Original Article

Fluctuating sexual selection and the evolution of a courtship strategy

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Fluctuating sexual selection caused by environmental heterogeneity can maintain variation in sexual signals. Sexual selection can also shape correlations among behavioral traits (behavioral syndromes) when certain behavioral combinations enjoy greater fitness than other combinations (i.e., under correlational sexual selection). Here, we tested the hypothesis that environmental heterogeneity in predation risk shapes the evolution of courtship tactics and behavioral syndromes of male water striders *Gerris gracilicornis*. Male *G. gracilicornis* use an intimidating form of courtship that depends on predation risk, and we predict that male courtship and related behavioral syndromes will be associated with the form of sexual selection under predation. We assayed 4 male behaviors: sex recognition sensitivity, exploration in a novel environment, intimidation, and boldness toward predators. We also estimated males' reproductive success in both the absence and presence of a predator. We found no predation-risk induced differences in linear (i.e., directional) sexual selection. Both treatments experienced directional selection favoring more intimidating courtship by males. Nonlinear sexual selection, however, varied with predation risk. Additionally, we found that sexual selection was not related to the behavioral syndrome structure. Overall, we demonstrate that sexual selection varies with predation risk in ways that might favor the spread of a novel courtship strategy (intimidating courtship) and the maintenance of alternative mating tactics in *G. gracilicornis*.

Key words: **fluctuating selection, *Gerris gracilicornis*, intimidating courtship, predation risk, sexual selection.**

INTRODUCTION

Sexual selection is responsible for the evolution of conspicuous, complex ornaments including bright color, exaggerated morphological structures, or elaborate courtship displays (Rieseberg et al. 2002; Ritchie 2007; van Doorn et al. 2009; Schluter 2009). In addition, the strength, direction, or form of sexual selection can change over time and space as biotic or abiotic environments vary. Spatial and temporal heterogeneity in environments, by altering the dynamics of female mate choice and male–male competition, can cause fluctuations in sexual selection (Siepielski et al. 2009; Siepielski et al. 2011; Siepielski et al. 2013; Miller and Svensson 2014). For example, different microhabitat structures and abiotic environmental factors alter the distribution and availability of potential mates, changing dynamics in the strength, direction, or form of sexual selection. Such fluctuations in sexual selections can contribute to maintaining variation in male sexual traits and reproductive tactics (Boughman 2002; Chaine and Lyon 2008; Cocroft et al. 2010; Miller and Svensson 2014).

Predation risk is a major ecological factor shaping the evolution of reproductive tactics in prey species (Lima and Dill 1990). Because predators exert selection on prey by exploiting mating signals or courtship displays of prey (Endler 1983; Zuk and Kolluru 1998; Haynes and Yeargan 1999), the regimes of sexual selection can change in response to the presence of predators, followed by the evolution of behavioral adaptations according to the strength of predation. Selection under predation favors individuals that tend to be careful in searching, courting mates with sexual traits that are less conspicuous to predators (Endler 1995). Thus, predation risk may shape suited of reproductive behaviors, and such behaviors might evolve plasticity to the immediate threat of predation.

Sexual selection can also explain the evolution of behavioral syndromes (but see Han and Brooks 2013a), suites of behaviors whose expression is correlated across situations (Sih et al. 2004a, 2004b). Such syndromes can be shaped by selection on certain combinations of traits (i.e., correlational selection sensu Price and Langen 1992; Falconer and Mackay 1996). The relationship between correlational selection and behavioral syndromes has been considered

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theoretically (Sih et al. 2004b; van Oers et al. 2005; Bell 2007), but a few empirical studies have tested their roles (but see Bell and Sih 2007; Adriaenssens and Johnsson 2013; Han and Brooks 2013a).

In order to study how fluctuating sexual selection across ecological circumstances (e.g., predation) contributes to evolution of reproductive tactics and behavioral syndromes, we studied an unusual reproductive tactic employed by the water strider *Gerris gracilicornis* (Heteroptera: Gerridae), in which males exploit predators' and females' sensory systems. Gerrid mating behavior is generally sensitive to predation risk as animals are vulnerable to attacks from the air or from beneath the water while they are mating or mate guarding (Sih et al. 1990; Sih and Krupa 1992; Sih and Krupa 1995; Krupa and Sih 1998; Moses and Sih 1998; Han and Jablonski 2010). Predation risk is especially important in *G. gracilicornis* (Han and Jablonski 2010), in which males that have mounted females produce courtship ripples on the water surface, attracting predators such as backswimmers nearby (Han and Jablonski 2009; Han and Jablonski 2010). The ripple signals threaten the female who, beneath the male, is at higher risk of attack from below (Han and Jablonski 2010). This stimulates her to permit copulation quickly to stop the male signaling (sensu "intimidating courtship," Han and Jablonski 2010). Because predation is closely related to the role of intimidating courtship in *G. gracilicornis*, we predict that selection on male intimidating courtship will be altered by cues of predation threat.

