



## Predators induce conditions for size-dependent alternative reproductive tactics in a water strider male



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Alternative reproductive tactics (ARTs) are commonly associated with differences in morphological, physiological and behavioural traits. The morphological differences can be associated with differences between ARTs in effectiveness of sexual display but the relationship has rarely been documented. We tested it using the Asian water strider *Gerris gracilicornis* (Heteroptera: Gerridae), in which males have two ARTs: a signalling courtship tactic and a nonsignalling tactic. Many *G. gracilicornis* males employ an intimidating signalling courtship tactic capitalizing on predators' behaviours and female responses to predators. The males produce courtship ripple signals on the water's surface by vibrating their middle legs after mounting the female. In this study we found that smaller males more often adopted a non-signalling than a signalling courtship tactic. We hypothesized that smaller males with shorter middle legs and weaker muscles may not be able to produce ripples that are sufficiently strong to attract predators from a distance, and therefore sexual selection favours nonsignalling tactics in smaller but not larger males. We created a  $2 \times 2$  experimental design to test the contribution of male body size to the intimidation effect under different levels of predation risk. We showed that only large males' ripple signals had an intimidation effect on females. We also found that females suffered a higher predation risk when large, but not small, males mounted them. The signal intensity of large males was also stronger than that of small males. Hence, we suggest that size-dependent effectiveness of the intimidation signalling maintains the size-dependent reproductive tactics in *G. gracilicornis*.

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Alternative reproductive tactics (ARTs) are widely observed in inferior males which subvert the female's preference for high-quality males by adopting nondisplaying or sneak mating tactics (Gross, 1996; Oliveira, Taborsky, & Brockmann, 2008; Shuster & Wade, 2003). ARTs are also commonly associated with discrete morphological traits (reviewed in Brockmann, 2008). Large males may develop conspicuous ornaments adapted for male-male competition or sexual display, whereas small males may develop only rudimentary ornaments. When these individual differences in body size or ornaments relate to the differences in reproductive behaviour, morphological differences can contribute to the evolution of ARTs (Aubin-Horth, Dodson, & Sinervo, 2004; Brockmann, 2002; Gross, 1996; Magellan, Pettersson, & Magurran, 2005; Moczek & Emlen, 2000). Large males with fully developed ornaments are usually territorial, engage in male-male competition and

exhibit courtship displays to attract females. In contrast, small inferior males with rudimentary ornaments are usually non-territorial, parasitizing superior males' courtship efforts, and attempting to copulate without courtship displays.

If body size or ornament size affects the characteristics of the courtship signalling, then the morphological differences may cause differences in effectiveness of sexual displays, creating sexual selection for morphology-dependent ARTs. For example, male guppies, *Poecilia reticulata*, increased courtship display when their tail length, which appears to affect the effectiveness of the courtship signal, was increased by manipulation (Karino & Kamada, 2009; Karino & Kobayashi, 2005). In insects, large male *Drosophila* were more successful in a vibrational courtship as a result of their larger wing area (Ewing, 1964). When the courtship effectiveness is size dependent and courtship is costly to produce, alternative non-signalling tactics might evolve in small males. Despite the effect of body size on ARTs, the relationship between body size (morphological phenotype), effectiveness of courtship behaviour (behavioural phenotype) and ARTs (reproductive tactics) is still poorly

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understood (but see Malavasi, Lindström, & Sundström, 2001; Malavasi, Torricelli, Lugli, Pranovi, & Mainardi, 2003).

Sneaker tactics arise from fitness benefits when inferior males avoid engaging in costly courtship displays (Brockmann, 2008). For example, when females prefer high-quality males which perform an elaborate courtship display, inferior males may subvert female mate choice and enjoy a mating advantage by adopting a non-displaying coercive tactic with less investment in ornaments (reviewed in Brockmann, 2008; Taborsky, Oliveira, & Brockmann, 2008). The sneaker tactic can arise as a 'best-of-a-bad-job' strategy when the fitness of sneaker small males is higher than that of signalling small males (e.g. when sexual selection acts against small males performing ineffective courtship display with rudimentary ornaments).

Here we tested the relationship between body size, courtship effectiveness and ARTs in an Asian water strider, *Gerris gracilicornis*. The males of this species use coercion to mount a female and the biomechanics of these initial struggles lead to size-assortative mating initiation (Han, Jablonski, Kim, & Park, 2010). After mounting a female successfully, a male produces courtship ripples on the water's surface by vibrating his middle legs (Fig. 1; Han & Jablonski, 2009). This unusual courtship behaviour is a form of male intimidation because the courtship ripple signals from males riding on top of a female attract aquatic predators such as notonectids (Hemiptera, Notonectidae), and the female is more vulnerable to predation because she is beneath the male (Han & Jablonski, 2009, 2010). Therefore females appear to be 'intimidated' by this potential increase in predation risk, especially in habitats with high predation risk, which the females can detect based on the perceived unsuccessful attack attempts by predators (Han & Jablonski, 2010). Therefore, females rapidly submit to copulation, to which males respond by stopping the intimidation signalling (intimidating courtship; Han & Jablonski, 2010). Typically a male continues signalling as long as the female does not allow intromission (Han & Jablonski, 2008, 2010). In contrast to this intimidating courtship strategy of the majority of males, we noted that the very small males tended to produce no such courtship

ripple signals after mounting the female (see Results), suggesting an existence of an alternative nonsignalling strategy in this species.

