Intimidating courtship and sex differences in predation risk lead to sex-specific behavioural syndromes

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Behavioural correlations between mating behaviour and antipredator behaviour are expected when sexual behaviour increases predation risk. However, the correlation would be different between males and females, particularly when the sexes experience different levels of predation risk. Here, we tested this idea using a water strider species, *Gerris gracilicornis* (Heteroptera: Gerridae). *G. gracilicornis* males employ an unusual intimidating courtship strategy capitalizing on predator behaviours and female response to predators. Since mounted females are more vulnerable than males to predatory attacks from below, we predicted that changes in female mating behaviours across predation contexts (boldness in a mating context) should be associated with female antipredator behaviours (boldness in a nonmating context), but that the correlation would be weaker for male mating behaviours. In a series of behavioural assays, we measured water striders' (1) mating behaviour in the absence of predators, (2) mating behaviour in the presence of predation risk, (3) boldness in the mating context (changes in female mating behaviour after predator attacks) and (4) boldness in the nonmating context. We found that females behaved more cautiously around predators in both the mating and nonmating contexts. We also found that females' boldness in the mating context was significantly correlated with their boldness in the nonmating context. In contrast to females, antipredator behaviour of males in the nonmating context did not correlate with their boldness in the mating context. Thus we suggest that sex differences in boldness across predation contexts appear to cause sex-specific behavioural syndromes.

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Over the last decade, a number of empirical studies have reported that individuals of the same population differ consistently in their behaviour (Bell, Hankison, & Laskowski, 2009). The repeatable parts of animal behaviour are often also correlated with each other across traits (Garamszegi, Markó, & Herczeg, 2012). These correlations are referred to as 'behavioural syndromes' (Dingemanse & Dochtermann, 2013; Dingemanse, Dochtermann, & Nakagawa, 2012; Dochtermann & Dingemanse, 2013; Sih, Bell, & Johnson, 2004a, 2004b). Behavioural syndrome studies have made great progress studying traits such as aggressiveness, boldness towards predators and activity in a novel environment (Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Yet, in spite of considerable research interest in behavioural syndromes, it remains unclear how sexual selection impacts their evolution (Schuett, Tregenza, & Dall, 2010), particularly the correlation between sexual and nonsexual behaviours (but see Han & Brooks, 2013, 2014, 2015). Because survival and reproductive success form the two major components of fitness, sexual selection can cause the emergence of a behavioural syndrome that is inherited via linkage disequilibrium (see review of Bell, 2007; van Oers, de Jong, van Noordwijk, Kempenaers, & Drent, 2005; Sih et al., 2004b). But how traits such as courtship behaviour and response to courtship relate to other nonsexual behaviours has received far less attention (but see Han & Brooks, 2013, 2014, 2015; Logue, Mishra, McCaffrey, Ball, & Cade, 2009).

Our understanding of behavioural syndromes involving antipredator behaviour has mostly come from studies of associations between predator avoidance or evasion and aggressiveness or exploration (see reviews Dingemanse & Réale, 2005; Sih & Bell, 2008). It remains unknown, however, whether this general

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behavioural syndrome of antipredator behaviour directly extends to mating behaviour. When sexual behaviour increases predation risk experienced by males, females or both sexes due to conspicuous sexual communication (Endler, 1980; Gwynne, 1989; Lima & Dill, 1990; Magnhagen, 1991), it is reasonable to expect the evolution of correlations between sexual behaviour and antipredator behaviour. First, life history trade-offs can explain the emergence of the behavioural correlation (Biro & Stamps, 2008; Réale et al., 2010; Stamps, 2007; Wolf, van Doorn, Leimar, & Weissing, 2007; Wolf & Weissing, 2010). Because of trade-offs between survival and reproduction, individuals active in mate search could tend to invest more in reproduction than in survival, and they are predicted to be less cautious towards predation threats (Roff, 2002). In addition to this view, a state-dependent explanation (Dingemanse & Wolf, 2010; Sih et al., 2015; Wolf & Weissing, 2010) also predicts the correlation between sexual behaviour and antipredator behaviour.

Regardless of whether sexual behaviour is positively or negatively correlated with risk-taking behaviour, the behavioural correlation between them is generally expected to be similarly expressed in both sexes (see Pruitt & Riechert, 2009; Sprenger, Dingemanse, Dochtermann, Theobald, & Walker, 2012). Although males' sexual behaviours (e.g. courtship) and females' sexual behaviours (e.g. preference) are not functionally equivalent, genetic covariation between male sexual trait and female preference may develop similar structures of correlations between sexual behaviours and antipredator behaviours in both sexes (see Fowler-Finn & Rodríguez, 2015), especially when sexual behaviours of both sexes are influenced by predation risk (see below). However, it is also possible that the correlation between sexual behaviour and antipredator behaviour differs between the sexes when males and females experience different returns from sexual behaviour and different forms and intensities of predation risk (e.g. Fresneau, Kluen, & Brommer, 2014). For example, when only males suffer an increased predation risk due to the presence of conspicuous displays (Andersson, 1994), we could expect the correlation between mating behaviour and antipredator behaviour in males but not in females. That is, males more active in reproduction are predicted to be less cautious to predators, whereas females would express their mating behaviours regardless of the level of predation they experienced. Likewise, we also expect correlations between sexual behaviour and antipredator behaviour in females when they suffer predation risk during mating.

