of the same sex that copulated only once, we tested for head width differences via t-tests. We used a Fisher's exact test to test whether first or second copulations were interrupted more frequently, using the fisher.test() function in R.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

Results

Description of Mating Behavior

Males mob newly emerged females, forming a mating ball (mean maximum no. of males ± SE: 9.72 ± 1.20; range: 3–21, $N = 25$ mating balls) within which males grapple with each other and the female for a variable length of time (mean duration while video recorded ± SE: 19.25 ± 2.91 s, $N = 17$ mating balls; see high-speed, high-definition video of mating aggregations and mating ball in Online Resources); females were occasionally observed to extend their sting. The only sounds heard during the mating ball were an occasional defensive buzz (see De Luca et al. (2014) for a description of defensive buzzes). Eventually the female left the mating ball with a single male clasped to her dorsum. Occasionally, male-male pairs also emerged from the mating ball (Supplementary Material). Females typically stopped walking, whereupon males would typically rock side to side and attempt to copulate. The period lasting from leaving the mating ball to copulating varied from several seconds to several minutes, but was not measured.

All mating pairs copulated at least once. Second copulations were interrupted by other males significantly more frequently than first copulations (Table 1; number of copulations interrupted: 1 of 11 first copulations; 4 of 5 second copulations). Pairs always broke up when interrupted.

Mating behavior consisted of clearly definable components arranged in a predictable sequence (Fig. 1). For all pairs observed, the first copulation was immediately preceded by a sideways rocking motion by the male (Fig. 1a and f; see high-speed, high-definition video in Supplementary Online Resource 1). Shortly after genital coupling began, males antennated the female (mean latency to antennation ± SE: 1.41 ± 0.14 s, *N* = 10 pairs). Antennation was brief (mean duration ± SE: 0.45 ± 0.02 s, *N* = 14 pairs) (Fig. 1b, c, and f). Then, as males raised their antennae, they flicked their hindwings forward 90° at regularly occurring intervals and simultaneously stroke-tapped the female on the sternum of her abdomen with the enlarged basitarsal spurs of one or both (simultaneously) of their hindlegs (Fig. 1c and f). The male's hindwing flicks were accompanied by sounds (see sound analysis below) and his thorax vibrated with each sound produced. In contrast, the female remained completely still (Supplementary Online Resource 1, 2). After a variable number of wing flicks (mean number ± SE: 18.5 ± 2.41; range: 6–32; *N* = 13 pairs) and hindleg stroke-taps (mean number ± SE: 89.63 ± 5.81; range: 81–111; *N* = 8 pairs), males immediately stopped copulating (Fig. 1d and f). Genital coupling for video-recorded pairs lasted an average of 7.40 ± 0.52 s (*N* = 14 pairs). Almost one third of pairs (31%) broke apart shortly thereafter (mean time after copulation ± SE: 1.88 ± 0.28 s, *N* = 10 pairs). In the remaining pairs, either copulation was repeated (38%) or the male rocked side to side without copulation occurring (31%). Pairs in which the male rocked tended to break apart (3 of 4 pairs); in one case, the male held on and rocked as the female wandered out of frame.

When a second copulation occurred (mean time between copulations ± SE: 2.1 ± 0.35 s, *N* = 5 pairs), they were longer (mean duration ± SE: 23.8 ± 2.21 s, *N* = 5 pairs), involved fewer components, and were not as stereotyped as first copulations. Males raised and lowered one or both antennae repeatedly and flicked their hindwings forward only occasionally, without the periodicity seen in the first copulation. Males did not engage in stroke-tapping behavior during second copulations.

Sounds Produced During Mating

As noted above, following the silent phase of copulation, which was short and nearly constant in duration, males produced regular and evenly spaced sounds until the end of the first copulation (Figs. 1 and 2). The duration of the full sequence of sounds thus reflects the duration of copulation. Sounds consisted of two components: long lower-pitch 'primary pulses' (mean duration ± SE: 141 ± 31 ms, *N* = 15 pairs) followed by short much higher-pitched secondary pulses (mean duration ± SE: 20 ± 1 ms; mean inter-pulse duration ± SE: 151 ± 16 ms; *N* = 15 pairs). For a first mating, each primary pulse was followed by zero to four secondary pulses (mean % of primary pulses in a vibration train with 0 secondary pulses ± SE: 9.69 ± 0.86; with 1 secondary pulse: 29.45 ± 3.73; with 2 secondary pulses: 56.53 ± 3.60; with 3 secondary pulses: 4.14 ± 1.05; with 4 secondary pulses: 0.19 ± 0.13; *N* = 43 pairs). We hereafter term the full sequence of pulses a 'vibration train'.