We hypothesize that size-dependent effectiveness of courtship signals in inducing females to copulate (i.e. intimidating females) contributes to the evolution of a nonsignalling strategy in small males. It is possible that larger males with longer middle legs and stronger muscles are able to produce stronger ripples, which attract predators from further away (allometry hypothesis). If so, we predict that the females will not respond to the intimidation signalling by mounted smaller males as strongly as to the large males. However, regardless of the intensity of intimidating courtship ripples, females mounted by large and heavy males are predicted to be more susceptible to predators' attacks and, therefore, more susceptible to mating attempts from large males (weight hypothesis). Previous research on water striders has shown that a female in mating tandem incurs higher predation risks than a single female (Arnqvist, 1989; Fairbairn, 1993; Rowe, 1994). This is known to be due to either decreased mobility because of the weight of the male or increased visibility. However, none of the studies has tested how the size of mounting males affects the predation risk of females during mating. Given that heavy individuals are more vulnerable to predators due to decreased mobility or increased visibility (Arnqvist, 1989; Fairbairn, 1993; Rowe, 1994), we suggest that a female's predation risk is less affected by a small male than by a large male mounted on top of her.

Here, we tested this hypothesis by asking the following questions. (1) Do smaller males employ the nonsignalling strategy more often than large males, and is there a size threshold below which only the nonsignalling strategy is observed in males? (2) Do smaller males mounted on females increase the predation risk to the females less than larger males? (3) Is the signal intensity (amplitude of waves) of smaller males weaker than that of larger males? (4) Do females respond less to the signalling by smaller than by larger males?

## METHODS

### *Study Species and Rearing Conditions*

*Gerris gracilicornis* is widely distributed in East Asia with a mating season between March and June. Insects were collected in creeks in Gwanak Mountain near Seoul National University. Males and females were put in separate rectangular, plastic basins (40 × 50 cm) and were fed ad libitum daily with surplus frozen crickets, *Gryllus bimaculatus*. Pieces of polyethylene sheets were provided as rest sites. All animals were individually marked on the thorax with small dots of enamel paint.

Notonectids, *Notonecta triguttata*, generalist predators that attack small insects fallen on the water's surface, were used as predators for water striders in the experiment. They detect prey at a distance using water vibrations and disturbances produced by prey (Lang, 1980). Notonectids were collected at pools near rice fields in the Kimpo region, South Korea, and individually kept in transparent, plastic, cylindrical boxes (diameter 10 cm, height 15 cm). Each individual received one frozen *G. bimaculatus* every day but was starved for 3 days before experiments (see below).

### *Size Distribution of Signalling and Nonsignalling Males*

To distinguish nonsignalling from signalling males, we observed the courtship behaviour of 192 males when we put males and females together in a basin. We classified a male as a 'signalling' male when he produced courtship ripples by vibrating his middle legs while attempting to mate with females (described in Han & Jablonski, 2009) and as a 'nonsignalling' male when he did not



**Figure 1.** A mating pair of *Gerris gracilicornis* and a notonectid predator (*Notonecta triguttata*). The male mounting the female is producing courtship ripple signals with his middle legs stretched forwards, parallel to each other (see Han & Jablonski, 2009). The predator is approaching the pair in response to the male's ripple signals.

produce courtship ripples during the precopulatory period (before genitalia intromission). Logistic regressions with binomial error were used to evaluate the relationship between body size and courtship behaviour (signalling versus nonsignalling). Prior to the observation, the body size (length from the tip of the anteclypeus to the most distal point of the last genital segment) of individual males and females was measured to the nearest 0.01 mm using digital photographs and Image J software (National Institutes of Health, Bethesda, MD, U.S.A.). Based on the result, we classified the signalling males that were either below the lower quartile (1.16–1.22 cm) or above the upper quartile (1.27–1.34 cm) as 'small' or 'large' signalling males (Fig. 2).

To test whether small males' nonsignalling courtship tactic depends on the size of the female, 18 nonsignalling males selected from the initial observation of mixed males and females were also paired with different sizes of females. Six males were paired with large females (body size > 1.49 cm), five with medium-sized females (1.45–1.49 cm) and seven with small females (<1.45 cm). Each male's courtship tactic (signalling/nonsignalling) was observed in an experimental basin (15 × 15 cm, water depth 5 cm). In addition, to test whether each male's courtship tactic was repeatable over time, each male was paired again with the same female on the next day and his courtship tactic was noted.

#### Relative Signal Intensity of Large and Small Males

To compare the relative amplitude of waves of courtship signals of large and small males, we recorded the courtship ripple signals of 12 large signalling males (1.27–1.34 cm) and 12 small signalling males (1.16–1.22 cm) using a similar method to that used by Wilcox and Kashinsky (1980). The recorder was composed of a small Styrofoam ball attached to the end of a voltmeter stylus, which was linked to an amplifier (Wilcox & Kashinsky, 1980; see Appendix 1, Fig. A1). The recorder converted the oscillatory movements of the Styrofoam ball on the water's surface to electronic signals. The electronic signals passed through the voltage amplifier and were saved in Waveform audio format. In addition, using the vibrations present on the water's surface both before and after the signals were recorded, the program Adobe Audition filtered noise in the recorded ripple signals. Spectrograms of recorded signals were analysed by the program Spectrogram (Visualization Software, <http://visualization-software-llc.software.informer.com/>).