Males of one Asian water strider species, *Gerris gracilicornis* (Heteroptera: Gerridae), employ an unusual courtship strategy (i.e. intimidating courtship, Han & Jablonski, 2009, 2010) that capitalizes on predator behaviour and female response to predators (i.e. a form of sensory exploitation, Han & Jablonski, 2010). A male mounted on a female produces courtship signals by repeatedly tapping the water surface until the female exposes her genitalia for intromission (Han & Jablonski, 2009), and these ripples can attract predatory aquatic insects such as notonectids (Hemiptera, Notonectidae). Because a mounted female is more susceptible than the male to predatory attack by notonectids approaching from below the water surface, courtship ripples are a form of threat males use towards females (Han & Jablonski, 2010). In response to male intimidating courtship, *G. gracilicornis* females protrude their genitalia and permit the courting male's attempt to copulate (Han & Jablonski, 2009). When the female allows the male to copulate, he ceases signalling. In a predator-free environment, females can delay intromission to terminate mounting males' mating attempts by resistance because males can be more easily thrown off if their genitalia are not inserted. Thus mating of *G. gracilicornis* is determined by females. However, in the presence of predators, females are not able to delay intromission because the ripple signals attract predators and the female is more vulnerable because of the mating

position (Han & Jablonski, 2010). Since female water striders become more sensitive to males' intimidating courtship ripples in the presence of predators (Han & Jablonski, 2010), the variation in the female's latency to genitalia protrusion can be regarded as a flexible mating strategy of females in response to predation risk. Similarly, since courtship signalling by males can increase male mating success more when predators are present (Han & Jablonski, 2010), males may intensify their courtship signals to increase the chance of attracting predators. Thus, contextual variation in male courtship in response to predation risk can also be regarded as a flexible mating strategy of males.

In this species, we predicted that the correlations between sexual and nonsexual responses to predation risk will be sex-specific because of the sex-specific effect of being sensitive to predators on their fitness. Because mating females, unlike males, are at a considerably greater risk of predation (Arnqvist, 1989; Fairbairn, 1993; Han & Jablonski, 2010; Rowe, 1994), we predicted that females should be more cautious to the predation risk in a mating context than males, and that female boldness to predation in a mating context could also be associated with their boldness in a nonmating context. That is, more risk-prone females (boldness in a nonmating context) are predicted to be less sensitive to intimidating courtship ripples of males (boldness in a mating context).

We predicted that, in contrast to females, sexual selection on boldness in a mating context should result in males being less cautious to predators than females. This is because males improve their mating success when they increase the risk of attracting predators by courting more intensely. We also predicted that this may lead to male boldness across mating and nonmating contexts being more weakly correlated than in females. That is, the change in male courtship intensity in response to the threat of predation (boldness in a mating context) is predicted not to be related to males' activity under predation risk (boldness in a nonmating context).

To examine sex differences in boldness correlations across mating and nonmating contexts, we measured antipredator behaviours and mating behaviours (male courtship intensity and female response to male courtship) of males and females in the absence/presence of a predator. Although *G. gracilicornis* males' sexual behaviours (e.g. courtship intensity) and females' sexual behaviours (e.g. female response to male courtship) are not functionally equivalent, the expressions of both sexual behaviours are affected by the level of predation (Han & Jablonski, 2010). Thus they could be considered as the same responses towards predators in a mating context. Based on the behavioural data, we also calculated an index of boldness in a mating context to quantify how the mating behaviour was affected by the experience of predatory attack. Then we compared boldness correlations across mating and nonmating contexts (i.e. syndrome structures) of males and females using structural equation modelling (SEM) and model comparison based on the Akaike information criterion (AIC).

METHODS

Collection and Rearing

Overwintered *G. gracilicornis* (130–150 individuals) were collected at Cheonseong Mountain, South Korea, and transported to the laboratory. They were then separated into two rectangular plastic containers filled with water (40×50 cm, water depth 5 cm) according to sex, and frozen crickets, *Gryllus bimaculatus*, were given as food every day. Pieces of floating Styrofoam were provided as resting sites. To avoid variation in each individual's mating experience, we let individuals of both sexes copulate randomly once a day after placing one male and one female in a small

container (20 × 30 cm, water depth 3 cm), then separated them again. We were able to distinguish the initiation and termination of copulation by eye (Han & Jablonski, 2009). In addition, since the container was so small that a female was not able to escape efficiently from a male's mating attempts, all the males and females were able to copulate easily. During the experiment, individuals mated after the behavioural assays.

Notonectids (Hemiptera, Notonectidae, *Notonecta triguttata*), generalist predators that attack small insects fallen on the water 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, 1980a). Notonectids were collected at pools near the rice fields in the Kimpo region, South Korea. They were reared individually in transparent, plastic, cylinder-shaped boxes (diameter 10 cm, height 15 cm). Each individual was fed with one frozen cricket every day but starved for 3 days before behavioural assays (see below).

Measuring Behavioural Syndromes

Each of 42 males and 43 females was subjected to three behavioural assays on 3 consecutive days: (a) day 1: assay 1, mating behaviour (male courtship intensity, female latency to genitalia protrusion); (b) day 2: assay 2, mating behaviour after the individual experienced predatory attacks; (c) day 3: assay 3, boldness towards predators in a nonmating context. Two females died between assay 2 and 3. Thus the sample sizes are different for different comparisons between assays. Below we describe the details.

Assay 1: mating behaviour

In assay 1 we measured male courtship intensity and female latency to genitalia protrusion. In each test, a focal individual was placed in a 20 × 30 cm tank filled with water up to 3 cm. After 3 min a randomly chosen individual of the opposite sex was introduced to the focal individual's tank.