An example of sounds (each primary pulse followed by a variable number of secondary pulses) produced during the first mating is presented in Fig. 2 (see Supplementary Online Resource 3 for an audio recording). The relative power of all frequencies between 0 and 22,050

Table 1. Analyses performed, with mean ± SE, sample sizes, parameter estimates, and 95% confidence intervals reported

Analyzed	Test statistic	*P* (analysis type)	Effect size	Mean ± SE, N	95% CI
Proportion of first versus second copulations interrupted	–	0.0128 (Fisher's Exact test)	0.038 (Odds ratio)	–	0.0005–0.752
Primary pulse versus hindwing flicking period	*W* = 68	0.474 (Wilcoxon signed-rank test)	0.148 (Rosenthal's *r*)	Pulse period: 0.291 ± 0.031 s, 15 males; hindwing period: 0.278 ± 0.014 s, 11 males	–0.047–0.023
Sweep male versus mating female head width	W = 465.5	<0.0001 (Wilcoxon signed-rank test)	0.535 (Rosenthal's *r*)	Males: 3.33 ± 0.019 mm, 83 males; females: 3.56 ± 0.019, 35 females	–0.293–0.160
Sweep versus mating male head width	$t_{124.5} = -5.74$	<0.0001 (*t*-test)	0.968 (Cohen's *d*)	Sweep: 3.33 ± 0.02 mm, 83 males; mating: 3.48 ± 0.02 mm, 52 males	0.103–0.212
Male versus female head width of mating pairs	$t_{31} = -0.769$	0.448 (Pearson's correlation)	0.137 (Pearson's *r*)	Males: 3.47 ± 0.046 mm; females: 2.85 ± 0.046 mm; 33 pairs	0.459–0.217
Primary pulse number versus copulation duration	$t_{62} = 10.17$	<0.0001 (Pearson's correlation)	0.791 (Pearson's *r*)	Pulse number: 25.78 ± 0.89; copulation duration: 6.91 ± 0.26 s; 43 pairs	0.676–0.868
Primary versus secondary pulse number	$t_{41} = 5.13$	<0.0001 (Pearson's correlation)	0.625 (Pearson's *r*)	Primary number: 25.95 ± 1.01; secondary number: 38.16 ± 1.71; 43 pairs	0.400–0.779
Primary pulse number in first vs second matings	$t_{6} = 6.081$	<0.0009 (paired *t*-test)	2.298 (Cohen's *d*)	first mating: 28.4 ± 1.6; second mating: 22.3 ± 2.0; 7 pairs	3.671–8.614
Male head width of first versus second matings	$t_{10.866} = -0.5556$	0.590 (*t*-test)	0.212 (Cohen's *d*)	first mating: 3.30 ± 0.05 mm, 6 males; second mating mm: 3.33 ± 0.03, 27 males	–0.171–0.102
Female head width of first versus second matings	$t_{8.2052} = 0.152$	0.883 (*t*-test)	0.065 (Cohen's *d*)	first mating mm: 3.56 ± 0.05, 7 females; second mating mm: 3.55 ± 0.03, 25 females	–0.124–0.142