To measure differences in the relative signal intensity between large and small males, each male was paired with the same female (body size: 14.4 cm, population average size). The female was freshly dead and attached to a thin wire that was also attached to a movable stick (see Appendix 1, Fig. A1). Because *G. gracilicornis* males are eager to initiate mating during the reproductive period, they will attempt copulation even with a dead individual. The experimental container (plastic, 40 × 50 cm and 15 cm high) had a recording space (acrylic plate, 10 × 50 cm and 5 cm high) separated

by a submerged partition (5 cm) and another partition (10 cm) about 3 mm above the water's surface (Fig. A1). Water was filled above 5 cm to allow ripple signals to transmit from the internal recording space to the external part and to dissipate without reflections (Fig. A1). The water depth was maintained the same during recording of ripple signals. Additionally, 'wave-breakers' made of aluminium foil attached along the inside walls of the experimental container further reduced the reflective signals (Fig. A1).

First, we placed a male in the recording space in the experimental container with the dead female. We then allowed the male to initiate attempts to mount the female to produce courtship ripple signals. After pairing, using the movable stick, we carefully moved the pair 3 cm from the Styrofoam ball at the end of the stylus. We then recorded three bouts of ripple signals after mounting males had produced courtship signals for 1 min.

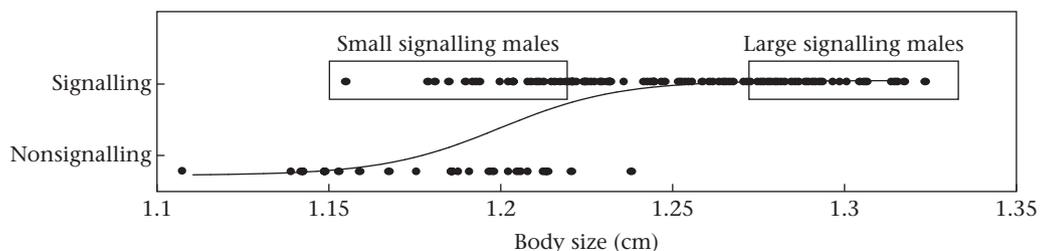
The basic unit of the males' courtship ripple signals consisted of a single tap on the water's surface with their middle legs. This behaviour created a short pulse on the water's surface, and several pulses were produced in bouts. Thus we regarded the highest amplitude of a pulse in three bouts of signals to represent the signal intensity of the male. The recorded signal amplitude was scaled in relative decibels (dB). Thus independent *t* tests were used to compare differences in the signal intensity between large and small males.

#### Predator Success in Attacks on Mating Females

The level of predation risk was assessed by the number of unsuccessful attacks of a notonectid before the first successful attack. Thus, we focused here on what happened after the predator had already detected the mating water striders.

Twenty-one females randomly paired with large and small signalling males were exposed to a single *Notonecta*. Nonsignalling small males were not used in this analysis. Each pair was exposed to the same notonectid individual for 2 consecutive days. On the first day, 11 females were individually paired with large signalling males (1.27–1.34 cm) and 10 with small signalling males (1.16–1.22 cm). Once a male had mounted, the pair was put in a plastic basin (30 × 30 cm) in which one starved notonectid was waiting. When notonectids detect a prey on the water's surface, they approach to grasp the prey with their forelegs (successful attack). However, the prey occasionally escapes before the notonectid grasps it (unsuccessful attack). In the experiment, the number of unsuccessful attacks by the notonectid was recorded until the notonectid successfully grabbed either member of the mating pair (usually the female). Females were separated from the predator soon after the successful attack.

A day after the first experiment, we paired the same females with males of a different size class to that of the first male, in order to keep the design balanced, and we replicated the assay. Males used on the first day of the experiment were not reused on the



**Figure 2.** Body size distribution of signalling males (courtship tactic) and nonsignalling males ( $N = 192$ ). Each dot denotes an individual male. The boxes in the figure represent size categories of large and small males used in the experiment.

second day. However, since the same females were used in this experiment over 2 days, paired *t* tests were used to compare the number of unsuccessful attacks between pairs with large and with small males.

#### *Female Response to Courtship under Different Risk*

To examine the contribution of male body size to the intimidation effect under different levels of predation risk, we created a  $2 \times 2$  experimental design and tested 16 experimental females under four conditions on 4 consecutive days. In each assay, we measured two variables that represent two aspects of the female's resistance to mating after the male has mounted her: (1) female latency until genitalia protrusion (duration in seconds from the moment a male attempted to mate until the successful intromission; during this whole time the signalling males continue producing signals on the water's surface); (2) female physical (or 'direct') resistance (whether the female attempted to dislodge the mounting male or not). The former may be viewed as indicating 'passive resistance' and the latter as indicating 'active/physical/direct resistance' by females to the male's attempts to mate (Han & Jablonski, 2010). Delaying genitalia protrusion by females can be a form of resistance to unwanted matings that delays and sometimes completely prevents copulation (see Brennan & Prum, 2012). To measure these behaviours, we followed the method outlined by Han and Jablonski (2010). On the first 2 consecutive days, females were tested with large and small males adopting a courtship tactic, followed by the predator assays. For the latter, the females were also tested with large and small males in the same manner but after they had experienced predatory attacks as described below.