The basic unit of the male courtship signalling consists of a single tap. The taps are produced in bouts separated by an interval between bouts (see details in Han & Jablonski, 2009) when no signalling occurs. We used the number of taps (signals) per bout as an index of male courtship behaviour (intensity of courtship). We measured the mean number of taps per bout (see details in Han & Jablonski, 2009) for 2 min starting at 1 min after the mounting male attached his genitalia onto the female's gonocoxae.

For females, we measured the latency until protrusion from the moment when a male grasps the female body part to the moment when a female protrudes her genitalia (more detailed methods are described in Han & Jablonski, 2010).

Assay 2: mating behaviour after predatory attack

Assays 2 and 3 were conducted in the same tank. An individual was introduced to one compartment of a larger tank (made of acrylic plate; 15 × 30 cm and 15 cm high; Appendix Fig. A1), with one *N. triguttata* predator. The large transparent tank was divided by an opaque partition (5 × 15 cm) into two separate compartments (15 × 15 cm). Neither water, water-borne chemicals nor predators could move between the two compartments (each of which was filled with water up to 4.8—5.0 cm). A second opaque plate (10 × 15 cm) was located above the first and could be lifted by the experimenter to allow the water strider to move from one compartment to the other.

In each test, the focal individual was introduced to the compartment housing a predator, and a randomly chosen individual of the opposite sex was introduced to the other 'experimental' compartment. When the focal individual was placed into the same compartment as a predator, it experienced predatory attacks, but the experimenter always interrupted successful predatory grasping to prevent the notonectid from piercing the cuticle of the water strider with its rostrum (more detailed methods described in Han & Jablonski, 2010). After being grasped by the predator once, the focal individual was allowed to escape to the experimental compartment housing the opposite sex, while the predator remained in the first compartment. Once the male mounted the female, we measured the male's intensity of courtship or the female's latency to protrusion in the same way as in assay 1.

To quantify how the mating behaviour was affected by the experience of predatory attack, we calculated an index of 'boldness in a mating context (δ)' using mating behaviour (the intensity of courtship or latency to protrusion) observed in assay 2 and a null expectation based on a regression (from the repeatability assays described in the Appendix 1) of the second day mating behaviour on the first day mating behaviour (more detailed methods are described in Appendix 2, Fig. A2). This index, δ, indicates how much the actual value on the second day is relatively far from the predicted value on the second day. In males, a positive δ score indicates a male courts more intensely after predator exposure than predicted, and he is bolder to predation risk in the mating context. In females, a positive δ score indicates a female's latency is longer after predator exposure than the prediction, and she is less sensitive to predation risk in the mating context.

Assay 3: boldness in a nonmating context

An individual was introduced to one compartment of the tank used in assay 2, with one *N. triguttata* predator. After the individual experienced a predatory attack, the partition was lifted to allow it to move to the experimental compartment, but a subsurface partition kept the predator in the first compartment. In the experimental compartment, no floating substrate was provided for the individual to rest. We observed its behaviour for 5 min and we measured (1) the number of strokes on the water (excluding strokes along the margin and towards the margin of the experimental compartment) and (2) grooming duration (duration of the focal individual washing itself with its legs). Then we used principal components analysis (PCA) to reduce the two variables to one composite measure of boldness, which summarized 54% of the total variation in male behaviours and 68% of the total variation in female behaviours (Appendix 2, Table A1).

Actively moving individuals (indicated by a high number of strokes) are more conspicuous to *Notonecta* predators which perceive surface vibrations (Lang, 1980a, 1980b). Therefore higher activity even soon after detecting a predator may be interpreted as boldness. Grooming activity is also a more risky behaviour because the individual is not supported on all legs, delaying the initiation of escape. Moreover, since water striders receive information on the water through tarsal vibration receptors on their legs (Goodwyn, Katsumata-Wada, & Okada, 2009; Lawry Jr., 1973; Murphey, 1971), they are less able to detect approaching predators well if some of their legs are not touching the water surface during grooming or mating. Therefore individuals that groom more soon after detecting a predator may be considered as bolder or less concerned with the possibility of a predatory attack.

Statistical Analysis

Effect of predator experience on behaviour

When original or transformed variables conformed to the assumptions of parametric statistical methods, we used *t* tests (for sex difference in δ and difference in male courtship intensity across contexts); otherwise we used the nonparametric Mann—Whitney *U* test (for sex difference in nonmating boldness) and Wilcoxon

matched-pairs test (for difference in female latency across contexts). Analysis of covariance (ANCOVA) was also used for comparing two regression slopes, i.e. (1) the slope from behavioural assays to measure repeatability and (2) the slope from behavioural assays to measure behavioural syndrome (see Appendix 2, Fig. A2).

Behavioural syndrome

Following the behavioural measurements, we estimated behavioural syndrome structures using SEMs after standardizing behavioural variables for each sex separately. Instead of focusing on pairwise behavioural correlations with their inherent problems (for a full discussion, see Dingemanse, Dochtermann, & Wright, 2010), it often helps to compare the fit of a priori considered SEMs (Bókony, Kulcsár, Tóth, & Liker, 2012; Dingemanse et al., 2010; Dochtermann & Jenkins, 2007). This alleviates the need to control for multiple testing because the test is conducted on the whole matrix.