In addition, because reproductive tactics may be associated with the expression of many other behavioral traits, predator-mediated sexual selection is also predicted to mould patterns of covariation among behaviors (i.e., behavioral syndromes; Bell 2007; Sih et al. 2004b; van Oers et al. 2005). Alternatively, behavioral syndromes of water strider males might arise via more proximate mechanisms, such as underlying cognitive architecture. Given that males vary in their tendency to use intimidating courtship after a predator attack (Han and Jablonski 2010), we predict that intimidation intensity will covary with males' sensitivity to detect a predator. Moreover, if male–male mounting behavior (Han and Brooks 2015b) is a consequence of male ability to distinguish males from females (Serrano et al. 1991; Harari et al. 2000; Serrano et al. 2000; Switzer et al. 2004; Bailey and French 2012; Burgevin et al. 2013), then recognition of sex may be correlated with cognition in other situations, including predator detection.

In this study, we examined sexual selection on male behaviors in the presence or absence of a predation threat. We tested for 1) fluctuations in sexual selection based on mating success of *G. gracilicornis* males under different levels of predation risk and 2) behavioral syndrome structures of males. We then tested the hypothesis that predator-mediated sexual selection shapes the unique intimidating courtship strategy discovered earlier in this species (Han and Jablonski 2010). We also determined if the observed sexual selection on the suite of behavioral traits matches the behavioral syndrome.

METHODS

Study species and intimidating courtship strategy

Gerris gracilicornis is a water strider widely distributed across East Asia. The male first mounts a female and then produces courtship ripple signals by tapping the water surface repeatedly (Han and Jablonski 2009). Males produce courtship taps in bouts, with an interval (2–3 s; Han and Jablonski 2009) between bouts (see details in Han and Jablonski 2009). We used the number of taps per bout as a measure of male courtship behavior because the index has

been known to be significantly repeatable over time among individuals ($N = 37$, $R = 0.63$, 95% confidence interval = 0.39–0.79; Han et al. 2015).

The courtship ripple signals made by male *G. gracilicornis* also attract predators nearby. Because predatory aquatic insects, such as notonectids (Hemiptera, Notonectidae), approach water striders from below the water surface, a mounted female is at higher risk of predation than the male. The male uses this fact to intimidate the female into mating, stopping his predator-attractant signaling once she acquiesces to mating (Han and Jablonski 2010).

Collection and rearing for the lab experiment

Gerris gracilicornis were collected at Cheonseong Mountain, South Korea. They were then placed in rectangular plastic rearing containers ($50 \times 40 \, cm^2$) with even sex ratio (10 males and 10 females). Frozen crickets (*Gryllus bimaculatus*) were given as food every day, and pieces of floating Styrofoam were provided as resting sites. Twenty-four hours prior to the mating assay, we placed the male with a single female until they copulated, and then separated them immediately, keeping them in single sex groups (20 individuals in each container, $50 \times 40 \, cm^2$).

Notonectids (Hemiptera, Notonectidae, *Notonecta triguttata*), generalist predators that attack small insects on the water surface, including gerrids, were used as predators for the experiment. They were separated individually and reared in transparent plastic cylinder-shaped boxes (diameter 10 cm, height 15 cm). Each individual was fed with 1 frozen cricket (*G. bimaculatus*) every day.

Overview of the sequence of experiments

In order to avoid carryover effect of predator experience on behavioral assays, we sequentially measured 1) mating success of males in predator-absent environment, 2) behavioral assays (sex recognition sensitivity, exploration ability, intimidation, and boldness toward predators during 5 consecutive days), and 3) mating success of males in predator-present condition.

Mating assays in predator-absent environment

In the mating assay, we measured mating success of water strider males in predator-absent environment. First, we divided the 54 males into 6 groups of 9 males, put each group of males together with 3 females in rectangular plastic containers ($50 \times 40 \, cm^2$) and recorded each male's mating success after an hour. The postmating guarding duration of *G. gracilicornis* males is much longer (e.g., up to 1 week) than other water strider species in the genus *Gerris* (Han et al. 2010), meaning that initial mating success is an important fitness determinant and proxy for lifetime reproductive success of males (Han and Brooks 2013a). In addition, during the mating season, when most female water striders are guarded by males, the sex ratio of single males to single females is usually between 1:1.5 and 5:1 (male:female; Han CS, unpublished data). Our experimental design therefore is within the range of possible intermale competition intensities that occur in the wild.

