



## Research

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# Traumatic mating increases anchorage of mating male and reduces female remating duration and fecundity in a scorpionfly species

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Traumatic mating is the male wounding his mate during mating using specialized anatomy. However, why males have evolved to injure their mates during mating remains poorly understood. We studied traumatic mating in *Dicerapanorpa magna* to determine its effects on male and female fitness. The sharp teeth on male gonostyli penetrate the female genitalia and cause copulatory wounds, and the number of scars on the female genitalia is positively related to the number of times females mated. When the injurious teeth were encased with low-temperature wax, preventing their penetration of the female's genitalia during mating, male mating success and copulation duration were reduced significantly, indicating the importance of the teeth in allowing the male to secure copulation, remain in copula and effectively inseminate his mate. The remating experiments showed that traumatic mating had little effect on the female mating refractory period, but significantly reduced female remating duration with subsequent males, probably benefiting the first-mating male with longer copulation duration and transferring more sperm into the female's spermatheca. The copulatory wounds reduced female fecundity, but did not accelerate the timing of egg deposition. This is probably the first report that traumatic mating reduces female remating duration through successive remating experiments in animals. Overall, our results provide evidence that traumatic mating in the scorpionfly helps increase the male's anchoring control during mating and provides him advantage in sperm competition, but at the expense of lowering female fecundity.

## 1. Introduction

Females of most animal species are polyandrous, mating with multiple males during their lifetime [1–3]. Polyandry often increases female reproductive success. Depending on the species, polyandry may increase female fecundity or offspring genetic diversity, or result in more sperm to maximize fertilization of eggs or obtaining more male-provided material benefits [1,2,4]. For males, however, polyandry reduces reproductive success. A female mating with multiple males causes sperm competition among the males and thus reduces paternity in the female's egg output [2,5,6]. These circumstances in polyandrous systems lead to intersexual conflict, which occurs when the sexes pursue reproductive success in ways that interfere with the reproductive success of the opposite sex [7–11]. Intersexual conflict generates evolutionary selection on the sexes for traits that reduce the negative fitness effects of the opposite sex's conflicting activity.

Traumatic mating is an extreme example of intersexual conflict. It involves the integument of the female being wounded during mating from puncture by

her mate's specialized morphology [12–18]. From the perspective of males, four non-mutually exclusive hypotheses have been proposed in the literature to explain why males evolve injurious structures to damage their mates. The hypothesis of transition to internal fertilization suggests that traumatic mating with insemination functions to achieve internal egg fertilization. For example, Strepsiptera females lack a gonopore. Piercing the female's integument with the penis to transfer sperm is the only way the male can achieve fertilization [17,19]. The hypothesis of physical anchorage states that the male's specialized structure penetrating the female's body allows the male to continue mating longer and more effectively inseminate the female against female activity including her resistance. Examples are the genital teeth of male leaf-cutting ants and the fangs of male wolf spiders [20,21]. The hypothesis of paternity benefits suggests that traumatic mating benefits the male by sperm competition advantage over male competitors and thus increases paternity percentage in a mate's egg output. In seed beetles, the sharp genital spines do not serve as an anchorage function to prolong copulation duration. Instead the spines cause damage and reduced lifespan of the female and an increased competitive fertilization success of the male [22–24]. The hypothesis of fecundity stimulation proposes that the wounded female's resource allocation to immunity and healing of the copulatory wound delays the female's remating to the reproductive benefit of the wounding male; or the wound itself or its associated infection accelerates female reproductive investment and egg laying that benefits the wounding male [16,25]. However, there is no unequivocal evidence to support the hypothesis of fecundity stimulation. Owing to the complexity of mating activities, why males injure their mates in various forms of traumatic mating remains poorly understood.

Traumatic mating involves potentially high costs to female fitness, because of the physical damage and associated risks of wound infection, sexually transmitted disease, and the immune responses and re-allocation of available resources needed for healing [16,26,27]. However, strong evidence for the negative fitness effects of traumatic mating on the female is scarce [16]. Seed beetles and bed bugs are probably the only groups that provide experimental evidence showing that female genital damage from mating increases mortality and reduces fecundity of the female [12,26–30].

Scorpionflies (Mecoptera, Panorpidae) are ideal animals to investigate intersexual conflict because of their diverse mating strategies and complex grasping structures used in mating [8,31–33]. In general, a male scorpionfly provides a salivary mass as the nuptial gift and grasps the female by highly modified abdominal and genital structures ([31–33], figure 1a). The basal process on each male gonostylus is an important genital clasper and holds the female genital segment to stabilize the female medigynium [33]. In several scorpionfly taxa, the paired basal processes and the separate paired median processes on the gonostyli possess sharp teeth [34]. In the taxa in which males have the sharp teeth, the genital segments of females often have conspicuous 'brown scars'. In *Dicerapanorpa* species, the scars occur on the female medigynium and subgenital plate and are melanized and often scabbed and extensive over the surface [35–37]. However, it remains unknown whether the female wounds

are inflicted by the male's sharp teeth during mating. Research on the fitness effects of the gonostylus teeth and the genital wounds of scorpionflies has not, to our knowledge, been reported in the literature.