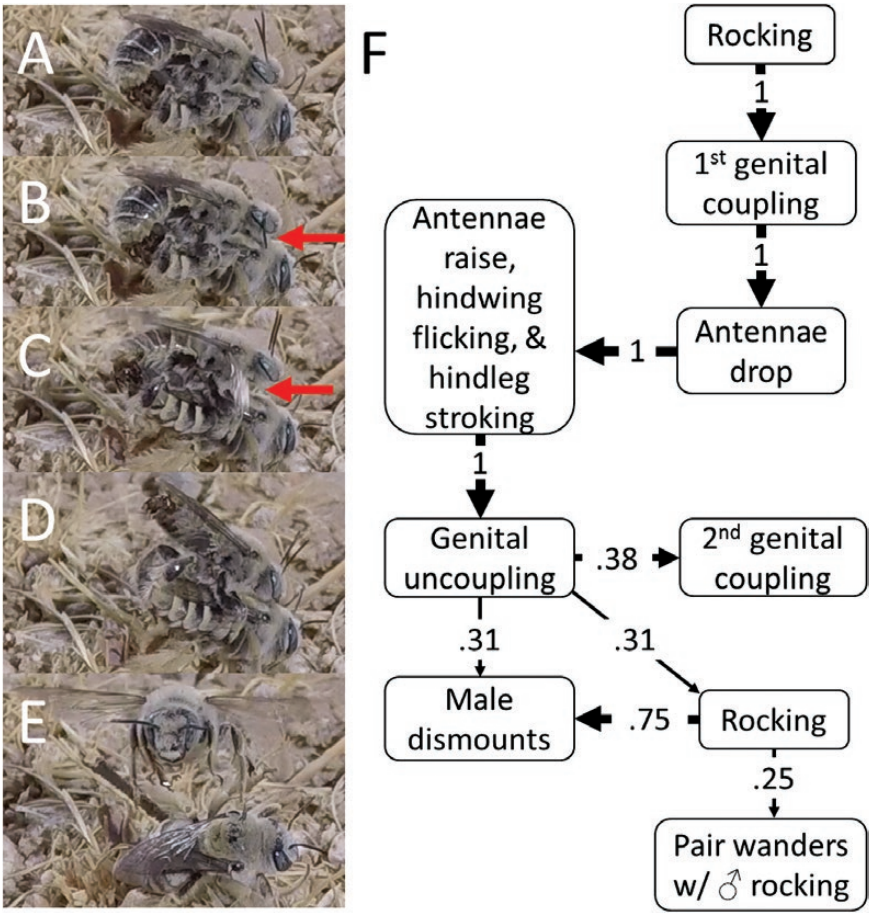

Fig. 1. (a–d) Phases in mating: (a) genital coupling, (b) antennae dropping and contacting the female indicated with the red arrow, (c) antennae raised and hindwing flicking (indicated with the red arrow), (d) genital uncoupling, and (e) male dismounts and male and female fly away. (f) Ethogram of the male mating behavior. Arrows indicate the transition from one behavioral component to another. The transition frequency is indicated by both the number and thickness of the arrow. We calculated values by dividing the average number of transitions for a particular component by the total number of transitions derived from a behavioral element. Thus, transition frequencies reflect only the transitions from a given component to any other component (i.e., all transitions from a given component add up to one). We report data from the averaged response of the full mating sequence for 11 pairs (1 pair dropped after the genital uncoupling component). Photo credit to Bruce Taubert.

Hz for the full spectrum of a 1-s sample is also shown. The fundamental frequency for the primary pulses is around 450 Hz and the first harmonic (double the fundamental frequency at ~912 Hz) is evident as a smaller peak (Fig. 2c). Additional fast Fourier transform analyses of secondary pulses (not shown) indicated that these contained much higher frequencies than primary pulses (ranging from 6,606 to 7,164 Hz).

The tempo of the male hindwing flicking corresponds well to the periodicity of the primary pulse sounds (Table 1). Small thoracic and tegular movements by the male that followed his hindwing flicks matched up in time with the secondary pulses, but constraints on video quality made quantification impossible for the majority of bees (Supplementary Online Resource 1).

Across all bees whose mating vibrations had been recorded, the number of primary pulses produced during the first copulation was bimodal (Fig. 4a; GFMM: log likelihood = −202.02, df = −424.84, BIC = −425.23; two normal components optimize the BIC: BIC likelihood ratio test: $P < 0.0034$).

Female-Biased Sexual Size Dimorphism

Male heads were on average 6.2% narrower than female heads, a numerically small but highly significant difference (Table 1). Variation in size was also significantly larger for males than for females (*F*-test: $F_{82,24} = 2.32$, $P < 0.008$), possibly a result of sexual selection (but see Blanckenhorn et al. (2006)).

Mating Males Were Relatively Large

Larger males procured newly emerged females more frequently than smaller males, relative to their frequency in the population: the heads of random sweep males were significantly smaller than those of mating males (Fig. 3; Table 1). Male head width was normally distributed for both random sweep and mating males (Fig. 3; Shapiro tests: sweep, $W = 0.975$, $P = 0.108$; mating, $W = 0.981$, $P = 0.560$).

Larger Males Engaged in Shorter Copulations

The duration of the vibration train (i.e., the duration of copulation; see section Sounds Produced During Mating) was significantly negatively correlated with male head width (Fig. 5a; Table 2). There was no significant association between vibration train duration and female head width, nor any significant interaction between male and female head width (Table 2). In addition, there was no evidence of assortative mating for size: we found no significant correlation between male and female head width for mated pairs (Table 1).