After this mating assay, we put each unmated male with a female until they copulated, in order to avoid variation in males' mating experience. We separated them after mating by their sex and put them back to the rearing container.

Behavioral assay

Each of 54 males was subjected to 4 behavioral assays: 1) sex recognition sensitivity, 2) exploration in a novel environment,

3) intimidation, and 4) boldness toward predators (risk-taking behavior). Each assay was conducted over 5 consecutive days (1 assay per day, but intimidation for 2 days) in the fixed sequence from (1) to (4) to guard against the carryover effect of predator experience (Bell 2013). To measure behaviors, we followed the method outlined by (Han and Brooks 2013a, 2013b, 2014, 2015a).

Sex recognition sensitivity

Water strider males sometimes exhibit same-sex mating attempts by mounting on males instead of females, and only dismount when, after attempting to copulate, they recognize their mistake. We call the speed with which males dismount a male their sex recognition sensitivity (after Han and Brooks 2013b). In male-biased populations, males are predicted to be more successful in mating it, and they are better at distinguishing females from males in their mating attempts. Sex recognition sensitivity shows repeatable among-male variation over time in laboratory studies (Han and Brooks 2013a, 2013b).

In order to measure sex recognition sensitivity, we presented the focal male with 3 other males in the focal male's home tank (22×15 cm^2) and recorded the behavior of the focal male for 5 min. We recorded the duration from the moment the focal male mounted another individual until the moment he dismounted (duration of the first mount). We follow Han and Brooks (2013a, 2013b) interpreting speed to recognize and dismount from a mounting attempt on another male as sensitivity to recognize the sex of a potential mate. Because all the males experienced 1 copulation in or after the mating assay, males had the same period of sexual deprivation. In the analysis, we multiplied the sex sensitivity scores by −1 so that higher behavioral scores mean higher sex sensitivity (males stay shorter on the back of other males).

Exploration in a novel environment

Gerris gracilicornis individuals are macropterous (long winged) and move among microhabitats along a creek. Males who cope with spatial changes in their nature environment are expected to move actively in a novel environment instead of standing still on the water. Such males would also spend less time grooming themselves because those males spend less time standing still on the water, which is when grooming happens. The novel environment consisted of a tank (40×50 cm^2; water depth, 5 cm) illuminated from above. The test male was transferred to the corner of the tank and left undisturbed for 30-s acclimation followed by a 5-min assays. He could move freely in the open novel environment, and the behavior was filmed with a video camcorder mounted above the tank. We measured 2 behaviors of males for 5 min: 1) the number of strokes (movement of middle legs to move forward) and 2) grooming duration (duration of the focal individual washing himself with legs).

Intimidation

To measure intimidatory courtship, we estimated male courtship signal frequencies in both predator-present and predator-absent conditions and compared them. If increasing the number of ripples per bout (i.e., courtship frequency, Han and Jablonski 2009) increases chances of attracting predators, higher signal frequency is predicted to be favored in riskier environment. Therefore, we measured 1) males' courtship signal frequency after the exposure to predators and 2) the relative increase in courtship signal frequency after predator attack.

Males' courtship signal frequency was measured in a 15×15 cm^2 arena, which was a part of a larger tank ($15 \times 30 \times 15$ cm^3, Supplementary Figure S1). The transparent large tank (made of acrylic plate, $15 \times 30 \times 15$ cm^3) was divided by opaque partition (5×15 cm^2; height × length) into 2 separate containers ($15 \times 15 \times 15$ cm^3) for blocking the transmission of chemicals, and sometimes predators, through the water between the 2 parts (each part was filled with water up to 4.8–5.0 cm). A second opaque plate (10×15 cm^2; height × length) was located above the first one and could be lifted by the experimenter to allow the water striders to move from one part to another.

Twenty-four hours later after the measurement of exploration ability, we recorded male courtship signal frequency in a predator-absent condition with no exposure to predators. A male and a female were placed separately in each compartment for the first 1 min as the acclimation period. Then, we allowed the male to move to female compartment and measured his courtship behavior. We used the number of taps (signals) per bout as the index of male courtship signal frequency.

Then, approximately 24 h later, we recorded males' courtship signal frequency in a predator-present condition after the exposure to predators. A male was introduced to 1 compartment with 1 notonectid (*N. triguttata*) predator and experienced predator's attack. The male was successfully attacked at least 1 time by notonectids (successful attack: backswimmers grasped males tightly). When males escaped from the attack of backswimmers (unsuccessful attack), we regarded 10 unsuccessful attacks as 1 successful attack (more detailed methods described in Han and Jablonski 2010). After the successful attack, we promptly separated the grasped male from the notonectid to prevent the notonectid from piercing the cuticle of the water strider with its rostrum. Then, predator-experienced male was allowed to move to female compartment, and we recorded his courtship signal frequency.