On the first 2 consecutive days, using females that had not been exposed to predators, we recorded females' mating behaviours after pairing them with large or small signalling males in a balanced order (control assay). On Day 1, eight females were individually paired with large signalling males (1.27–1.34 cm) and eight with small signalling males (1.19–1.22 cm). On Day 2, for the balanced design, each female was paired with a male of the opposite size, and we replicated the observation. Prior to the experiment, we screened each male's reproductive tactic, and nonsignalling small males were not used in this analysis.

For the test, a transparent basin (acrylic,  $15 \times 30$  cm) was divided by an opaque acrylic plate ( $15 \times 15$  cm), the lower 5 cm of which was fixed and submerged and the upper 10 cm was movable (see Appendix 1, Fig. A2). The submerged portions of the compartments were totally separated to block the transmission of chemical signals through the water between them. But, as the upper partition could be moved up and down, individuals could move between the two compartments. For each observation session, we put a male and a female in each compartment for 3 min to adapt to the experimental basin environment. We then induced a male to move to the female compartment and observed their behaviour (female latency until genitalia protrusion and female direct resistance). If the experimental individuals appeared to be stressed after the adaptation period, we terminated this test and put them aside in their basin for further acclimation, after which we tried to use them again. If they continued to be stressed or not interested in mating, they were no longer considered subjects of the experiment.

On the next 2 consecutive days, we used the same females that were tested on the first 2 days, and measured females' latency to copulation after the experimental manipulation of predation risk (predator assay). We exposed the females to predators prior to the assay, and recorded their mating behaviours after pairing them with large or small males in a balanced order. On Day 3 (the first day of the predator assay), eight females were paired with large

males and eight with small males. On Day 4 (the second day of the predator assay), each female was paired with a male of the opposite size. The experimental basin was the same as that used in the assay without a predator. A male and a female were put in each compartment for the acclimation. During the acclimation period notonectids were shown to females in the female compartment. To experience high predation pressure, a female had to be successfully attacked (i.e. grasped tightly) at least once by a notonectid. When females escaped (unsuccessful attack), we regarded 10 unsuccessful attacks as one successful attack (see Han & Jablonski, 2010). After the successful attack we promptly separated the grasped female and the notonectid. When the acclimation period was over, we removed the partition and let the female move to the male's side. Males and females were returned to their home containers after each observation. Males used in an assay were not reused in another assay.

This  $2 \times 2$  factorial experimental design allowed us to test the interaction effect between male body size and recent predator experience of females on female mating behaviours. A linear mixed model approach was used to examine the effect of factors on log-transformed female latency until genitalia protrusion. Tukey's honestly significant difference (Tukey HSD) was used to compare assays. A binomial generalized linear mixed model approach with logit link was used for female direct resistance (female resist (1) or not (0) during the observation). Post hoc comparisons were performed using chi-square tests with Holm *P* adjustment in the R package 'phia' (De Rosario-Martinez, 2013). In each model, we included female identity as a random factor and predator experience, male body size and their interaction as fixed effects.

One may argue that a more rigorous design would be to use new and different females and males in each test. However, the repeated measures design, in which the same individuals were tested repeatedly across assays, allowed us to decrease between-assay variation due to between-individual variation in behavioural types (see Han & Jablonski, 2010). The variable latency until female genitalia protrusion showed low within-individual variance and high between-individual variance (temporal consistency, Han & Jablonski, 2010), indicating that the repeated measures design was more appropriate for calculating female response to male body size and to predator experience.

#### *Ethical Note*

Experiments on insects do not require approval from the ethics committee of Seoul National University, Korea. However, our design did seek to minimize the number of individuals used and our protocols were designed to minimize disturbance of the animals. In the assay, water striders were exposed to notonectid predators, and this could result in injury. To prevent this, water striders were immediately separated after the predator's attack (see above).

## RESULTS

### *Size Distribution of Signalling and Nonsignalling Males*

Male *G. gracilicornis* had two discrete reproductive tactics: a courtship tactic (signalling males, Fig. 1) and a nonsignalling tactic (nonsignalling males). Larger males were more likely to exhibit a courtship tactic during the precopulatory stage (Fig. 2). A signalling male tapped the water's surface with his middle legs stretched forwards when he grasped a female's body (see details in Han & Jablonski, 2009). A nonsignalling male, however, did not produce ripples during mounting periods. It took much longer (several hours) for nonsignalling small males to achieve mating (see Appendix 2). Although we did not record the female that each male

mounted, males did not change their reproductive tactics, signalling or nonsignalling, according to the trait (e.g. size) of females that they mounted.