We used four behavioural variables (described above) to construct six a priori hypotheses of behavioural syndrome structures (models 1–6, Fig. 1). A priori hypotheses of syndrome structure were based on the behavioural ecology of *G. gracilicornis* (Han & Jablonski, 2010) and behavioural syndrome literature (Johnson & Sih, 2007; Stamps & Groothuis, 2010; Wilson & Godin, 2009). Model 1 represented a null model with the absence of covariance between behaviours (null model). Model 2 represented the mating behavioural syndrome that mating behaviours were linked across contexts (mating linked syndrome, Stamps & Groothuis, 2010). Model 3 represented a link between boldness in a nonmating context and mating behaviour before predatory attacks, independent of other behaviours (linked mating behaviour and boldness). This correlation can emerge on the basis of individual differences in state (Dingemanse & Wolf, 2010; Wolf & Weissing, 2010). Model 4 represented boldness syndrome independent of mating behaviours (boldness syndrome, e.g. Johnson & Sih, 2007; Wilson & Godin, 2009). Model 5 represented mating behaviours linked across contexts independently from boldness syndrome (independent mating and boldness syndromes, Han & Jablonski, 2010). Last, model 6 represented mating behavioural syndrome linked with boldness in a nonmating context (linked mating syndrome and boldness).

The comparison between models was achieved by comparing AIC values with the optimal model (the lowest AIC). The difference between a model's AIC and the optimal model's AIC is referred to as ΔAIC. Models with ΔAIC more than 2 are considered to lack explanatory power relative to the optimal model. Statistical analyses for SEMs were performed using AMOS 21.0, SPSS Inc. (Arbuckle, 2006).

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

Effect of Predator Experience on Behaviour

In the presence of predators, females were more cautious in the nonmating context than males (Fig. 2a, b; Mann–Whitney U test: number of strokes: $U = 694.5$, $z = 1.52$, $P = 0.13$; grooming: $U = 570$, $z = 2.65$, $P < 0.01$; male $N = 42$, female $N = 41$). There was also a significant sex difference in PC score of boldness (Mann–Whitney U test: $U = 551$, $z = 2.82$, $P = 0.004$).

Moreover, females' index of boldness in the mating context (δ) was lower than that of males (Fig. 2c; $t_{83} = -2.07$, $P = 0.04$). This indicated that female responsiveness was more dramatically reduced by the presence of a predator than male courtship. When we compared the slope for the repeatability experiment with the slope for the behavioural syndrome experiments, there was no significant difference in males (ANCOVA: $F_{1,74} = 0.57$, $P = 0.45$; Appendix 2, Fig. A2), but there was a marginally significant difference in females (ANCOVA: $F_{1,64} = 3.63$, $P = 0.06$; Appendix 2, Fig. A2). In addition, when we also compared mating behaviours before and after exposure to predators (assays 1 and 2), females significantly decreased their latency to genitalia protrusion after exposure to predators (assay 1 and 2 comparison: $N = 43$, $Z = 2.54$, $P = 0.01$) but males' courtship intensity did not change across predation contexts (assay 1 and 2 comparison: $t_{41} = -0.01$, $P = 0.99$). Thus all the results indicated that males were not sensitive but females were sensitive to predation risk in the mating context.

Behavioural Syndrome

SEM analyses showed that males and females had different behavioural syndrome structures (Table 1, Fig. 3). For males, the model that best explained the behavioural data predicted that mating behaviours before/after predatory attacks (assay 1 and 2) covaried with boldness in a nonmating context (assay 3)
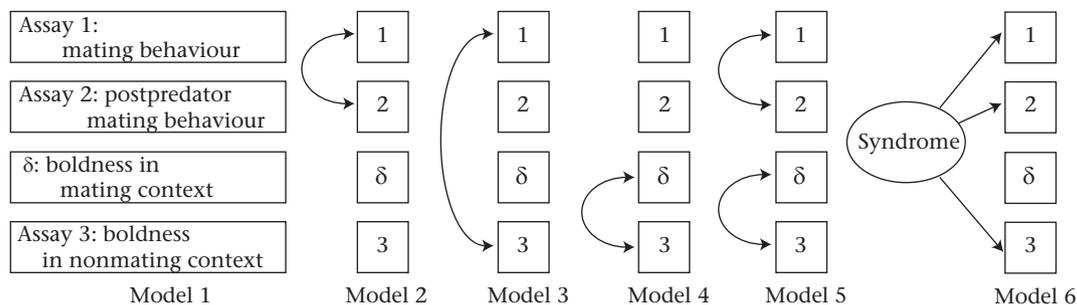