Boldness toward predators

Using the same experimental tank in the intimidation assay (Supplementary Figure S1), we measured males' response toward predators. A male was introduced to 1 compartment with 1 notonectid (*N. triguttata*) predator and experienced predator's attack as we did in an intimidation assay (see above). The partition was then lifted to allow the male to move to the experimental compartment. In the experimental compartment, no shelter was floated on the water for the male to escape from the open habitat. We observed behaviors, 1) the number of strokes on the water and 2) grooming duration for 5 min.

Actively moving individuals (indicated by high number of strokes) are more conspicuous to *Notonecta* predators that perceive surface vibrations. Therefore, higher activity even soon after detecting a predator may be interpreted as boldness. Grooming activity is also a more risky behavior because the individual is not supported on all legs, delaying the initiation of escape. Because water striders receive information on the water tarsal vibration receptors on their legs (Murphey 1971; Lawry 1973; Perez Goodwyn et al. 2009), they are not able to detect approaching predators and escape from them well if some of their legs are out of touch with the water surface (Han and Jablonski 2010). Thus, predator vigilance can be affected by the amount of attention simultaneously being focused on other activities such as foraging or grooming. Therefore, males insensitive to the presence of predators are expected to be more active on the water surface and spend more time for grooming themselves when they stand still on the water.

Mating assays in predator-present environment

In order to measure mating success of males in predator-present environment, males and females were put together after

experiencing predator notonectids' attack before mating assay. We divided males to groups composed of the same individuals at the previous mating assay and let them experience predatory attacks. In order to make individuals experience predatory attacks, we put an individual into the same container with a notonectid predator, but the experimenter always interrupted a successful predatory grasping to prevent the notonectid from piercing the cuticle of the water strider with its rostrum (more detailed methods described in Han and Jablonski 2010). We then put them together in rectangular plastic containers ($50 \times 40\,cm^2$) and then recorded their mating success after an hour. We started with 54 males, but 4 males died during the boldness assay (see above) and 2 males escaped before the second mating assay in a predator-present environment conducted. Thus, 6 new males were added to the group during the mating assay, but they were not included in the analyses. That explains why sample sizes differ in the calculation of behavioral correlations (see Table 1; Supplementary Table S2).

Table 1

The model comparison describing the effect of risk level on linear/nonlinear selection gradients

	Models	df	AIC	AIC weight
1	Treatment + linear terms	8	108.7	0.02
2	Treatment + linear terms + treatment × linear terms	12	112.7	0.00
3	Treatment + linear terms + nonlinear terms	18	106.1	0.08
4	Treatment + linear terms + nonlinear terms + treatment × (linear + nonlinear terms)	32	101.4	0.89

The initial mating success, a response variable, was expressed as 0 (failure) or 1 (success) and was modeled with a Binomial error distribution. The linear terms in the model description indicate all linear terms of 4 behavioral variables (sex sensitivity, exploration, intimidation, and boldness). The nonlinear terms indicate all the quadratic terms of 4 behavioral variables and all the interaction terms between behavioral variables. The treatment in the model description indicates the effect of exposure to predatory attacks (the absence/presence of predators). Male IDs and experimental subgroups were included as random factors in all model structures. The candidate models were compared using AIC scores and AIC weights.

Statistical methods

Sexual selection gradients

We adopt multivariate selection analyses (Lande and Arnold 1983; Phillips and Arnold 1989) in order to detect selection acting on multiple traits and combination of traits (i.e., correlational selection).

To estimate the effect of behavioral traits on mating success in different predator-present conditions, we used generalized linear mixed model (GLMM) and Laplace methods to approximate the likelihood to estimate GLMM parameters (Bolker et al. 2009). Mating success was expressed as 0 (failure) or 1 (success) and was modeled with a binomial error distribution. The linear and nonlinear (quadratic and interaction) terms of standardized behavioral traits (sex recognition sensitivity, exploration, intimidation, and boldness) were added to the model as the covariates. Experimental subgroups were added as a random factor.