All the males below 1.15 cm body length did not produce courtship ripples. The proportion of signalling males increased as male body size increased (Fig. 2; logistic regression:  $P < 0.001$ ). All the males over 1.25 cm produced courtship ripples (Fig. 2). All the nonsignalling males did not produce courtship ripple signals even when we repeatedly exposed them to the same females after 24 h ( $N = 18$ ). In addition, they employed the nonsignalling tactic regardless of female body size. Although it was not explicitly tested here, males' reproductive tactic (signalling/nonsignalling) appears to be fixed over their lifetime regardless of population density, sex ratio, social isolation or male age (C.S. Han, personal observation).

#### Relative Signal Intensity of Large and Small Males

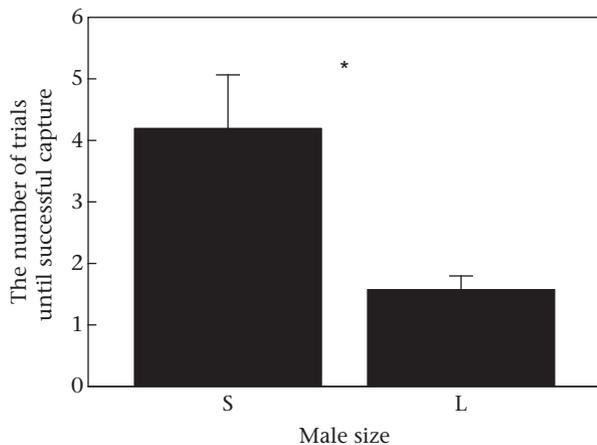
The relative signal intensity of large males was significantly stronger than that of small males ( $t_{22} = 3.97, P < 0.001$ ); the highest relative amplitude of courtship ripple signals of large males (mean  $\pm$  SD =  $-9.63 \pm 0.62$  dB) was higher than that of small males (mean  $\pm$  SD =  $-10.67 \pm 0.66$  dB).

#### Predator Success in Attacks on Mating Females

Large *G. gracilicornis* males were 1.15 times as heavy as small males (mean weight of large males  $\pm$  SE =  $0.030 \pm 0.001$  g,  $N = 20$ ; mean weight of small males  $\pm$  SE =  $0.026 \pm 0.002$  g,  $N = 15$ ). The notonectids made more unsuccessful attempts to grasp a female paired with a small signalling male than a female paired with a large signalling male (Fig. 3;  $N = 21, t = 2.84, P = 0.01$ ). That is, females suffered higher predation risk during the precopulatory period when larger signalling males attempted to mate and mounted the female. In addition, the proportion of attacks that were successful on the first attempt was also significantly higher when large males mounted the female ( $\chi^2 = 4.67, P = 0.03$ ).

#### Female Response to Courtship under Different Risk

Female behavioural responses depended on a combination of previous predation exposure and male body size (Fig. 4, Table 1; size-dependent intimidation effect). Females allowed copulation sooner if they experienced predatory attacks than if they did not



**Figure 3.** Effect of male size on the number of unsuccessful capture attempts of predators until a successful capture. S: small male; L: large male. Error bars indicate SEs. \* $P < 0.05$ .

when courted by large signalling males (Fig. 4a; Tukey HSD:  $P = 0.005$ ) but not when courted by small signalling males (Fig. 4a; Tukey HSD:  $P = 0.99$ ). In the absence of predation exposure, there was no difference between small and large males in their efficiency at intimidating females (Fig. 4a; Tukey HSD:  $P = 0.83$ ), but after experiencing predatory attacks, females accepted mating attempts of large signalling males sooner than those of small signalling males (Fig. 4a; Tukey HSD,  $P = 0.04$ ).

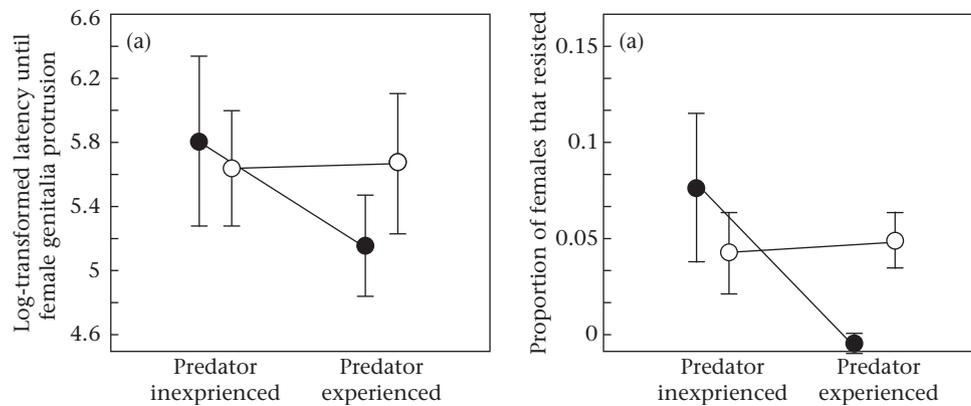
This size-dependent effect was also seen in the analysis of female direct resistance behaviour (Fig. 4b, Table 1). After experiencing predatory attacks, females tended to decrease direct resistance when courted by large signalling males (Fig. 4b;  $\chi^2 = 4.81, P = 0.06$ ). Only one of 16 females physically directly resisted large signalling males. However, direct resistance behaviour of females courted by small signalling males was not influenced by predator experience (Fig. 4b;  $\chi^2 = 0.25, P = 0.62$ ). In the analysis within each assay (predator-inexperienced/predator-experienced assays), female direct resistance did not differ between small and large males (Fig. 4b; predator-inexperienced assay:  $\chi^2 = 0.92, P = 0.34$ ; predator-experienced assay:  $\chi^2 = 3.71, P = 0.11$ ).