We focus in this study on the occurrence of female genital damage, the effects of the male's gonostylus teeth on male fitness, and the reproductive cost to the female of copulatory wounds in *Dicerapanorpa* scorpionflies. We hypothesize that: (i) the male gonostylus teeth pierce the female medigynium and subgenital plate during copulation and leave brown scars; (ii) male scorpionflies pierce their mates to enhance anchoring control (hypothesis of physical anchorage) and to increase their sperm competition advantage to improve paternity (hypothesis of paternity benefits); (iii) the healing of copulatory wounds delays female remating with a new male, and the infection of copulatory wounds promotes the female to lay eggs more quickly (hypothesis of fecundity stimulation); and (iv) the genital wounds caused by the teeth damage the female reproductive fitness by reducing her number of offspring.

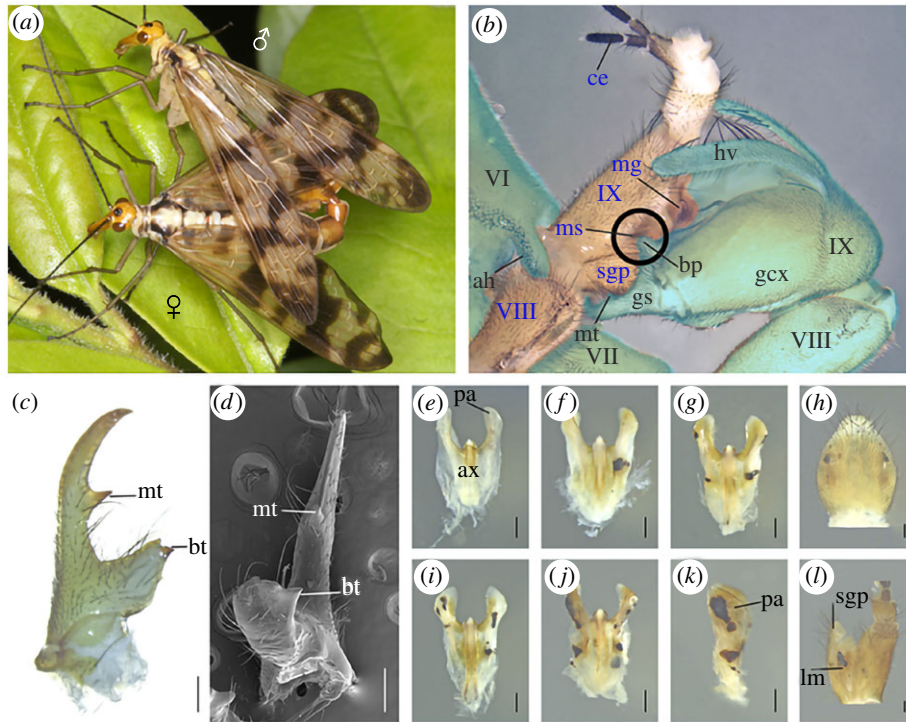
To test these hypotheses, the copulatory mechanism and female wounds of *Dicerapanorpa magna* [38] were investigated. The anchoring experiment was done to examine the enhanced anchoring control caused by the basal and median gonostylus teeth. The remating experiment was designed to test the effects of copulatory wounds on prolonging the female mating refractory period (time between matings) and reducing female remating duration. The experiment of female oviposition was used to determine whether copulatory wounds reduce female pre-oviposition period, fecundity or hatching rate. It was found that traumatic mating in the scorpionfly enhances the male's anchorage and control during mating and reduces the female remating duration and fecundity.

## 2. Material and methods

### (a) Study animals

Adults of *D. magna* were collected from Huoditang (33°25' N, 108°27' E, elev. 1500–2000 m) in the Qinling Mountains, central China from late April to late June in 2018 to 2020. The adults were checked immediately to confirm whether the female genitalia had brown scars and whether both the basal and median teeth of male gonostyli were sharp. Females without melanized scars were treated as virgins because they lacked sperm in their spermatheca (5/5, 100% without sperm), while the females with melanized scars had sperm in their spermatheca (5/5, 100% with sperm). This shows that females with melanized scars had mated. A total of 1000 age-unknown adults (480 female, 370 virgins; 520 male, 510 with sharp teeth) were captured at the spring emergence of the scorpionfly. Of these, 363 virgin females and 504 males with sharp teeth were used in the experiments.

Virgin females as well as males of unknown mating status were transported to the laboratory. All adults were identified with red, black, purple or blue marks on the forewings. Laboratory temperatures were kept at  $20 \pm 5^\circ\text{C}$  during the day and  $15 \pm 3^\circ\text{C}$  during the night, with a photoperiod of 14 D : 10 L and relative humidity of  $80 \pm 10\%$  [33]. Before being allocated to an experiment, 20–22 males or females were maintained in a single-sex cage ( $40 \times 40 \times 60$  cm), which contained fresh twigs of trees to simulate the natural environment and five dead mealworm larvae (*Tenebrio molitor*) as food. Twigs and food were replaced every 3 days to ensure freshness.



**Figure 1.** Genital coupling and genitalia of *Dicerapanorpa magna*. (a) A pair in copula (Photo by Wen Zhong). (b) Genital coupling to show the male basal teeth anchoring the female medigynium (black circle); the terminal end of male is tinted in smoky blue. (c,d) Male gonostyli in ventral and mesal aspects. (e–g), (i–k) Scars on female medigynium: (e) virgin female; (f) mated once; (g) mated twice; (i) mated three times; (j) mated multiple times; (k) mated multiple times, in lateral aspect. (h,l) Copulatory wounds on female subgenital plate. Ah, anal horn; ax, axis; bp, basal process; bt, basal tooth; ce, cercus; gcx, gonocoxite; gs, gonostylus; hv, hypovalve; lm, lateral membrane; mg, medigynium; ms, melanized scar; mt, median tooth; pa, posterior arm; sgp, subgenital plate; VI–IX, abdominal segments VI–IX. Scale bars, 0.2 mm. (Online version in colour.)