At first, to test the effect of risk level on overall selection gradient, we compared 4 models with and without the interaction effect between treatments and linear/nonlinear terms of standardized behavioral traits (sex recognition sensitivity, exploration, intimidation, and boldness) (Table 1). If the interaction effect between treatments and linear/nonlinear terms improved the fit of the model, it would indicate that recent experience of predation risk changed the fitness landscape. After the assessment of the effect of predation risk, in order to find behavioral traits affecting mating success from the selected model, further model selection was conducted on the basis of stepwise backward selection by removing nonsignificant linear or nonlinear terms (Tables 2 and 3). We used Akaike's information criterion (AIC) to assess whether each term or class of terms (in the case of linear and nonlinear selection gradients) improved the overall fit of the model, and the model with the lowest AIC value is considered best supported. The analysis was performed using the package lme 4 in the program R 2.14.1. Also, to visualize fitness landscape for interactions effects (i.e., correlational selection), we used a cubic spline fitting procedure using the software STATISTICA 8 (Statsoft).

Behavioral syndrome

Correlations between behavioral traits were assessed using Spearman's rank nonparametric correlations. Behaviors other than

Table 2

The model comparison describing the effect of behaviors on initial mating success in a predator-absent environment, and a selected model of initial mating success as a function on behaviors and a combination of behaviors

	Models	AIC	AIC weight
1	Linear terms + interaction terms + quadratic terms	50.9	0.009
2	Linear terms + interaction terms	49.2	0.02
3	S + B + E + S × B + S × E + B × E	45.1	0.16
4	**S + E + S × E**	**41.9**	**0.81**

Variables	Estimate (SE)	z	P
Intercept	−2.23 (0.86)	−2.59	0.01
Sex sensitivity	2.19 (1.72)	−1.27	0.20
Exploration	0.85 (0.83)	1.02	0.31
Sex sensitivity × exploration	**−5.19 (2.36)**	**2.20**	**0.03**

The initial mating success, a response variable, was expressed as 0 (failure) or 1 (success) and was modeled with a Binomial error distribution. The linear terms in the model description indicate all linear terms of 4 behavioral variables (sex sensitivity, exploration, intimidation, and boldness). The quadratic terms indicate all the quadratic terms of 4 behavioral variables. The interaction terms indicates the interaction between behavioral variables. The best model is indicated in bold. Experimental subgroups were included as random factors in all model structures. B, boldness toward predators; E, exploration in a novel environment; S, sex recognition sensitivity.

Table 3

The model comparison describing the effect of behaviors on initial mating success in a predator-present environment, and a selected model of initial mating success as a function on behaviors

	Models	AIC	AIC weight
1	Linear terms + interaction terms + quadratic terms	67.2	0.004
2	Linear terms + quadratic terms	60.0	0.15
3	**I + E + I^2 + E^2**	**56.6**	**0.84**

Variables	Estimate (SE)	z	P
Intercept	−0.19 (0.51)	−0.38	0.70
Intimidating intensity	0.32 (0.45)	0.72	0.47
Exploration	0.17 (0.40)	0.43	0.69
Intimidating intensity^2	**−0.88 (0.41)**	**−2.13**	**0.03**
Exploration^2	**0.60 (0.29)**	**2.09**	**0.04**

The initial mating success, a response variable, was expressed as 0 (failure) or 1 (success) and was modeled with a Binomial error distribution. The linear terms in the model description indicate all linear terms of 4 behavioral variables (sex sensitivity, exploration, intimidation, and boldness). The quadratic terms indicate all the quadratic terms of 4 behavioral variables. The interaction terms indicates the interaction between behavioral variables. The best model is indicated in bold. Experimental subgroups were included as random factors in all model structures. E, exploration in a novel environment; I, intimidation intensity; SE, standard error.

sex recognition sensitivity were summarized and standardized using Principal component analysis. We retained component scores for principal components (PCs) with eigenvalues >1.0. Statistical significance was inferred if P values remain significant after the procedures for controlling false discovery rate (Benjamini and Hochberg 1995; Storey and Tibshirani 2003). Because Bonferroni adjustment has been shown to be overly conservative (e.g., Benjamini et al. 2001; Nakagawa 2004; Narum 2006), we used a false discovery rate (Benjamini and Hochberg 1995; Benjamini et al. 2001; Storey and Tibshirani 2003; Dochtermann 2010) to account for 6 correlations (α < 0.0204 for 6 correlations).

Because of inherent problems of pairwise behavioral correlations (see Dingemanse et al. 2010), it often helps to compare the fit of a priori considered structural equation models (Dochtermann and Jenkins 2007; Dingemanse et al. 2010; Bókony et al. 2012). This alleviates the need to control for multiple testing because the test is conducted on the whole matrix. However, because this study measured nonlinear selection acting on the combinations of behaviors, the measurement of pairwise behavioral correlations is appropriate in order to compare the pattern of behavioral correlation with correlational selections (see Han and Brooks 2013a). Thus, we present pairwise behavioral correlations for behavioral syndrome structure.