## DISCUSSION

Our results suggest that the alternative, nonsignalling reproductive tactic observed in the small *G. gracilicornis* males might have arisen from the weak intimidation effect of small males' courtship ripples on female mating behaviour. Typically, *G. gracilicornis* males coerce females to copulate by their unusual species-specific intimidating courtship which attracts potential predators (Fig. 1; Han & Jablonski, 2010). However, some small males do not produce courtship ripple signals after mounting females (Fig. 2). Instead they may stimulate females by probing their external genitalia (gonocoxal plates) with the phallus (e.g. Fairbairn, Vermette, Kapoor, & Zahiri, 2003; Han & Jablonski, 2009) or producing chemical signals (Andersen, 1982). Here we showed that only large males' ripple signals had an intimidation effect on females in the presence of predators: predator-experienced females rarely resisted and allowed intromission quickly only when they were courted by large males. Because females paired with the large males were more vulnerable to predators than females paired with small males, the results are consistent with the previously documented notion that females are sensitive to predation risk and are more susceptible to intimidation by predator-attracting male courtship in conditions of high predation risk. Additionally, in the absence of predators (hence in the low predation risk situation as perceived by the female), this difference between small and large males in their efficiency at intimidating females into copulating was absent. This indicates that predator presence (and its perception by females) is crucial for the differentiation between small and large males with respect to the benefit from signalling. This may lead to the two different mating tactics, intimidation signalling in large males and nonsignalling mating tactics in small males in the presence of predators.

#### Hypotheses for Nonsignalling: Body Weight and Allometry

We suggest that size constraints on courtship may have shaped the ARTs of *G. gracilicornis* (e.g. Karino & Kobayashi, 2005; Karino & Kamada, 2009). Our results are consistent with the notion that the size-dependent intimidation effect on females is related to the effect of the signalling male's size on female predation risk. We suggest that size-dependent intimidation effects (decreased latency until copulation and resistance to mating in the presence of predators) might be directly linked to the increased success of a



**Figure 4.** Effect of experience with predators and the size of males on (a) log-transformed female latency to copulate and (b) proportion of females that resisted male mating attempts. Males were categorized as large (closed circles) or small (open circles). Error bars indicate 95% confidence intervals (a) and SEs (b).

**Table 1**

Generalized linear mixed model of female mating behaviours (latency until genitalia protrusion/resistance) as a function of predator experience, male body size and their interaction

| Variables      | Latency until genitalia protrusion |       |       | Resistance    |       |      |
|----------------|------------------------------------|-------|-------|---------------|-------|------|
|                | Estimate (SE)                      | t     | P     | Estimate (SE) | Z     | P    |
| Predator       | -0.65 (0.20)                       | -3.33 | 0.005 | -3.30 (1.47)  | -2.25 | 0.02 |
| Size           | -0.17 (0.20)                       | -0.86 | 0.40  | -0.98 (0.97)  | -1.01 | 0.31 |
| Predator* size | 0.68 (0.28)                        | 2.46  | 0.03  | 3.81 (1.78)   | 2.14  | 0.03 |

predator's attacks on tandem mating pairs with large males. This is probably caused by the heavy weight of mounting males, which, regardless of the intensity of intimidating ripples, increases the risk of capture by attacking predators as shown in our results (Fig. 3). This 'weight hypothesis' proposes that females paired with large males might not be able to escape efficiently from attacks of notonectids because the heavy load decreases the females' mobility. Therefore, because larger and heavier mounting males decrease females' mobility and make females more vulnerable to predator attacks, females may allow larger males to mate quickly in order to silence the male preventing him from attracting predators.

However, the weak intimidating courtship effect of small males on females may also be linked to an effect of the male's morphology on the strength of the ripple signals (allometry hypothesis) and consequently on the increase in predation risk due to signalling. The weak intensity (amplitude) of courtship ripple signals of small males could be caused by their shorter middle legs and weaker muscles. This may decrease the predation risk of females by decreasing the distance at which predators can detect them. Additionally, the strong intensity of ripples from large mounting males may not only attract predators from larger distances, but also disrupt the females' ability to detect ripples from approaching predators. Water striders perceive ripples of the water's surface through tarsal vibration receptors on their legs (Goodwyn, Katsumata-Wada, & Okada, 2009; Lawry, 1973; Murphey, 1971), and they can also detect approaching conspecific individuals or predators with these organs. However, when large males produce strong courtship ripples around mounted females (Fig. 1), the females are likely to miss the cues from approaching predators leading to an increase in predation risk.

In both hypotheses, the difference in predation risk between females mounted by large versus small males can only be observed if predators are present. Therefore it is not surprising that females in our experiments were less sensitive to the weight difference or

courtship intensity difference between large and small males and equally resisted both types of males in the absence of predators. Given that the allometry and weight hypotheses are not mutually exclusive, we suspect that females may determine the moment when they allow copulation on the basis of both cues: body weight and the intensity of courtship ripples (which depend on male size), taking into account the presence or absence of predators.