### (b) Occurrence of injury and female wounds

Sixty-three virgin females and 63 males of unknown mating status were randomly allocated to six cages to keep the sex ratio of 1 : 1 (10 or 11 adults for each sex in one cage) for two weeks. This allowed the opportunity for multiple matings. Mating behaviours were observed and recorded every 30 min for 24 h. Additionally five pairs in copula were frozen with a carbon dioxide aerosol sprayer [33]. Females with different numbers of mating times (eight females mated once; seven females mated twice; eight females mated three times) and the copulating pairs were dissected under a Nikon SMZ1500 microscope. The female genital scars and any injury by the sharp teeth to the female genitalia were observed using light microscopy (QImaging Retiga 2000R Fast 1394 Digital camera equipped on the microscope). The number of scars and the amount of surface area they covered with different numbers of mating times were measured with IMAGEJ v. 1.8.0.

For scanning electron microscopy, male gonostyli with basal and median teeth were dehydrated in a graded ethanol series, freeze-dried for 3 h, coated with gold in a sputter coater and examined in a Hitachi S-3400N scanning electron microscope at 5 kV.

### (c) Anchoring function of sharp teeth

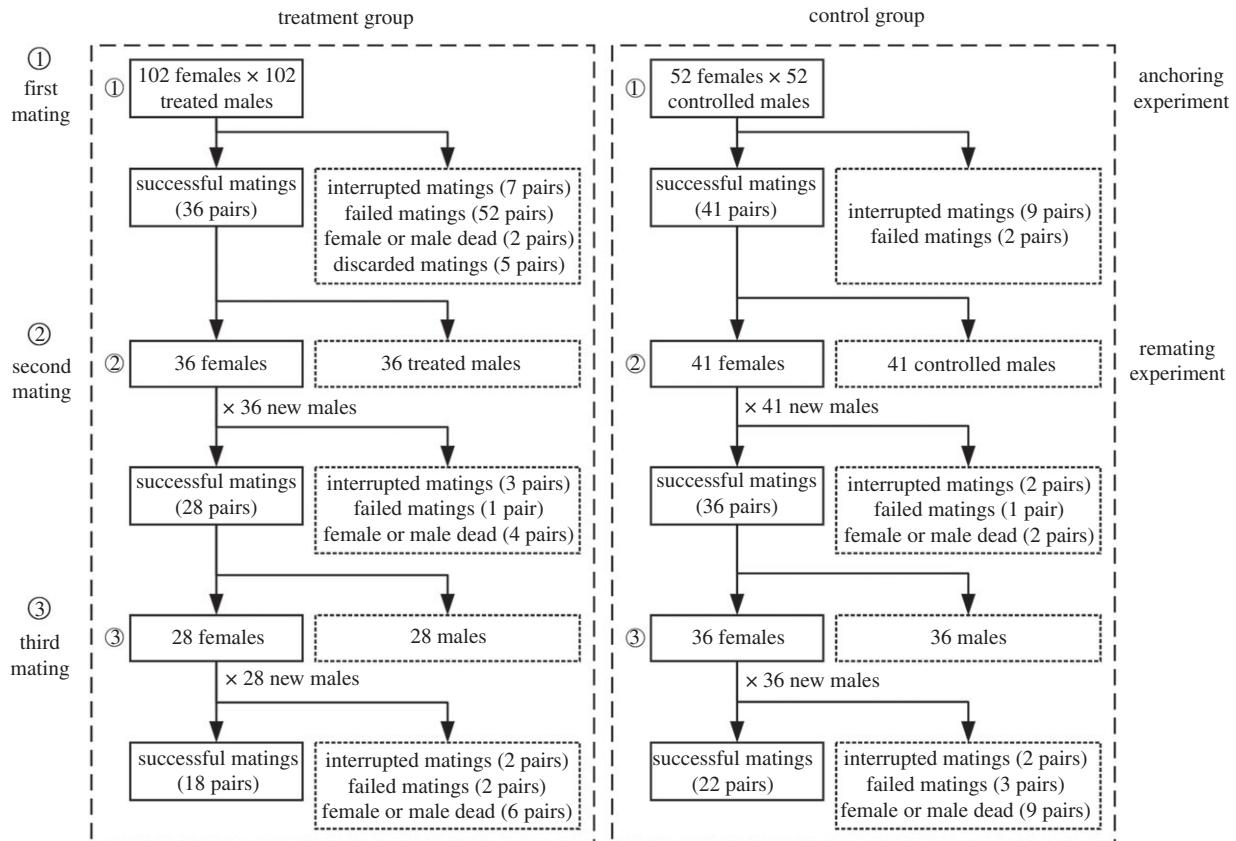
The basal and median teeth of gonostyli of 102 males were encased with low-temperature wax Dichen (Shengkun, Taizhou, China) in the treatment group, after the males had been anesthetized with diethyl ether. Distal parts of gonostyli of 52 males were encased with the wax in the control group (electronic supplementary material, figure S1). To reduce impact on males we used the wax to encase the teeth instead of cutting them off [39–42]. Then 102 and 52 virgin females were allocated to the treatment (11 cages) and control groups (6 cages), respectively, to keep the sex ratio of 1 : 1, 10 females + 10 males in one cage, or fewer for the remaining.