Primary Pulse Number Correlates With Male But Not Female Size for Mating Pairs

As with vibration train duration (see section Larger Males Engaged in Shorter Copulations), the number of primary pulses produced during the first mating was strongly significantly negatively correlated with male head width (Fig. 5b; Table 3). There was, however, no significant

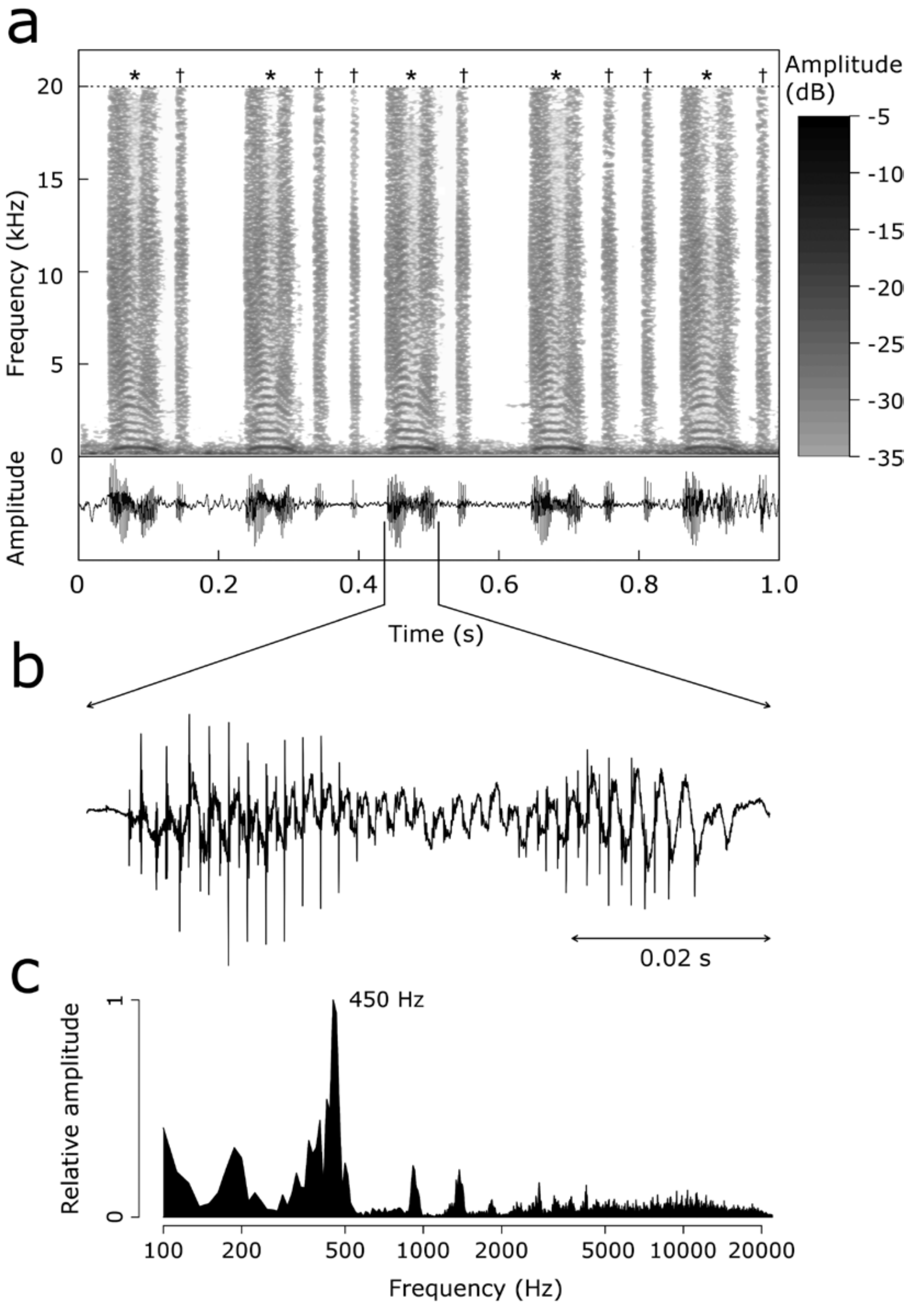

Fig. 2. Frequency spectrogram and amplitude oscillogram of a 1-s portion of sounds typically produced by copulating pairs of *D. rinconis* during their first mating. (a) The frequency spectrogram shows each primary pulse (indicated by *) followed by a variable number of secondary pulses (indicated by †). (b) A zoomed section of the vibration train. (c) A fast Fourier transform analysis (FFT) of the zoomed section of the vibration train is shown in the lower right. It has a peak sound frequency of 450 Hz.

association between primary pulse number and female head width, nor any significant interaction between male and female head width on primary pulse number (Table 3). Furthermore, the number of primary pulses produced during the first mating does not appear to be modified independently of copulation duration (Fig. 4b; Table 1). Although secondary and primary pulse number were significantly correlated (Table 1), there was no significant correlation between secondary pulse number and male or female head width, nor any interaction (Table 4).