Here, the 'mate choice' mechanism via female resistance cannot account for our results. Water strider females vigorously struggle to free themselves from unwanted males (Arnqvist, 1997). If females are less resistant to males that they prefer, reduced female resistance against large males when predation risk is high might indicate female preference for large males. However, theoretical models and empirical evidence predict that female preference decreases with increasing costs of mate choice such as predation risk (Crowley et al. 1991; Godin & Briggs, 1996; Hubbell & Johnson, 1987; Pomiankowski, 1987; Pomiankowski, Iwasa, & Nee, 1991; Real, 1990). Given the longer latency to copulation and increased female resistance when predation risk is low, a mate choice mechanism would rather work under low than high predation risk. However, even when risk is low, our previous research (Han et al. 2010) showed that females expressed similar intensity of physical resistance against smaller or larger males (relative to female size), giving no suggestions that relatively smaller or larger males are preferred. Hence reduced female resistance against large males when predation risk is high is unlikely to reflect female mate preference for large males.

#### Predation Risk and Courtship Modification

The presence of predators can lead to changes in courtship behaviour of prey species including evolution of novel signals, loss of courtship or modification of courtship characteristics (Zuk & Kolluru, 1998), followed by a divergence of populations (Verrell, 1991) or population-specific ARTs (Cade, 1975). This scenario can be applied to *G. gracilicornis* males. Although selection in the presence of predators operates to maintain the intensity of courtship ripple signals (Han, Brooks, & Jablonski, 2015), our results implied that inefficiency of courtship ripple signals of small *G. gracilicornis* males could lead to their adopting a nonsignalling reproductive tactic. Moreover, only large *G. gracilicornis* males vary the intensity of courtship ripple signals according to the risk of predation (Han & Jablonski, 2010). In response to predators, large males increased their signal intensity to increase the intimidation effect, whereas small males did not (Han & Jablonski, 2010). The different responses in males might also be due to size-dependent

effectiveness of their courtship ripple signals. Therefore the mating system of *G. gracilicornis* provides evidence that the presence of predation risk contributes to the evolution and maintenance of size-dependent ARTs as well as the courtship strategy (i.e. intimidating courtship, Han & Jablonski, 2010).

### The Cost of Signalling

Although the two hypotheses ('weight' and 'allometry') can explain why the signalling of smaller males has a weaker intimidation effect on females, we still need to determine why there are nonsignalling males among the smallest males in the population. Although courtship ripples produced by smaller males are probably weaker, small signalling males can copulate sooner than nonsignalling males (see Appendix 2), and predators can still be attracted to small males' ripples. For the smallest males, the costs of signalling are predicted to outweigh the benefits of a low degree of intimidation, and their optimal strategy in this case is to cease signalling at all. Thus, in addition to the two hypotheses discussed above, we need to consider additional possible mechanisms to prove the cost of signalling.

Although the energetic costs of signalling should be considered in this context, they are not the only costs that males experience. For example, the nonsignalling strategy might evolve to reduce the costs of harassment from other males when small males mount females and attempt to mate (harassment reduction hypothesis). Mating pairs of water striders experience some harassment because males are indiscriminate in attempting to copulate (Chang & Sih, 2013; Eldakar, Dlugos, Wilcox, & Wilson, 2009; Han & Brooks, 2013a, 2013b, 2014, 2015; Han et al. 2015; Sih, Chang, & Wey, 2014; Sih & Watters, 2005). Even when a female is already mounted by a male, other males frequently harass and try to mount the pair. This usually disturbs the normal mating process and may prevent the mounted male from inserting his genitalia properly. Occasionally this harassment attempt may even result in a takeover of the female. Small males, which are not heavy and do not have strong forelegs to grasp females, are likely to be more disturbed and more frequently disengaged by other males' harassment. Therefore, the nonsignalling ART would be especially beneficial for dwarf males, which in this manner avoid paying the costs of harassment due to attraction of other males to the pair.

### Conclusion

In conclusion, we have provided evidence that size-dependent courtship effectiveness can arise and maintain ARTs in *G. gracilicornis* males. In the presence of predators, females respond to courtship ripple signals from large males. Their responses to small males, however, are not different across risk levels. In addition to the low benefit of intimidation (poor courtship effectiveness), courtship ripple signals may increase the cost of other males' harassment to small males. All of these mechanisms can explain why the smallest males do not produce ripple signals. Thus our study highlights how size-dependent courtship effectiveness can drive size-dependent ARTs, represented here by an increasing probability of males using the signalling tactic with increasing male body size. Our results are consistent with previous studies showing that males that are inferior in male-male competition and less attractive to females employ ARTs that maximize mating success under the given constraints (Gross, 1996; Oliveira et al. 2008; Shuster & Wade, 2003). However, more evidence for the fitness benefit and cost of alternative tactics of small males is essential to understanding the evolution and maintenance of ARTs in *G. gracilicornis* males.