*Dicerapanorpa* males start copulation through seizing the female with the notal organ and the paired anal horns (figure 1b) without a salivary mass until the copulation has been maintained for several hours [36]. Thus the mating of *Dicerapanorpa* can be divided into a pre-gift-providing stage (from genital coupling until a salivary mass is provided by the male) and a post-gift-providing stage (from salivary mass providing to complete separation of mating partners). The pre- and post-gift-providing stages together constitute the entire copulation duration (from genital coupling to complete separation of mating partners) [36]. We observed and recorded the mating success, duration of the pre-gift-providing stage, and overall copulation duration of copulating pairs every 30 min continually for 24 h from the beginning to the end of the experiment. We predicted that the mating success, duration of the pre-gift-providing stage, and overall copulation duration would be reduced in the treatment group compared with the control group.

Once a mating ended, the male and the female were weighed using an electronic balance WT2002 K (accurate to 0.01 g, Wantai, Changzhou, China). Then the genitalia of both sexes were observed after maintaining the adults individually for 4 h. In the treatment group, five copulating pairs were discarded and not used in the subsequent experiments because the low-temperature wax fell off the teeth and melanized scars were present on the female genitalia (electronic supplementary material, table S1). The experimental time line is illustrated in figure 2.

To check whether the manipulation of low-temperature wax reduces male mating ability, 21 untreated males (blank control, the gonostylus unencased with low-temperature wax) and 21 virgin females were randomly allocated to two cages to keep the sex ratio of 1 : 1 (10 or 11 adults for each sex in one cage). The mating success, duration of the pre-gift-providing stage, and overall copulation duration were recorded.

To score mating success, we defined a failed mating as a male that attempted but failed to establish genital coupling with a



**Figure 2.** The time line of anchoring and remating experiments.

female; interrupted mating as genital coupling established but later interrupted by other adults (both male and female can interrupt a mating); and successful mating as genital coupling established and not interfered by other adults.

When either all the males or all the females have mated, mating success  $p$  can be calculated as follows:

$$p = \frac{N_s + N_i}{N_s + N_i + N_f},$$

where  $N_s$  is the number of successful matings,  $N_i$  is the number of interrupted matings and  $N_f$  is the number of failed matings.

#### (d) Mating refractory period and remating duration

Successful mating females of the anchoring experiment (36 in the treatment group and 41 in the control group) were allocated to 36 and 41 new untreated males, respectively, to record the rematings of the females (the second mating of the female), including the mating refractory period and remating duration.

Copulating pairs of failed (one pair in both the treatment and the control groups) and interrupted matings (three pairs in the treatment group and two pairs in the control group) were discarded. Copulating pairs of successful mating (28 in the treatment group and 36 in the control group) were weighed and maintained individually for 4 h (figure 2 and the electronic supplementary material, table S2). Then these females were randomly allocated to 28 and 36 new untreated males to allow re-mating (the third mating of the female) to take place as described above (the numbers of copulating pairs of failed, interrupted and successful matings in figure 2 and the electronic supplementary material, table S3).

To further determine whether the genital scars reduced the remating durations of females, we artificially wounded the medigynia of 40 virgin females with a heated needle. Based on our observation, marked scars can be found within 2 h after that manipulation. In 4 h after visible scars formed, these wounded

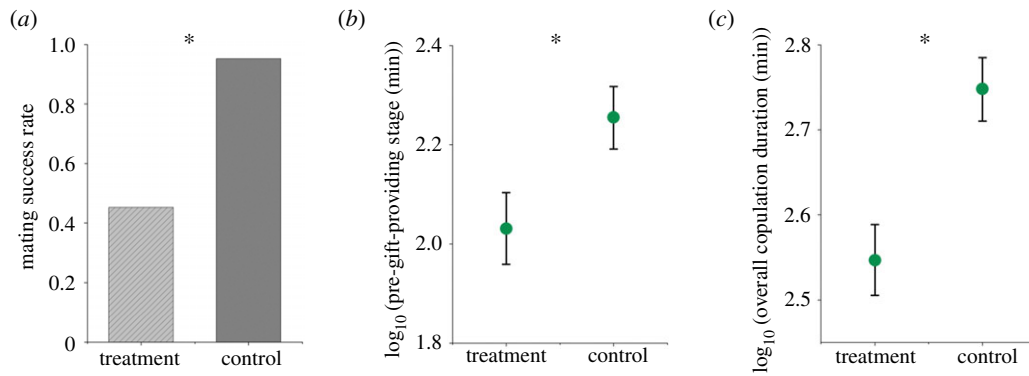
virgin females were randomly allocated to 40 untreated males (four cages, 10 females + 10 males in one cage) to allow mating to take place, and copulation durations were recorded.