Rematings Involved Fewer Primary Pulses, But Remating Individuals Were Not Larger

Captive pairs occasionally mated twice. Pairs made significantly fewer primary pulses during their second mating than during their first mating (Table 1). In terms of head width, neither males nor females mating twice differed from individuals of their sex that mated only once (Table 1).

Both males and females held in captivity also readily copulated with multiple additional novel males or females, respectively, after the original pair broke up (Tables 5 and 6). In addition, we recorded on video a single noncaptive female copulating twice in quick succession, with a different noncaptive male on each occasion.

Discussion

Scramble competition polygyny has been characterized as a 'race to locate females' (quote from Herberstein et al. (2017)). This is an apt

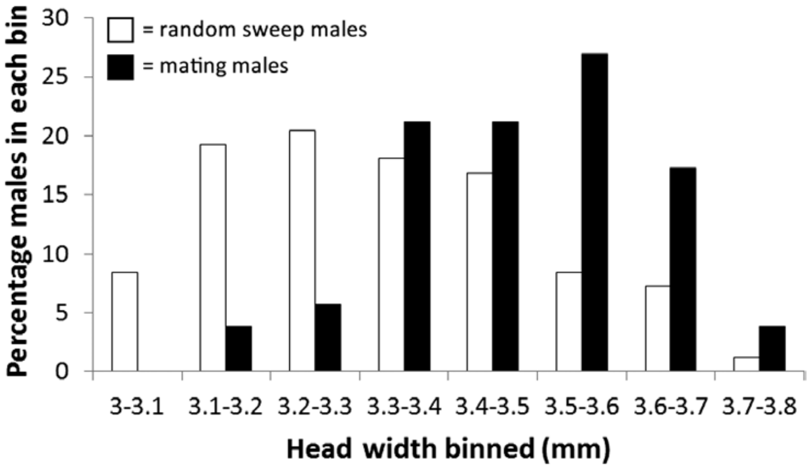

Fig. 3. Head width distribution of random sweep males and mating males, binned by every 0.1 mm. *N* = 83 and 52 males for sweep and mating samples, respectively.

Table 2. Parameter estimates for the model relating the duration of the vibration train to male and female head width, via a linear model with a post hoc Type II ANOVA

Predictor	*F*	*P*	Cohen's f^2
Male head width	7.745	0.0099	0.220
Female head width	0.018	0.894	0.0005
Male:female head width	2.899	0.101	0.078

Model R_2 = 0.299, $F_{3,26}$ = 3.696, $P < 0.025$, Bonferroni correction α-value = 0.017, Cohen's f^2 = 0.427.

Table 3. Parameter estimates for the model relating the number of primary pulses during the first mating to male and female head width, via a linear model with a post hoc Type II ANOVA

Predictor	*F*	*P*	Cohen's f^2
Male head width	21.373	<0.0001	0.457
Female head width	0.072	0.790	0.001
Male:female head width	0.814	0.375	0.016

Model R_2 = 0.475, $F_{3,26}$ = 7.84, $P < 0.0007$, Bonferroni correction α-value = 0.017, Cohen's f^2 = 0.905.