Figure 1. Six candidate models of behavioural syndrome structures. Model 1: the null model, no relation between behaviours. Model 2: mating behaviours linked, independent of boldness in a mating or nonmating context. Model 3: boldness in a nonmating context linked with mating behaviour before predatory attacks, independent of other behaviours. Model 4: boldness in a mating context linked with boldness in a nonmating context, independent of mating behaviours. Model 5: mating behaviours linked and boldness behaviours linked, but mating behavioural syndrome is independent of boldness syndrome. Model 6: mating syndrome linked with boldness in a nonmating context. 1: mating behaviour before the individual experiences predatory attacks; 2: mating behaviour after the individual experiences predatory attacks; 3: boldness towards predators in a nonmating context; δ: boldness towards predators in a mating context.
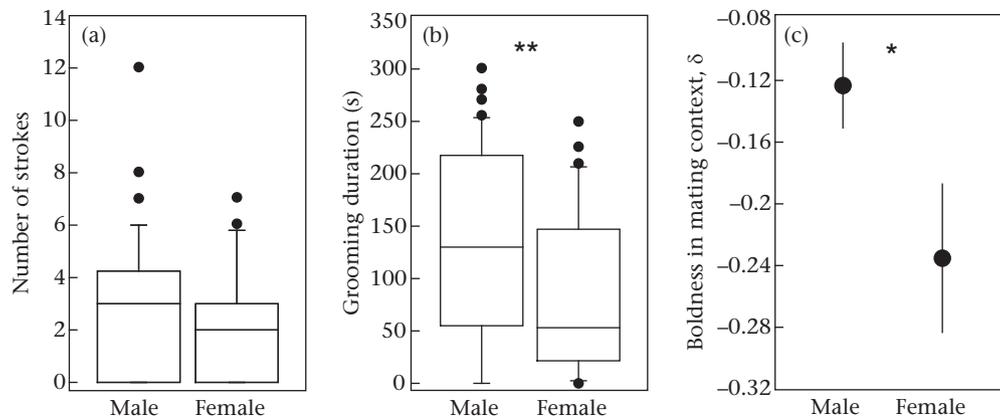
Figure 2. Sex differences in boldness to predation risk in (a, b) a nonmating context and (c) a mating context. (a,b) Boxes indicate the 25% and 75% quartiles. The median is marked as a solid line. Outliers are black dots outside the error bars which cut off the lower and upper 10% of the distribution. (c) More negative δ values indicate that individuals are more cautious to predators. Error bars indicate standard errors around the mean. *$P < 0.05$; **$P < 0.01$.

independent of boldness in a mating context (δ; model 6, linked mating and boldness syndromes, Fig. 3, Table 1). In contrast, for females, the best model was a two-functional context syndrome (model 5, independent mating and boldness syndromes, Fig. 3, Table 1). In this model, mating behaviours before/after individuals experienced predatory attacks linked together, and this was independent from a boldness syndrome (δ; boldness in a mating context) which covaried with assay 3 (boldness in a nonmating context).

DISCUSSION

Our results provide evidence for the presence of sex-specific behavioural syndromes in a water strider *G. gracilicornis*. Female boldness to predation risk in a mating context (changes in female responsiveness to males after predator attacks) was associated with their boldness towards predators in a nonmating context (Fig. 3, Appendix 2, Fig. A3). Hence, females that behaved more cautiously around predators in a nonmating context submitted more quickly to intimidating male courtship regardless of their underlying level of sexual responsiveness (female latency to genitalia protrusion; Fig. 3, Appendix 2 Fig. A3c, d). Males, on the other hand, showed a contrasting pattern. Males that courted most intensely tended to show the greatest boldness in response to predation threat in a

nonmating context (Fig. 3, Fig. A3a, b). But those same males did not change their courtship behaviour any more or less than other males in response to the threat of predation.

Female *G. gracilicornis* have more to lose from being bold in a mating context. Depending on the species, female water striders suffered predation in 75% (*Gerris odontogaster*) to 100% (*G. gracilicornis*) of predatory attacks on mating pairs (Arnqvist, 1989; Fairbairn, 1993; Han & Jablonski, 2010; Rowe, 1994). Since *G. gracilicornis* females that are courted by males are exposed to dramatically increased predation risk due to attraction of predators, they should be more cautious and especially sensitive to predation risk in the mating context. Thus risk-averse females acquiesce quickly to males' mating attempts and mate with courting males in order to stop their intimidating courtship.

Male *G. gracilicornis*, on the other hand, have more to gain from being bold in both the mating and nonmating contexts. A water strider male mounted on a female in a typical mating position is at lower risk during a predatory attack than a single male (Arnqvist, 1989; Fairbairn, 1993; Han & Jablonski, 2010; Rowe, 1994). In addition, boldness in performing intimidating courtship also delivers reproductive benefits to males because females acquiesce

Table 1
Model comparison results for six candidate models using structural equation models

Model	Discrepancy	k	AIC	ΔAIC	Model weight
Males					
6	167.68	7	181.68	0	0.95
2	179.10	5	189.10	7.42	0.02
5	178.19	6	190.19	8.51	1.35×10^{-2}
3	180.31	5	190.31	8.63	1.27×10^{-2}
1	190.87	4	198.87	17.19	1.76×10^{-4}
4	189.95	5	199.95	18.27	1.02×10^{-4}
Females					
5	78.85	6	90.85	0	0.88
6	80.90	7	94.90	4.05	0.12
2	96.75	5	106.75	15.90	3.11×10^{-4}
4	101.24	5	111.24	20.39	3.30×10^{-5}
1	119.13	4	127.13	36.28	1.17×10^{-8}
3	118.69	5	128.69	37.84	5.36×10^{-9}

k is the number of parameters estimated in a model. Model discrepancy is the bootstrapped ($N = 1000$) maximum-likelihood discrepancy between the model and the data. Models are described in Fig. 1.
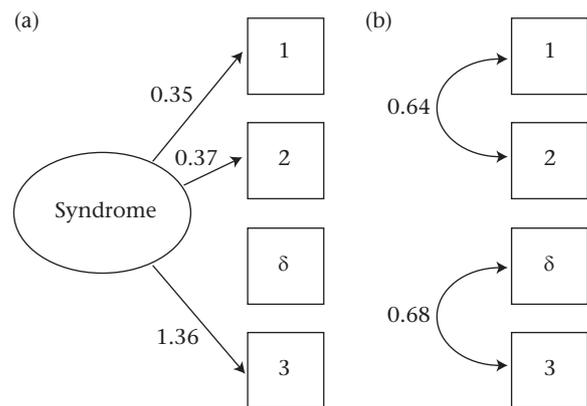

Figure 3. The most supported structural equation models of syndrome structures in (a) males and (b) females. Numbers in (a) associated with the arrows are standardized effects of the underlying syndrome structure on behaviours. Numbers in (b) associated with the arrows indicate correlations between behaviours. 1: mating behaviour before the individual experiences predatory attacks; 2: mating behaviour after the individual experiences predatory attacks; 3: boldness towards predators in a nonmating context; δ: boldness towards predators in a mating context.

and mate with males that perform intimidating courtship, especially under high predation risk (Han, Brooks, & Jablonski, 2015). Last, males that are more exploratory in nonmating contexts may perform better in scramble competition for females. Thus bolder males are generally favoured even in the presence of predators.