#### (e) Female oviposition

We randomly allocated 80 virgin females (0.08–0.09 g of total body mass) and 80 untreated males to eight cages (10 females + 10 males in one cage) to allow the females to mate twice and recorded the copulation durations. We only recorded the oviposition situation of the females whose copulation duration lasted for 400–600 min. If the overall copulation duration of a twice-mating female was less than 400 min, the females were discarded owing to insufficient copulation duration (in total, five females in the treatment group and two females in the control group) (electronic supplementary material, table S4). Once the copulation duration reached 600 min, the copulating pair was artificially interrupted immediately to avoid excessive copulation duration. Then we artificially aggravated wounds inflicted on mated females' medigynia with a heated needle in the treatment group. The mated females in the control group were not manipulated as such. After that, all females were maintained individually in plastic cups with soil and food, and were checked every day to record the pre-oviposition period and fecundity. After oviposition, the females (29 in the treatment group and 37 in the control group) were removed from plastic cups. The deposited eggs were checked every day to record the egg stage duration. The larval number hatched was recorded every day to calculate the hatching rate of eggs. We stopped checking the eggs when no more hatching took place in four weeks.

#### (f) Statistical analyses

All statistical analyses were performed with R v. 4.0.3 [43]. Spearman's rank correlations were used to test the relationships between the number of mating times and the number and area



**Figure 3.** Effects of the encasement of gonostylus teeth on mating success and copulation duration of *Dicerapanorpa magna*. (a) Mating success, (b) pre-gift-providing stage and (c) overall copulation duration were significantly reduced. Dots and error bars represent means and 95% confidence intervals of log-transformed values of copulation durations, respectively. \* $p < 0.05$ .

of female scars. For the experiment of the anchoring function of gonostylus teeth, linear mixed models (LMMs) (the lmer function in the lme4 package [44]) were used to analyse the effects of teeth-encasement on copulation durations, with male weight as covariate, and cage number as random effect. The copulation durations were log-transformed to attain normality. A generalized linear mixed model (GLMM) with a logit link function (the glmer function in the lme4 package [44]) was used to examine the effect of teeth-encasement on copulation success (binary variable), with male weight as covariate, and cage number as random effect. LMMs and a GLMM were also used to test whether the manipulation of low-temperature wax influenced the mating ability of males using the same method as mentioned above.

For the experiment of the mating refractory period and remating duration, LMMs (the lmer function in the lme4 package [44]) were used to assess the effects of teeth-encasement on the female refractory periods and the effects of copulatory wounds on the female mating durations with subsequent males, with male weight as covariate, and cage number as random effect. The copulation durations were log-transformed to attain normality.

For the experiment of female oviposition, one-way ANOVA was used to assess the influence of artificial wounds on the female pre-oviposition period, fecundity, egg period and hatching rate.

### 3. Results

#### (a) Occurrence of injury and female wounds

During mating, male *D. magna* pierced the female medigynium and subgenital plate with basal teeth of gonostyli. Occasionally, the median teeth also injured the female subgenital plate (figure 1a–d). Both the number (Spearman's rank correlation,  $r = 0.907$ ,  $n = 23$ ,  $p < 0.001$ ) and the area of scars ( $r = 0.715$ ,  $n = 23$ ,  $p < 0.001$ ) were positively correlated with the number of mating times by females (figure 1e–l and the electronic supplementary material, figure S2).

#### (b) Anchoring function of sharp teeth

The teeth-encasement significantly affected the male mating success (GLMM,  $\chi^2_1 = 20.118$ ,  $p < 0.001$ ), duration of pre-gift-providing stage (LMM,  $F_{1,76} = 22.465$ ,  $p < 0.001$ ) and overall copulation duration (LMM,  $F_{1,76} = 52.679$ ,  $p < 0.001$ ). Consistent with prediction, when the male gonostylus teeth were encased (the treatment group), the mating success

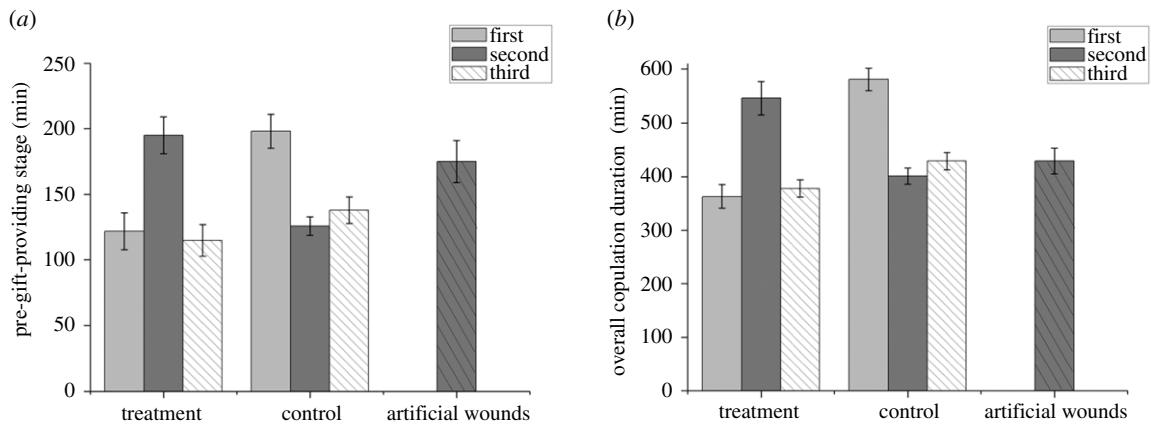
(45.2%, 43 of 95), pre-gift-providing duration ( $121.67 \pm 68.47$  min, mean  $\pm$  s.d.,  $n = 36$ ) and overall copulation duration ( $365.83 \pm 98.04$  min,  $n = 36$ ) were all significantly decreased compared with the control group (mating success 96.2%, 50 of 52; pre-gift-providing duration  $199.39 \pm 96.18$  min,  $n = 41$ ; overall copulation duration  $579.75 \pm 158.84$  min,  $n = 41$ ) (figure 3 and table 1). The male weight had no significant effects on the mating success (GLMM,  $\chi^2_1 = 0.024$ ,  $p = 0.876$ ), pre-gift-providing duration (LMM,  $F_{1,76} = 2.076$ ,  $p = 0.150$ ), and overall copulation duration (LMM,  $F_{1,76} = 1.447$ ,  $p = 0.229$ ). The manipulation of low-temperature wax had no significant effect on the male mating ability (mating success, GLMM,  $\chi^2_1 = 0.0546$ ,  $p = 0.815$ ; pre-gift-providing duration, LMM,  $F_{1,57} = 0.618$ ,  $p = 0.390$ ; overall copulation duration, LMM,  $F_{1,57} = 0.026$ ,  $p = 0.872$ ).