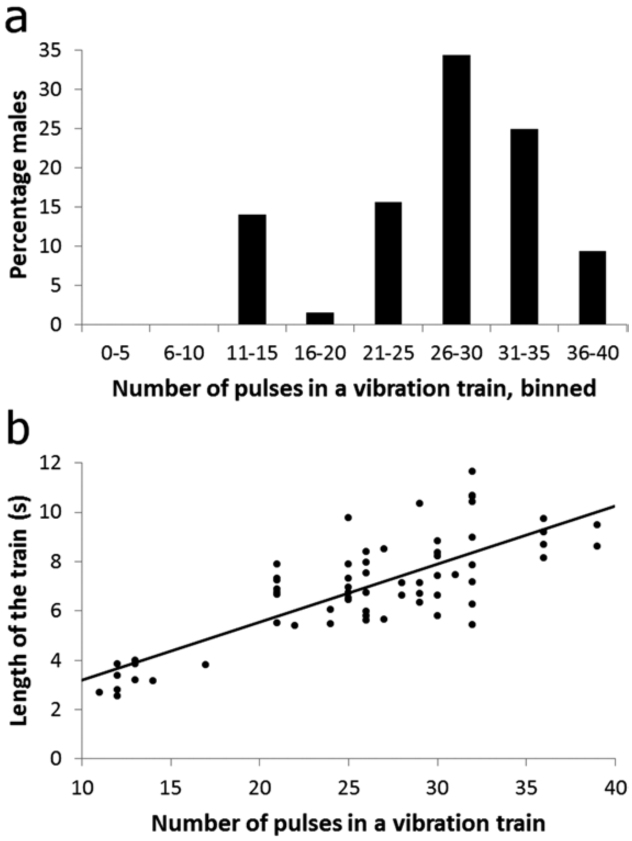

Fig. 4. How the number of primary pulses in the first copulation relates to the duration of copulation (vibration train duration) and distribution among males. (a) The percentage of males in pairs producing a given number of primary pulses in the first vibration train, binned by every 5 pulses. *N* = 64 pairs. (b) The number of primary pulses in a vibration train graphed against the duration of a vibration train, measured in seconds. *N* = 64 males.

description of what happens in *D. rinconis* aggregations where literally thousands of males are searching simultaneously for newly emerging females in a relatively confined area (see video in Supplementary Online Resource 4). So intense is the competition that we only very rarely observed the beginning of a mating ball. *D. rinconis* males also engaged in intense physical competition after locating females (i.e., within the mating ball). As with the 'race' to find females, the intense interference (or, 'contest') competition is imposed by the strongly male-biased operational sex ratio within mating balls (up to 21:1).

In quantifying the sex ratio, courtship behavior, and multiple mating, our results support and extend prior evidence of a pattern of scramble competition polygyny in *D. rinconis* (see Ordway (1987) and Neff and Simpson (1992)). Scramble competition polygyny is characterized by nonmonopolizable females and by competitive mate searching by males (Thornhill and Alcock 1983, Berghänel et al. 2010, Baena and Macías-Ordóñez 2015). Under such conditions, males are not expected to guard mates but to search and acquire as many mates as possible (Schwagmeyer 1988, Herberstein et al. 2017). Consistent with this expectation, *D. rinconis* females were observed emerging from burrows only rarely. Males frequently entered burrows, but almost always emerged shortly afterwards and flew away, suggesting emerging females were rarely found and that males were not guarding female pupae or imagos until they emerged as adults. Precopulatory mate guarding may be disfavored because

Table 4. Parameter estimates for the model relating the number of secondary pulses during the first mating to male and female head width, via a linear model with a post hoc Type II ANOVA

Predictor	F	P	Cohen's f^2
Male head width	1.029	0.329	0.055
Female head width	0.842	0.376	0.055
Male:female head width	0.506	0.489	0.033

Model R_2 = 0.1437, $F_{3,13}$ = 0.73, P = 0.554, Bonferroni correction α-value = 0.017, Cohen's f^2 = 0.144.

Table 5. Remating of focal females with different males

Female	Number of copulations
1	3
2	2
3	4
4*	3
5*	5
6*	3

Each copulation was with a different male.

*Females allowed to completely groom themselves after their first copulation.

Table 6. Remating of focal males with different females

Male	Number of copulations	Number of rejections*
1	2	15
2	2	7
3	4	4
4	4†	9
5	1	3
6	1	29
7	3	3
8	2	12

Each copulation was with a different female.

*Male attempted to copulate but did not initially (or sometimes, ever) succeed.

†Male copulated twice with each of two females, for a total of four copulations.

emerging pairs are quickly swarmed by males and the guarding male may not get the mating anyway.

Neither did postcopulatory mate guarding occur; mating pairs broke apart and took flight nearly immediately after copulation (compare with Neff and Simpson (1992), who observed postcopulatory mate guarding in *Diadasia*). It may benefit both males and females if the latter left the nest site. Given that females are capable of mating again, as our results indicate, a rapid departure might reduce the likelihood of other males finding the pair and taking over the female. At the same time, the male has an earlier opportunity to look for another female. Furthermore, captive males readily sequentially mated with multiple females, suggesting that free flying males might similarly optimize their fitness. *Diadasia* scramble competition polygyny thus shares characteristics of both a 'prolonged searching polygyny' and an 'explosive mating assemblage' (Emlen and Oring 1977, Thornhill and Alcock 1983, Herberstein et al. 2017): males must patrol to find rare females, but interference competition predominates and both sexes can mate multiply, all within a narrow time window (a breeding season of 6–11 d; Neff and Simpson 1992; S.L.B., A.L.R., and D.R.P., personal observation).