Sex-specific Selection and Behavioural Syndrome

We further suggest that sex differences in boldness may follow from sex-specific selection on risk taking and boldness in a mating context. Not only do male and female mating behaviours respond differently to predation threat, and relate differently to a more general boldness in the presence of predators, but the mean levels of boldness in a nonmating context differ between males and females. Sex-dependent selection on mating-related boldness may have driven the evolution of sex differences in boldness in nonmating contexts. If boldness in a mating context is associated with boldness in a nonmating context, such as via pleiotropic genetic variation, then selection on boldness in the mating context could also indirectly operate on boldness in a nonmating context.

Sex-specific selection on boldness could, therefore, have far-reaching sex-dependent effects on behavioural syndromes that relate to boldness and antipredator behaviour. Sex-specific selection operates on the traits shared between the sexes, often leading to the evolution of sexual dimorphism in those traits (Bonduriansky & Chenoweth, 2009; Hedrick & Témeles, 1989; Williams et al., 2008). Sex-specific selection can lead to intralocus sexual conflict (Bonduriansky & Chenoweth, 2009; Rice & Chippindale, 2001), imposed when male and female optimal trait expressions differ. If sex-specific selection favours sexual dimorphism in behavioural traits such as boldness towards predators, boldness and other correlated traits that are part of the same behavioural syndrome (Bell, 2007; van Oers et al., 2005) could be a target of intralocus sexual conflict.

Sex-specific behavioural syndromes might also arise as more complex products of the interaction between sex-specific selection on boldness and pace-of-life syndrome (Réale et al., 2010). The pace-of-life syndrome hypothesis proposes that individual behavioural variation is associated with life history strategies and physiological traits (e.g. metabolic rates; Réale et al., 2010). Individuals with a 'fast' lifestyle are characterized by high metabolic rates (Lovegrove, 2003; Symonds, 1999; Wiersma, Muñoz-Garcia, Walker, & Williams, 2007), fast growth, early reproduction (Biro & Stamps, 2008), high aggressiveness and more risk taking (Wolf et al., 2007). In contrast, individuals with a 'slow' lifestyle show the opposite patterns. Moreover, quantitative genetic studies also show that pace of life could be an important predictor of courtship and reproductive effort (Lailvaux, Hall, & Brooks, 2010). Although we should be cautious about generalizing the pace-of-life syndrome hypothesis, under the fast–slow life history continuum (e.g. Bielby et al., 2007; Gaillard et al., 1989; Jones et al., 2008) or pace-of-life syndrome (Réale et al., 2010), a slow–fast metabolic continuum might underpin the correlation between reproductive behaviour and other behaviours such as activity, exploration and boldness. Thus the combination of pace-of-life syndrome and sex-specific selection can explain female boldness across contexts, and male behavioural correlation between courtship intensity and boldness.

Coercion and Choice

The pathways by which female choice and resistance to coercion evolve remain the subject of heated debate (Brennan & Prum, 2012; Cameron, Day, & Rowe, 2003; Eberhard, 2005; Kokko, 2005; Kokko, Brooks, Jennions, & Morley, 2003; Kokko, Jennions, & Brooks,

2006). In the mating system of *G. gracilicornis*, delaying genitalia protrusion by females can be viewed as a form of resistance to unwanted matings that delays and sometimes completely prevents copulation (see Brennan & Prum, 2012). In this it is also a form of female choice or mating bias because it favours the boldest males that court most intensely, especially under predation risk. There can be little doubt that this mating bias imposes sexual selection on male intimidating courtship and boldness under predation risk. Females that acquiesce to intimidating courtship benefit directly by avoiding the costs of predation. Much can be learned about the evolution of mate choice by considering and quantifying the costs a female incurs from extra matings relative to the costs of resisting male courtship. Further, the question of whether females can also benefit indirectly from mating with more intimidating or persuasive males remains to be assessed.

Our findings have shown the possibility that an intimidation mating system and sex-related differences in predation risk generate sex-specific behavioural syndromes in the water strider *G. gracilicornis*. We highlight that the mechanisms to generate and maintain behavioural correlations are likely to be based on ecological factors and life history characters (Møller & Garamszegi, 2012). Also, given the prevalence of sex-specific selection in the wild (Cox & Calsbeek, 2009), we predict that many species with sexually dimorphic behaviours would express a sex-specific behavioural syndrome.

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Appendix 1. Repeatability

Here we describe tests for temporal consistency of mating behaviour (male courtship intensity and female response) in a predator-free environment, and of nonmating boldness (i.e. exploration and grooming behaviour) in the presence of a predator.