RESULTS

Sexual selection gradients

Predator exposure had no effect on the linear selection response surface (models 1 and 2, ΔAIC = +4.0, χ^2 = 4.07, degrees of freedom [df] = 4, P = 0.40, Table 1). Regardless of predator treatments, directional selection acted on intimidation intensity (Table 4). That is, males that signaled more (as indicated by the "intimidation PC1") enjoyed greater mating success. But there was no effect of other behavioral traits on mating success.

However, there were significant differences in nonlinear selection between predator treatments (models 3 and 4, ΔAIC = −4.7, χ^2 = 32.67, df = 14, P = 0.003, Table 1). In the absence of predators, significant correlational selection on sex recognition sensitivity and exploration ability was detected (Table 2, Figure 1). However, in the presence of predators, males that intimidated females at intermediate intensity enjoyed greatest mating success (Figure 2b).

Table 4

Linear selection gradients for 4 behavioral traits

Variables	Estimate (SE)	z	P
Intercept	−1.35 (0.47)	−2.86	0.004
Treatment	0.66 (0.55)	1.20	0.23
Sex sensitivity	−0.26 (0.33)	0.77	0.44
Boldness	0.57 (0.33)	1.72	0.09
Intimidation	**0.69 (0.33)**	**2.11**	**0.03**
Exploration	−0.23 (0.33)	−0.69	0.49

The significant term is indicated in bold. SE, standard error.

In addition, given that disruptive selection on exploratory behavior, extreme activity patterns, such as inactive or hyperactive, are likely to be favored over intermediates in predator-present environments (Figure 2a).

Behavioral syndrome

Exploration PC1 (eigenvalue = 1.25) obtained from the factor loadings showed that active males on the water spent less time grooming but inactive males spent more time grooming (Supplementary Table S1). Thus, males that had lower exploration PC1 scores spent more time grooming but explored a smaller area, whereas males that had higher scores on exploration PC spent less time grooming but they were more active. Boldness PC1 (eigenvalue = 1.40) obtained from the factor loadings showed that active males on the water spent more time grooming after the exposure to predators (Supplementary Table S1). In contrast to the factor loading for exploration ability, boldness PC1 showed positive correlation between movements and grooming. Thus, bold males that had higher boldness PC1 scores spent more time grooming and explored a larger area. Shy males that had lower scores on boldness PC1 spent less time grooming and they were less active. In addition, intimidation PC1 (eigenvalue = 1.36) obtained from the factor loadings indicated that males who produced high signal frequency after experiencing predators showed the relative large increase in signal frequency after predatory attacks (Supplementary Table S1).

Male sex recognition sensitivity was marginally correlated with boldness toward predators (Table 5). That is to say, males with poor sex recognition sensitivity tended to be less sensitive to predators. When we

calculated correlations between behavioral variables originally used to calculate PCs, sex recognition sensitivity was correlated with grooming duration after predator attack (Supplementary Table S2).

DISCUSSION

The form of directional sexual selection on males' intimidating courtship did not fluctuate according to the presence of predators, and thus play a role in shaping and maintaining intimidating courtship of males *G. gracilicornis*. In contrast, nonlinear sexual selection fluctuated with the level of predation risks. Combining with directional selection, stabilizing selection appears to contribute to the maintenance of intimidating courtship. Disruptive selection also appears to play a role in the maintenance of alternative reproductive tactics in *G. gracilicornis* males through its effect on exploration in the presence of predators (e.g., Smith 1993; Brodie et al. 1995; Rueffler et al. 2006; Hendry et al. 2009).
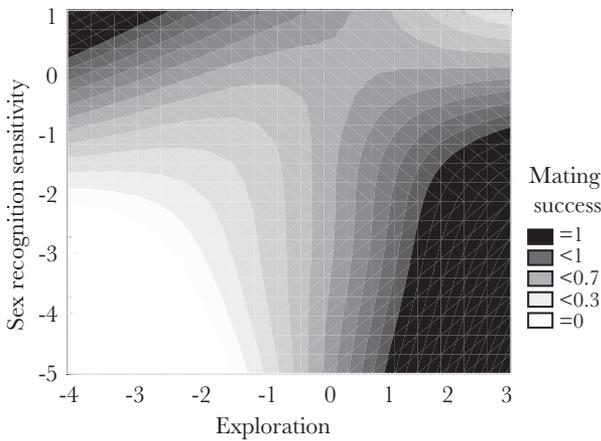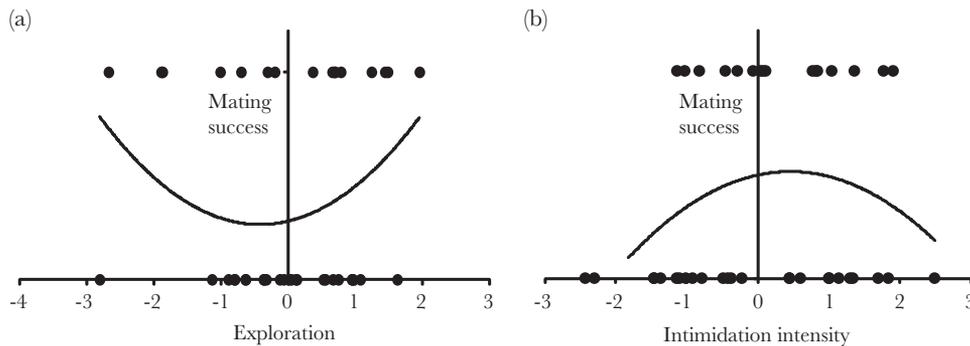