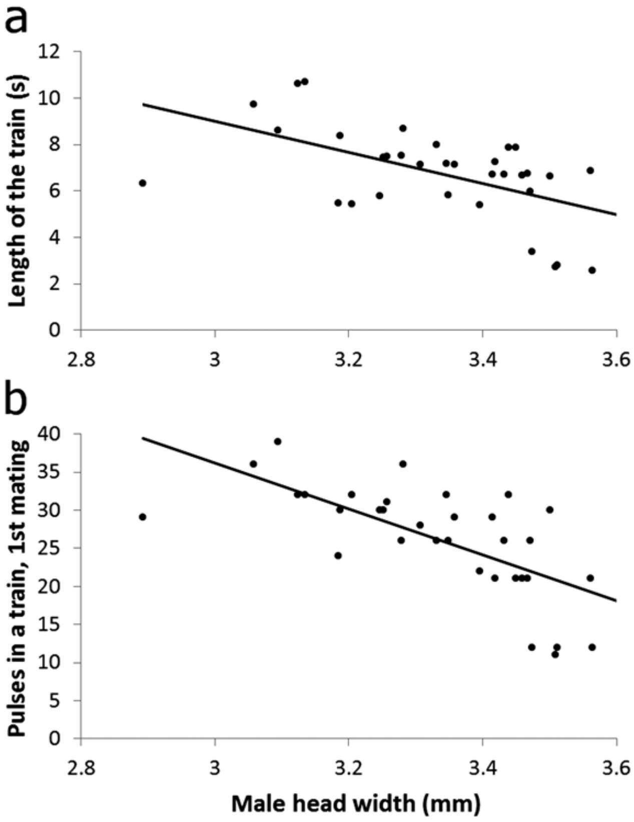

Fig. 5. The relationship between male body size (head width) and duration of the first copulation (vibration train duration) or number of primary pulses in the first copulation. (a) Male head width plotted against the duration of the first copulation vibration train. (b) Male head width graphed against the number of primary pulses made during the first copulation. N = 33 males.

A Large Male Advantage for Mate Acquisition

A strong male-biased operational sex ratio is also expected to select for traits allowing males to compete effectively with other males. Indeed, we found that mating males were larger on average than searching males (though still smaller than females; see also Neff and Simpson (1992)). It is common, however, to uncover a small male advantage in taxa under scramble competition, presumably because smaller males have better mobility/agility and are thus better able to acquire mates (e.g., Székely et al. 2004, Kelly et al. 2008, Herberstein et al. 2017; but see Fairbairn and Preziosi (1994) and references within; Bertin and Cézilly 2003). How body size affects the outcome of aggressive competition in scramble competition polygyny has rarely been examined (see Baena and Macías-Ordóñez (2015) and Herberstein et al. (2017)). A large male advantage in a scramble competition system could reflect enhanced prowess in physical competition or enhanced capacity to locate females, the latter being a consequence of mobility and perceptivity being potentially improved by body size (Schwagmeyer 1988, Herberstein et al. 2017). To disentangle these hypotheses, it would be useful to determine whether *Diadasia* males within mating balls are also larger than searching males, and whether mating males are on average larger than males within mating balls (Alcock 2013). We predict that bees in mating balls will be larger than searching males, reflecting a strong advantage of large body size in locating these ephemeral (<46 s on average) mating balls.

Weak Sperm Competition and Evidence for Female Choice

In scramble competition polygyny, long copulations trade off against locating and acquiring additional matings (Parker et al. 1999, Mazzi et al. 2009, Herberstein et al. 2017). On this account, larger *Diadasia* males, which are more likely to acquire mates, should invest less effort in each mating in order to increase mating frequency. Although we find that male *Diadasia* body size is negatively associated with copulation duration, copulation duration is short to begin with (<12 s) and decreases in duration are likewise small. Although this pattern is unlikely to provide substantial benefits in terms of enabling larger males to locate additional matings (unlike when copulation duration and decreases are long: see Holwell et al. (2016)), if males transfer sperm throughout copulation, shorter copulations would allow larger males to conserve sperm for subsequent matings. Additionally, the negative association between male body size and copulation duration may reflect female choice in some way, such as females permitting larger males to transfer sperm earlier in copulation (e.g., Fedina and Lewis (2007)). To test this, future work should examine how soon after genital coupling sperm transfer occurs and whether sperm transfer begins sooner with larger males. In this context, it is intriguing to note that the pronounced basitarsal spur used by the male in stroke-tapping behavior shows striking diversity within the genus *Diadasia* (see Supplementary Material). Although mating in other *Diadasia* species is poorly understood (see Neff et al. (1982), Ordway (1987), and Guardado Torres (1996) for brief accounts), we might expect the use of stroke-tapping to be correlated with the size and prevalence of the basitarsal spur and possibly accompanying female morphology for the spur to interact with.