Methods

We assessed temporal (short-term, day-to-day repeatability) consistency of (1) male intensity of courtship, (2) female latency to protrusion and (3) male and female risk-taking behaviour towards predators. Each behaviour was recorded twice, with a 24 h interval between observations. Different individuals were used for each behavioural assay. Repeatability (i.e. temporal consistency) of

behaviours was assessed as the proportion of total phenotypic variance due to between-individual variance (Lessells & Boag, 1987). We calculated 95% confidence intervals (CI) of repeatabilities for each index by parametric bootstrapping ($N = 1000$ simulation iterations; Faraway, 2006; Nakagawa & Schielzeth, 2010).

Courtship intensity (male mating behaviour)

The tests were conducted in the same manner as in the 'Behavioural assays to measure behavioural syndromes' section in the main text. We tested 37 males.

Latency to protrusion (female mating behaviour)

The tests were conducted in the same manner as in the 'Measuring behavioural syndromes' section in the main text. We tested 26 females.

Boldness in a nonmating context (males and females)

The tests were conducted in the same manner as in the 'Measuring behavioural syndromes' section in the main text. We measured (1) the number of strokes on the water and (2) grooming duration for 17 males and 17 females.

Results

Repeatability of male courtship intensity and female latency to protrusion were high (male ($N = 37$): $R = 0.63$, 95% CI $= 0.39–0.79$, $P = 0.001$; female ($N = 26$): $R = 0.70$, 95% CI $= 0.44–0.85$, $P = 0.001$). This indicates that males produced a consistent number of signals (taps per bout) and females permitted copulation within a consistent latency over the short term (24 h).

When individuals recognized the presence of predators through predator attacks, grooming duration was highly repeatable in both sexes (male ($N = 17$): $R = 0.73$, 95% CI $= 0.41–0.88$, $P = 0.003$; female ($N = 17$): $R = 0.75$, 95% CI $= 0.41–0.90$, $P = 0.002$). Female grooming behaviour did not change over the short term (24 h interval; Wilcoxon test: $T = 43$, $N = 17$, $P = 0.86$). However, males tended to decrease their grooming behaviour over time (Wilcoxon test: $T = 32.5$, $N = 17$, $P = 0.07$). This indicates that males became less bold on the water after the repeated experience of predator attacks. On the other hand, in contrast to high repeatability of grooming behaviour, the number of strokes on the water was not significantly repeatable (male ($N = 17$): $R = 0.41$, 95% CI $= 0.0–0.78$; female ($N = 17$): $R = 0.0$, 95% CI $= 0.0–0.61$) and did not change over time in either sex (Wilcoxon test: male: $T = 35.5$, $N = 17$, $P = 0.78$; female: $T = 5$, $N = 17$, $P = 0.25$).

Appendix 2. Additional methods and results

Table A1
Principal component analysis on boldness towards predators

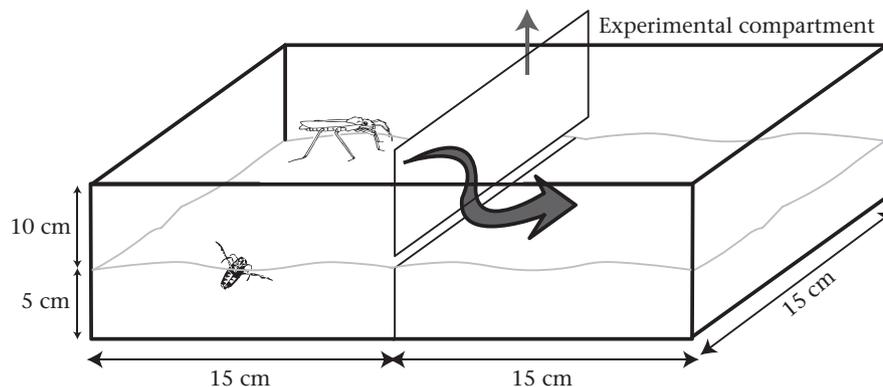| Principal components	Behaviours in boldness assay	Loading	% Variation explained	Eigenvalue
Boldness (male)	Grooming	0.68	54	1.08
	Stroke number	0.68		
Boldness (female)	Grooming	0.61	68	1.35
	Stroke number	0.61		