#### (c) Mating refractory period and remating duration

The injury to the female medigynium during mating had no significant effect on the female mating refractory period (time interval between the first and second matings, LMM,  $F_{1,63} = 0.244$ ,  $p = 0.628$ ). The interval between the second and third matings did not differ significantly between the treatment and control groups (LMM,  $F_{1,63} = 1.047$ ,  $p = 0.354$ ) (electronic supplementary material, figure S3). The male weight did not significantly influence the copulatory intervals (the first and second matings, LMM,  $F_{1,63} = 0.425$ ,  $p = 0.514$ ; the second and third matings, LMM,  $F_{1,63} < 0.001$ ,  $p = 0.996$ ).

However, the injury to the female medigynium had significant effects on the female remating durations (pre-gift-providing duration, LMM,  $F_{1,63} = 19.619$ ,  $p < 0.001$ ; overall copulation duration, LMM,  $F_{1,63} = 16.703$ ,  $p < 0.001$ ). The pre-gift-providing duration ( $195.71 \pm 76.61$  min,  $n = 28$ ) and overall copulation duration ( $546.43 \pm 165.44$  min,  $n = 28$ ) in the treatment group were significantly longer than those in the control group (pre-gift-providing duration,  $126.11 \pm 44.41$  min,  $n = 36$ ; overall copulation duration,  $401.86 \pm 90.06$  min,  $n = 36$ ) (figure 4 and table 1).

The injury to the female medigynium significantly influenced the copulation duration of the female with a subsequent mating male for the first mating in the control group (pre-gift-providing duration, LMM,  $F_{1,76} = 19.177$ ,  $p < 0.001$ ; overall copulation duration, LMM,  $F_{1,76} = 38.108$ ,  $p < 0.001$ ) and the second mating in the treatment group (pre-gift-providing duration, LMM,  $F_{1,45} = 17.862$ ,  $p < 0.001$ ; overall copulation duration, LMM,  $F_{1,45} = 10.991$ ,  $p < 0.001$ ).



**Figure 4.** Influence of copulatory wounds on remating durations of female *Dicerapanorpa magna*. (a) Duration of pre-gift-providing stage and (b) overall copulation duration of the successful mating in the first, second and third mating, and in mating of virgin females with artificial wounds (mean  $\pm$  s.e.; in the second mating,  $n = 28$  for treatment group,  $n = 36$  for control group; in the third mating,  $n = 18$  for treatment group,  $n = 22$  for control group;  $n = 12$  for artificial wounds).

**Table 1.** Duration of pre-gift-providing stage and overall copulation duration of *Dicerapanorpa magna* females in three matings and artificial wounding experiments.

order	treatment	pre-gift-providing stage (min)				overall copulation duration (min)			
		<i>n</i>	mean	$\pm$ s.d.	$\pm$ s.e.	<i>n</i>	mean	$\pm$ s.d.	$\pm$ s.e.
first	treatment group	36	121.67	68.47	11.41	36	365.83	98.04	16.34
	control group	41	199.39	96.18	15.02	41	579.75	158.84	24.80
	blank control	17	223.82	117.67	28.54	17	575.88	147.05	35.67
second	treatment group	28	195.71	76.61	14.48	28	546.43	165.44	31.27
	control group	36	126.11	44.41	7.40	36	401.81	90.06	15.01
third	treatment group	18	115.28	50.63	11.93	18	398.89	67.97	16.02
	control group	22	138.63	49.79	10.62	22	429.09	76.80	16.35
artificial wounds on virgins		12	175.00	58.85	16.98	12	429.16	86.07	24.85

In the control group, the pre-gift-providing duration ( $126.11 \pm 44.41$  min,  $n = 36$ ) and overall copulation duration ( $401.81 \pm 90.06$  min,  $n = 36$ ) of the second mating were significantly shorter than those of the first mating (pre-gift-providing duration,  $199.39 \pm 96.18$  min,  $n = 41$ ; overall copulation duration,  $579.75 \pm 158.84$  min,  $n = 41$ ) (figure 4 and table 1). In the treatment group, the pre-gift-providing duration ( $115.28 \pm 50.63$  min,  $n = 18$ ) and overall copulation duration ( $398.89 \pm 67.97$  min,  $n = 18$ ) of the third mating were also significantly shorter than those of the second mating (pre-gift-providing duration,  $195.71 \pm 76.61$  min,  $n = 28$ ; overall copulation duration,  $546.43 \pm 165.44$  min,  $n = 28$ ) (figure 4 and table 1).