### Acknowledgments

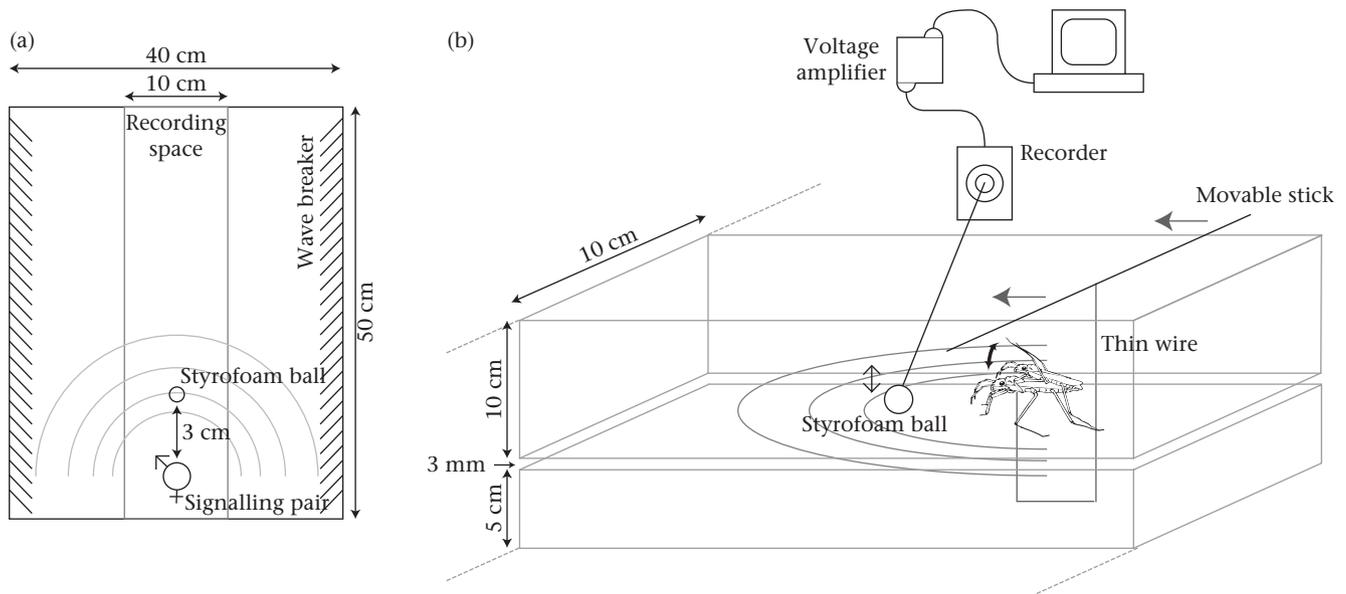
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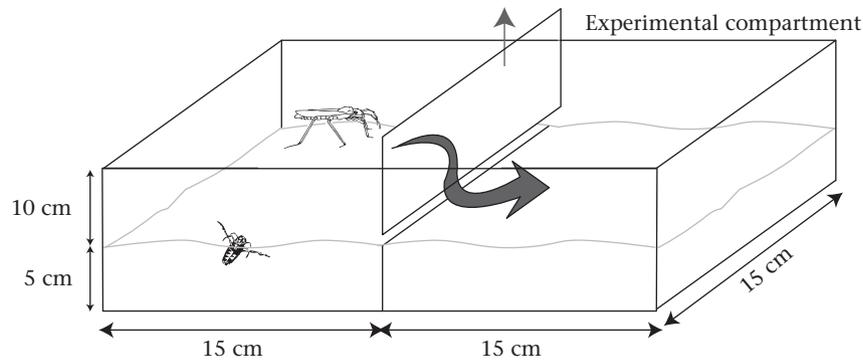
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## Appendix 1. Supplementary methods



**Figure A1.** (a) The upper view of the experimental box for measuring the amplitude of courtship ripple signals. The ‘wave-breaker’ attached along the inside walls of the experimental container reduces the reflective signals. (b) The side view of the experimental container. The oscillatory movements of the light Styrofoam ball produce electronic currents in the recorder. Through the low-frequency voltage amplifier, the electronic currents are recorded in the computer as digitalized ripple signals.



**Figure A2.** A schematic figure of the experimental arena.

## Appendix 2. Nonsignalling small male copulation latency

### Methods

To estimate how long it took nonsignalling small males to achieve mating, we presented the focal nonsignalling male with one female in a basin ( $15 \times 15$  cm, water depth 5 cm) with a Styrofoam shelter and recorded the behaviour by a series of spot checks every 10 min for 12 h. We recorded whether a male mounted a female and whether a male copulated with a female. After mounting a female, *G. gracilicornis* males copulate with females for 20–25 min (Han et al. 2012) and remain on a female's back (postmating guarding) for many hours up to 2 days (Han et al. 2010). This

indicated that making 10 min spot checks was a reasonable method to detect the focal male's mating status. In the assay, eight males were individually paired with females randomly selected from a laboratory population.

### Results

All of the eight nonsignalling small males successfully mounted females within 20 min. However, only one female allowed the nonsignalling male to copulate within 2 h. Four nonsignalling small males were successful at mating within 10 h. Females did not permit the other four males to copulate within 12 h.