Figure A1. A schematic figure of the experimental tank used in the measure of boldness in a mating context (assay 2) and boldness towards predators (assay 3).
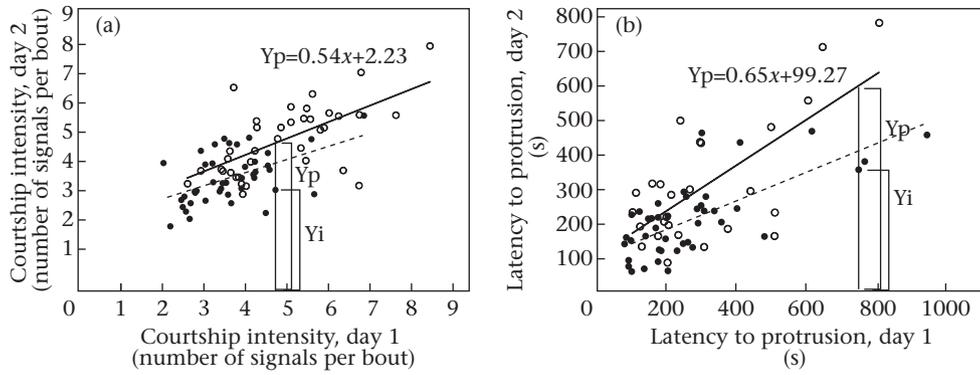
Figure A2. Methods to measure the level of change in mating behaviour of (a) males and (b) females after the experience of predatory attack (boldness in a mating context, δ). First, we used the regression between each individual's behaviour on the first day and that on the second day as the null expectation when no predator-induced change occurred in the repeatability assays. Next, we calculated the difference between the null expectation (Yp; behaviour value predicted from the regression formula) and the behaviour observed on the second day after the exposure to predators (Yi; behaviour value of individual i observed in assay 2 to measure behavioural syndrome). We then expressed the degree of intimidation by predators as the proportional difference between this null expectation (Yp) and behaviour observed in assay 2 (Yi) which is given by δ = (Yi−Yp)/Yp. Thus this index δ indicates how much the observed behaviour value on the second day is relatively far from the predicted value on the second day. A positive index indicates an individual's score of behaviour after predator exposure is larger than the prediction, and the individual is less sensitive to predation risk in a mating context. Open circles indicate behaviour variables in assays to measure repeatability (control for regression formula, no predator experience) and closed circles indicate behaviour variables in assays to measure behavioural syndrome (predator experience on day 2).
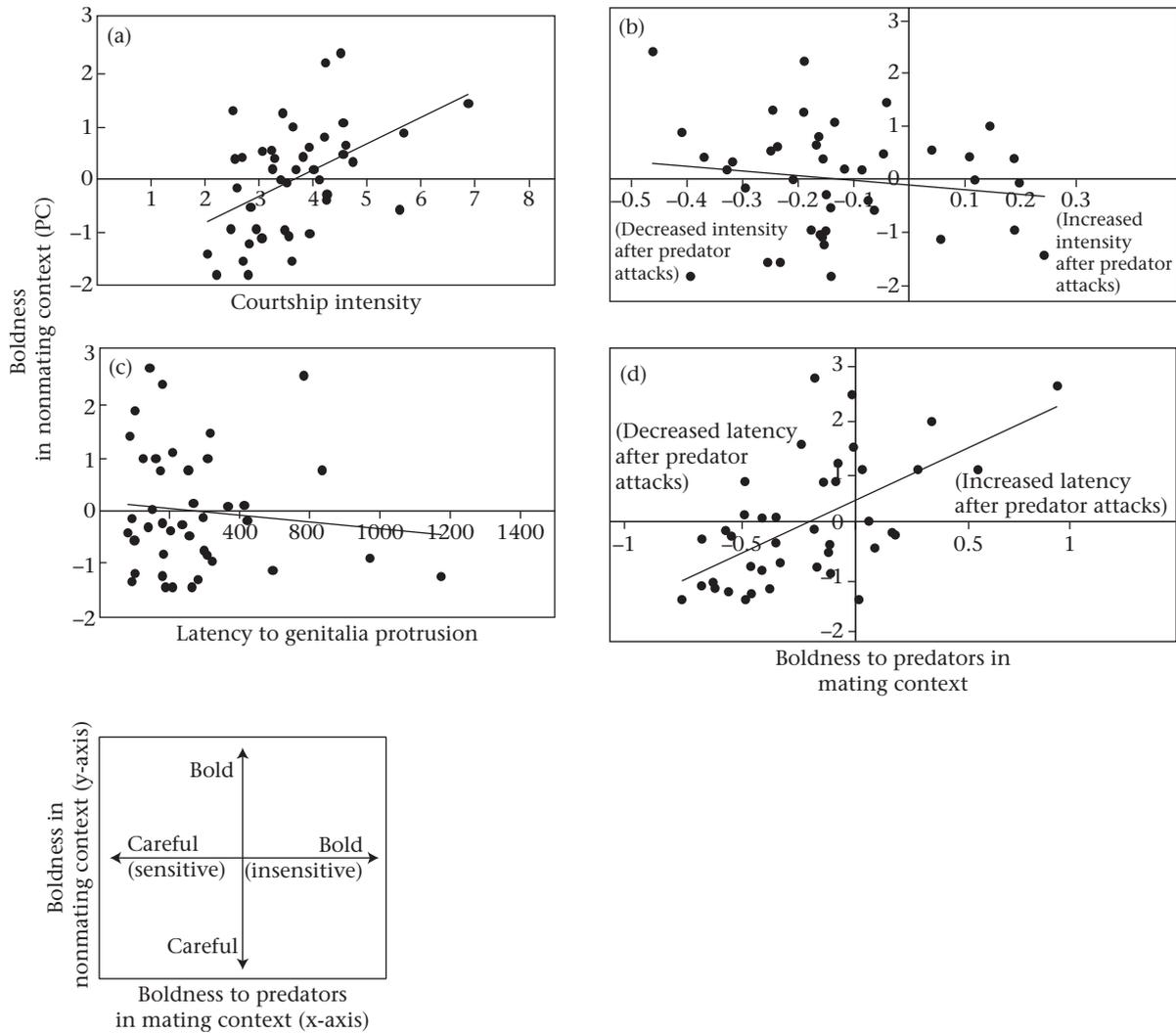

Figure A3. Behavioural correlations between behaviours in mating and nonmating contexts. (a, b) Correlations of male boldness in a nonmating context with (a) mating behaviour (courtship intensity) and (b) male boldness in a mating context (δ). (c, d) Correlations of female boldness in a nonmating context with (c) mating behaviour (latency to genitalia protrusion) and (d) female boldness in a mating context (δ). In (b) and (d), individuals less cautious to predators in a mating context have larger values along the x-axis, and bolder individuals in a nonmating context have larger values along the y-axis.