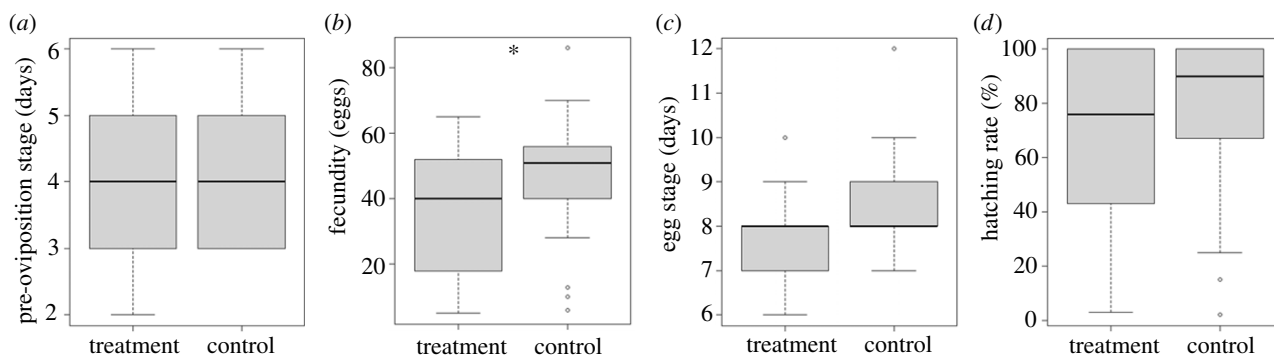
When the medigynia of virgin females were injured by a heated needle, the pre-gift-providing duration ( $175.00 \pm 58.85$  min,  $n = 12$ ) was slightly shorter than that of the control group ( $199.39 \pm 96.18$  min,  $n = 41$ ), but not significantly (LMM,  $F_{1,52} = 0.406$ ,  $p = 0.487$ ) (figure 4a and table 1). The overall copulation duration of the artificially wounded group ( $429.16 \pm 86.07$  min,  $n = 12$ ), however, was significantly shorter than that of the control group ( $579.75 \pm 158.84$  min,  $n = 41$ , LMM,  $F_{1,52} = 13.3504$ ,  $p < 0.001$ ) (figure 4b and table 1). The male weight had no significant effects on the male copulation durations (pre-gift-providing duration, LMM,  $F_{1,52} = 0.375$ ,  $p = 0.540$ ; overall copulation duration, LMM,  $F_{1,52} = 0.619$ ,  $p = 0.431$ ).

#### (d) Female oviposition

Copulatory wounds had no significant effects on the female pre-oviposition period (ANOVA,  $F_{1,65} = 1.536$ ,  $p = 0.22$ ), egg stage duration (ANOVA,  $F_{1,65} = 2.982$ ,  $p = 0.089$ ), and hatching rate (ANOVA,  $F_{1,65} = 2.946$ ,  $p = 0.091$ ). The pre-oviposition period (mean  $\pm$  s.d.,  $4.34 \pm 1.28$  d,  $n = 29$ ), egg stage duration ( $7.93 \pm 0.84$  d) and hatching rate ( $64.72\% \pm 34.38\%$ ) in the treatment group were similar to those of the control group (pre-oviposition period,  $4.00 \pm 0.97$  d,  $n = 37$ ; egg stage duration,  $8.32 \pm 0.97$  d; hatching rate,  $77.94\% \pm 28.20\%$ ) (figure 5a,c,d). The wounds, however, significantly reduced the female fecundity (ANOVA,  $F_{1,65} = 6.223$ ,  $p = 0.015$ ). The egg number per female in the treatment group ( $36.31 \pm 18.12$  eggs,  $n = 29$ ) was significantly fewer than that in the control group ( $46.75 \pm 15.87$  eggs,  $n = 37$ ) (figure 5b).

## 4. Discussion

The present study reported traumatic mating in the insect order Mecoptera, to our knowledge for the first time. The sharp teeth of male gonostyli pierce the female genital segment and leave scars. When the sharp teeth are encased by wax, the male mating success and copulation duration are



**Figure 5.** Influence of copulatory wounds on female oviposition of *Dicerapanorpa magna* between treatment ( $n = 29$ ) and control groups ( $n = 37$ ). (a) Pre-oviposition stage duration, (b) fecundity, (c) egg stage duration and (d) hatching rate. \* $p < 0.05$ .

reduced significantly. Copulatory wounds have no significant effect on the female remating propensity, but reduce the female remating durations with subsequent males. This is probably the first report that traumatic mating reduces the female remating duration through successive remating experiments in animals. The number of sperm transferred is positively correlated with male copulation duration in scorpionflies of the genus *Panorpa* [45,46], which are close phylogenetic relatives of *Dicerapanorpa*. Hence, traumatic mating in *Dicerapanorpa* may increase the stored sperm proportion of the first-mating male in the female mate's spermatheca. Copulatory wounds do not accelerate the investment of the female causing egg deposition more quickly, but do reduce female fecundity significantly. Traumatic mating of *Dicerapanorpa* scorpionflies probably evolved because it enhances male control during mating and increases the first-mating male's chance of winning sperm competition but at the expense of reducing the number of offspring produced by the female.