Figure 1

The fitness surface of nonlinear selection in a predator-absent environment. The fitness surface of the 2 major axes of nonlinear selection, exploration and sex recognition sensitivity, of male water striders in a predator-absent environment. Larger values (close to zero) of sex recognition sensitivity indicate that males more rapidly to recognize that they have mounted another male or a mated pair and dismount. Smaller values of exploration mean that males spent more time in grooming but less active, whereas larger values of exploration mean that males were more active but spent less time in grooming.

Evolution of intimidating courtship

The presence of directional selection for more taps per bout in intimidating courtship, both when predators were absent and present, suggests that intimidating courtship is an important fitness component in *G. gracilicornis* populations, possibly even when notonectid predators are rare or absent. The observed pattern of consistent directional selection is expected to cause further evolutionary elaboration of male courtship and mating signals (Andersson 1994). In natural situations, lack of cues of predator presence may often be not a good indication of their actual absence. Therefore, females' sensitivity to intimidating signals regardless of whether they detected predators or not might be an adaptation to this uncertainty.

Although the strength of linear selection toward more intimidating courtship did not vary with predator presence/absence, in the presence of predators, this selection became somewhat stabilizing, with the highest intensities of intimidation resulting in lower mating success. This suggests some optimization of intimidating courtship rates, although its effects on selection remain to be assessed in larger studies, preferably in the field, of this behavior. Because in our experiments both sexes might have changed their behavior independently due to their prior exposure to a predator, we cannot determine here the relative contributions of males and females' behavioral plasticity to creating the stabilizing selection on the intimidating courtship intensity.

The changes in nonlinear selection on intimidating courtship between assays where predators were present and absent, raises the possibility that selection on this behavior might vary between microhabitats. In nature, where predation risk may vary spatially and temporally, changes in the selection operating on intimidating courtship might occur over small spatial scales. Although we found fluctuations in nonlinear selection across the risk of predation, a small number of our sample size is likely to result in the high error in the estimation of nonlinear selections. We might fail to detect nonlinear selection even when it is present because of small sample sizes (see Kingsolver et al. 2001). Therefore, although it is cautious to make strong conclusions from relatively small sample sizes, we can still hypothesize that in natural conditions, where predators are undoubtedly experienced by both sexes, the selection in the context of mating success (i.e., sexual selection) have an effect on the structure of intimidating courtship.

Figure 2

The fitness surface of (a) disruptive selection on exploration and (b) stabilizing selection on intimidation intensity of male water striders in a predator-present environment. Smaller values of exploration mean that males spent more time in grooming but less active, whereas larger values of exploration mean that males were more active but spent less time in grooming. Larger values of intimidation indicate that males produced high-frequency signals and increased their signal frequency after the exposure to predators.

Table 5

The structure of behavioral syndrome among behaviors, sensitivity to distinguish the correct sex (sex recognition sensitivity), exploration in a novel environment (exploration), boldness toward predators (boldness), and intimidation intensity (intimidation)

	R_s	n	P
Sex sensitivity—intimidation	0.11	46	0.45
Sex sensitivity—boldness	**−0.30**	**46**	**0.04**
Sex sensitivity—exploration	−0.09	48	0.56
Intimidation—boldness	−0.15	44	0.34
Intimidation—exploration	−0.13	45	0.39
Boldness—exploration	−0.10	45	0.53

When P values were adjusted for multiple comparisons (a false discovery rate B–Y adjustment, α < 0.02 for 6 correlations), there were no significant behavioral correlations (R_s values, Spearman's rank nonparametric correlations). However, the correlation between sex sensitivity and boldness was marginally correlated (indicated in bold).