Larger males (which acquire more matings) might also mate for shorter durations to reduce predation risk during mating (e.g., Sih et al. (1990)). Likewise, smaller males might risk predation and mate longer, given that they are less likely to mate. Mating pairs on the ground do not fly and are fully exposed at the open, unvegetated sites typical of *Diadasia* nesting aggregations. Indeed, we observed significant levels of predation on walking *Diadasia* by birds (cactus wrens, curve-billed thrashers, gilded flickers, Gila woodpeckers, and roadrunners) and lizards (e.g., desert spiny lizard and Sonoran spotted whiptail). If mating pairs are at greater risk than individual bees, this might explain in part our observation that the body size of mating males at our study site declined significantly across days in our 2016 survey (see Supplementary Material). Removal of larger males from the site due to selectively high predation on mating pairs may eventually permit smaller males to acquire matings (see Alcock (1995) and Oliveira et al. 2016). However, the decrease in mating duration with body size was small and future work will be required to investigate its impact on predation risk.

Large males might further enhance their fitness by mating selectively with larger and presumably more fecund females (e.g., Harari et al. (1999) and Hoefler (2007)). Such a pattern would constitute size-assortative mating (Harari et al. 1999, Hoefler 2007). We did not find evidence of size-assortative mating in *D. rinconis*. This is probably not surprising. In explosive breeding systems like *Diadasia,* where operational sex ratio is skewed, the more abundant sex is expected to be less choosy (Harari et al. 1999, Izzo et al. 2012). This lack of male choosiness is perhaps further reflected by the 3% of pairings emerging from mating balls in which a male was paired with another male. Size-assortative mating is also less likely when females are capable of mating multiply and when copulations are relatively short (Fairbairn 1990, Fairbairn 2007, Mobley et al. 2013, McDonald and Pizzari 2016), as we observed in *D. rinconis*. Polyandry weakens effects of male choice because ejaculates must compete postcopulation (sperm competition) and because selection for cryptic female choice is increased; shorter copulations are less costly, which relaxes selection for partner choice.

Function of Male Vibratory Courtship

Although we have presented large male advantage as a consequence of male-male competition within the mating ball, it could conceivably be due to female *Diadasia* choosing larger males within the ball. We suggest that female choice is more likely after a pair emerges from the mating ball and might be mediated by male vibratory courtship behavior (which occurred only during copulation). Primary pulse number was instead strongly negatively correlated with male body size. Because the cessation of male vibratory courtship coincided exactly with genital uncoupling, we propose that pulses stimulate female *Diadasia* to continue copulating. Other effects, such as stimulation of sperm uptake and sperm use, are also possible (Firman et al. 2017). Pulses may additionally dissuade searching males from breaking apart copulating pairs (e.g., a jamming signal, Miranda 2006): compared with first copulations, second copulations, which involved fewer pulses, were disrupted by intruder males significantly more frequently. Alternatively, second copulations may be more likely to be disrupted because the pair is together for longer and thus more likely to be discovered by intruder males.

In conclusion, we suspect that the copulatory vibrations of *D. rinconis* males may mediate cryptic female choice in *D. rinconis*. Although female *Diadasia* might also exhibit mate choice before copulation, as captive females often thwarted copulation attempts and readily remated, natural pairs were always observed to copulate. Future work needs to determine the extent of multiple mating by females in nature. Furthermore, we were not able to examine some aspects of the male vibratory courtship behavior on which female choice might operate. Specifically, although the interpulse interval was highly consistent within and between individuals, equipment limitations made it impossible to compare the amplitude of pulses (a presumably costly component of the copulatory behavior; see Conrad et al. (2010)) across bees. Future work might also focus on uncovering the basis for the bimodal distribution in vibration train duration, which could indicate a hidden alternative male mating strategy or different consequences of female choice. Finally, although female choice is often thought to drive selection for complex male courtship behavior (Eberhard 2017, Firman 2017), our results suggest that those behaviors might simultaneously influence choices of competing males.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Authors' Contributions

All authors conceived this research, designed experiments, and collected data. A.L.R. and S.L.B. conducted analyses. A.L.R. wrote the paper and all authors participated in revisions.

Data Availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

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