The primary function of genital spines, hooks or teeth in male insects is to aid the males in firmly anchoring the female [16,20,21,40]. Similarly, the gonostylus teeth of male scorpionflies increase the male's mating control over the female by increasing mating success and copulation duration. In fact, the basal processes of male scorpionflies in various forms clamp the female during mating, including the cupped and truncated processes [33,34]. We believe that the teeth of gonostyli are a morphological adaptation of basal processes that functions to control the female during mating.

Consistent with previous studies [16,28,29], copulatory wounds played little role in affecting the female mating refractory period and female acceleration of reproductive investment. This phenomenon may be explained by three possible reasons. First, copulatory wounds are usually small, and are quick to scab, reducing the risk of infection. Thus they may not cue likely death of the female and thereby do not stimulate acceleration of female terminal investment [47,48]. Second, females can evolve thickened or sclerotized adaptations to patch or resist the male damage [49–51]. Third, seminal fluid may provide nutritive compensation to minimize the cost to female longevity resulting from the physical damage [16,28].

Our results are probably the first to report the phenomenon that traumatic mating can reduce the female remating duration of animals. Notably, although the copulation duration of successive mating is reduced, there is no sustained

decrease with the increase of mating times, at least over the range of matings in our study. This implies that the reduction of remating duration is passively affected by copulatory wounds, rather than actively reduced by the female.

Cryptic male mate choice may be an alternative hypothesis to explain the reduction of the remating duration caused by melanized scars. Previous studies, including in *Panorpa*, found that male insects can assess the amount of rival's sperm transferred and adjust their investment accordingly with decreasing amounts of investment with more sperm from rival matings [6,52]. In *Dicerapanorpa*, copulatory wounds inflicted by the first-mating male may serve as a cue by which subsequent males evaluate the mating situation of the female. If a male judges from scars that the female has already mated, he may strategically reduce mating duration and hence sperm investment to save resources.

In fact, the reduction of female remating duration with subsequent males, in turn, gives the first-mating male a sperm competition advantage. Female scorpionflies randomly use the sperm stored in the spermatheca [45,46]. The number of male sperm transferred is positively related to copulation duration. Therefore, the paternity of male scorpionflies can be decided by their copulation duration to a great extent [45,46]. For male scorpionflies that engage in traumatic mating, the sharp teeth function to prolong copulation duration, and the genital wounds reduce the copulation duration of the female with subsequent rivals. Both methods are probably beneficial for the first-mating male to increase his ratio of sperm in his mate's spermatheca, and thus increase his advantage in sperm competition.

The evolution of injurious mating structures in many species may be related to sperm competition. Besides *Dicerapanorpa* scorpionflies, in which the genital damage caused by the sharp teeth can reduce the female remating duration, males of other animals have also evolved injurious structures that increase their paternity. For example, traumatic insemination in the spider *Harpactea sadistica* evolved through sperm competition as a means for males to circumvent female choice mechanisms by bypassing the female genital tract and sperm storage organs [14]. The male seed beetle *Callosobruchus maculatus* has evolved sharp genital spines that stimulate the female to receive more seminal fluid, which increases male competitive fertilization success [16,22–24]. In general under polyandry, the high sperm competition faced by males may result in the evolution of male injurious structures to avoid sperm competition or to increase the

chance of winning sperm competition to obtain the higher paternity percentage.

The phenomenon that copulatory wounds reduce female fecundity is also reported in other invertebrates [26,29], and even vertebrates [53]. This is one of the most common negative effects of traumatic mating [12,26–29]. However, male scorpionflies secrete one or two columnar salivary masses as a nuptial gift to the female during mating [36], differing greatly from the well-studied bed bugs, seed beetles, fruit flies and sea slugs, which lack any edible gift-providing behaviour for mating [12–18]. Nuptial feeding is beneficial for the female to improve the quality and quantity of offspring [45,54]. Although the male *Dicerapanorpa* scorpionflies have nuptial feeding behaviour during traumatic mating, the fecundity of the female is reduced significantly, suggesting that genital damage inflicts a major adverse effect on the female reproductive fitness.

In conclusion, the research we conducted identified the male fitness effects and the female cost of traumatic mating in a scorpionfly species: such mating anchors the mating male, increasing copulation duration and sperm transfer, but at the expense of reducing female fecundity. Our research reported here is possibly the first to find the negative effect of genital damage on the female remating duration. Based on

this, we propose a hypothesis that copulatory wounds in this species are a cue to males that the female has already mated. The perception of this cue in turn, we hypothesize, causes a subsequent male to reduce the sperm investment of his mating. Also we hypothesize that this phenomenon results in the first-mating male obtaining the greatest advantage in sperm competition. This research may provide new insights into understanding the evolution of traumatic mating.

**Data accessibility.** Supporting data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.bnzs7h49h> [55]. The data are provided in the electronic supplementary material.

**Authors' contributions.** X.T.: data curation, formal analysis, investigation, software, writing—original draft; P.-Y.W.: investigation; M.-Z.J.: investigation; R.T.: writing—review and editing; B.-Z.H.: conceptualization, funding acquisition, project administration, resources, writing—review and editing. All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

**Competing interests.** We declare we have no competing interests.

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