Evolution of alternative reproductive tactics

Disruptive selection can generate phenotypic diversification within populations and may lead to emergence of alternative life histories and alternative reproductive strategies (Gross 1985; Emlen and Nijhout 2000; Sinervo and Svensson 2002; Mendoza-Cuenca and Macías-Ordóñez 2010). The selection gradients for exploration ability (in predator-absent and predator-present environments) suggest that disruptive selection on exploration (activity level) may shape alternative strategies of *G. gracilicornis* and that in predator-absent conditions this selection on exploration is additionally correlated with selection on sex recognition sensitivity with 2 behavioral phenotypes favored: low exploration with good sex recognition sensitivity or high exploration with poor sex recognition sensitivity.

All these results indicate that, in habitats with temporal or spatial variation in predator presence, sexual selection may favor 2 distinct exploratory strategies in *G. gracilicornis* males. One is a sit-and-wait strategy when males decrease their activity but distinguish the sex carefully, chase females, and attempt to mate with them. Another strategy is to explore actively in the habitat and indiscriminately attempt to mate with passing individuals. Behavioral observations of male *G. gracilicornis* indicated that some of small males tended to be less active and did not produce postmounting courtship signals (Han and Jablonski 2016). Thus, we suggest a possibility that alternative reproductive tactics of *G. gracilicornis* males are generated by the disruptive sexual selection that favors extremely high or low activity (e.g., Gross 1985; Mendoza-Cuenca and Macías-Ordóñez 2010).

Absence of a behavioral syndrome

We failed to find a significant behavioral syndrome structure (Table 5) even when correlational selection in the context of mating success exerted on sex recognition sensitivity and exploration in a predator-absent environment (Table 2). The marginally significant behavioral correlation between sex recognition sensitivity and response toward predators was also not matched with the observed correlational selection. This indicates that sexual selection as measured in our lab assays is not related to the behavioral syndrome structure (e.g., Han and Brooks 2013a).

To measure behavioral syndrome, we should have conducted each assay on each individual repeatedly to quantify individual behavioral consistency and to separate "among-individual" and "within-individual" correlations. Behavioral correlations at the phenotypic level are the product of the joint influences of "among-individual" and "within-individual" correlations (Dingemanse et al. 2012; Brommer 2013; Dingemanse and Dochtermann 2013), and behavioral syndromes refer to among-individual correlations rather than simple unpartitioned phenotypic correlations (Dingemanse and Dochtermann 2013). The among-individual correlation is the correlation between each individual's average phenotype across multiple behaviors, that is, the correlation between the repeatable parts of behavioral traits. However, because we took only a single measurement per individual, we could not partition behavioral correlations at the phenotypic level into among-individual correlations and within-individual correlations. Therefore, it is necessary to collect repeated measures on behaviors for all individuals to calculate behavioral syndromes (among-individual correlations) especially when behavioral repeatabilities are low (low among-individual variance and high within-individual variance in behaviors).

There is also a lack of behavioral syndrome when the functional context (e.g., predation) in which one behavior is expressed is not related to the expression of another behavior. As male intimidating courtship ripples have a role in attracting predators approaching from below the water surface and threatening females (Han and Jablonski 2010), we predicted an association between male intimidation intensity and male response to predators. However, there was no correlation between them (Table 1). This indicates that the expression of male intimidation is unrelated to males' recognition of the presence of predators. It is also supported by our recent results that males do not adjust their intimidation intensity according to the level of predation risk (Han et al. 2015). Hence, males express similar level of intimidation regardless of the differing levels of predation risk (Han et al. 2015), explaining a lack of correlation between intimidation and behavioral response to predators.

CONCLUSIONS

In conclusion, we provide evidence on how a novel courtship strategy and alternative reproductive tactics of male water striders *G. gracilicornis* evolve and are maintained in heterogeneous environments. We also suggest that the form of sexual selection on males could fluctuate according to the presence of predators in the population. Correlational and disruptive sexual selection in our results imply that sexual selection might not explain the evolution of behavioral syndromes but play a role in retaining variation in traits. Because studies on a cause and a consequence of fluctuating sexual selection with environmental conditions changing over time and space are much needed (Siepielski et al. 2011, 2013; Miller and Svensson 2014), future studies should explore the effect of rate of fluctuation (rate of changing selection over time) or a balance between fluctuating sexual and natural selection on the evolution of sexual traits. Finally, this empirical study using water striders also provides insight into the way behavioral syndrome is generated and maintained in a population. We suggest that future studies consider the proximate mechanisms as a more likely basis of the emergence of behavioral syndrome in males *G. gracilicornis*.

SUPPLEMENTARY MATERIAL

Supplementary material can be found at http://www.beheco.oxfordjournals.org/

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