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SEXUAL CONFLICT AND ANTAGONISTIC COEVOLUTION ACROSS WATER STRIDER POPULATIONS

Jennifer C. Perry[1,2,3] and Locke Rowe[1]

[1]*Department of Ecology and Evolutionary Biology, University of Toronto, 25 Willcocks St. Toronto, ON, M5S 3B2, Canada*

[2]*E-mail: jennifer.perry@zoo.ox.ac.uk*

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Microevolutionary studies have demonstrated sexually antagonistic selection on sexual traits, and existing evidence supports a macroevolutionary pattern of sexually antagonistic coevolution. Two current questions are how antagonistic selection within-populations scales to divergence among populations, and to what extent intraspecific divergence matches species-level patterns. To address these questions, we conducted an intraspecific comparative study of sexual armaments and mating behaviors in a water strider (*Gerris incognitus*) in which male genitals grasp resistant females and female abdominal structures help ward off males. The degree of exaggeration of these armaments coevolves across species. We found a similar strong pattern of antagonistic coevolution among populations, suggesting that sexual conflict drives population differentiation in morphology. Furthermore, relative exaggeration in armaments was closely related to mating outcomes in a common environment. Interestingly, the effect of armaments on mating was mediated by population sexual size dimorphism. When females had a large size advantage, mating activity was low and independent of armaments, but when males had a relative size advantage, mating activity depended on which sex had relatively exaggerated armaments. Thus, a strong signal of sexually antagonistic coevolution is apparent even among populations. These results open opportunities to understand links between sexual arms races, ecological variation, and reproductive isolation.

KEY WORDS: ***Gerris incognitus*, intraspecific comparative study, sexually antagonistic coevolution, sexually antagonistic trait, sexual size dimorphism, water striders.**

The sexes are expected to have conflicting evolutionary interests within nearly all sexually reproducing species (Arnqvist and Rowe 2005). Under some conditions, this conflict is expected to generate sexually antagonistic coevolution (SAC), which may produce continual exaggeration of male persistence and female resistance traits (an arms race), cycles of trait exaggeration, or gradual trait de-escalation and retreat (Parker 1979; Holland and Rice 1998; Gavrilets et al. 2001; Rowe et al. 2005; Hoyle and Gilburn 2010).

A full understanding of the influence of conflict on the evolution of sexually antagonistic traits (SATs) requires studies at several levels. Understanding the function of SATs requires within-population studies that assess selection and the costs and benefits of trait expression across a range of ecological contexts (Chapman et al. 2003; Rowe and Day 2006; Fricke et al. 2009). At the macroevolutionary level, testing the hypothesis that conflict drives coevolutionary divergence in SATs requires comparative studies of trait evolution. Finally, population-level studies of

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[3]Current address: The Edward Grey Institute, Department of Zoology, University of Oxford, South Parks Rd., Oxford OX1 3PS, United Kingdom.

divergence in sexual armaments are needed to link microevolutionary processes to the species-level outcomes.

Subsets of these data are now available in a few systems. For example, within-population studies have revealed the function and selective consequences of male grasping and female antigrasping structures of water striders (e.g., Arnqvist and Rowe 2002b) and the harmful male genitalia and toughened female tissue in *Callosobruchus* seed beetles (Rönn et al. 2007) and bedbugs (reviewed by Siva-Jothy 2006). Furthermore, interspecific comparative studies in both water striders and seed beetles have shown that male and female SATs coevolve, with imbalances in the degree of armaments predicting the mating outcomes for a given species (Arnqvist and Rowe 2002a; Rönn et al. 2007). Evidence of coevolution can also be detected at the population level in a very few species, including the grasping male tarsal pads and smooth or ridged female dorsal surfaces of diving beetles (Bergsten et al. 2001), as well as population divergence driven by sexual conflict in experimentally evolving dung flies (Martin and Hosken 2003, 2004). However, there is currently no system in which a comprehensive assessment of the influence of sexual conflict at all three levels is available.

Some of the more complete evidence for SAC comes from functional, economic, and comparative studies of water striders (reviewed by Arnqvist and Rowe 2005). In these animals, the functions of grasping and antigrasping traits are well understood (reviewed by Rowe et al. 1994; Arnqvist 1997). These structures are closely correlated across species of *Gerris* water striders, such that species with highly exaggerated females also tend to have more exaggerated males, whereas in other species the traits in both sexes are reduced (Arnqvist and Rowe 2002b). Moreover, the link between sexual morphology and mating outcomes has been well established: in species where males have an advantage in grasping morphology, mating rates increase because males are better able to maintain their grip on struggling females (Rowe and Arnqvist 2002; Arnqvist and Rowe 2002a).

In this model system, population-level data have the potential to link the existing microevolutionary and interspecific studies. Yet, there is currently little known about the coevolution of SATs among water strider populations. These data are needed because it is not always possible to predict the outcome of sexual selection at one level based on patterns at other levels (e.g., Blanckenhorn et al. 2006; Johnson and Wade 2010). One reason is that the trajectory of selection measured within populations does not necessarily predict the coevolutionary pattern among groups. This may occur when the coevolutionary process is overwhelmed by environmental variation and alternative forces of selection or through the masking effect of behavioral plasticity. In the water strider *G. odontogaster*, much of the interpopulation variance in male grasping structure size can be explained by ecological factors (predation pressure and population density; Arnqvist 1994). Furthermore, female resistance to remating in water striders—which generates sexual selection on male grasping traits—is very sensitive to ecological variation (reviewed by Rowe et al. 1994). A second reason is that distinct processes may influence the outcome of SAC at the species and population levels. Population divergence in SATs driven by SAC may be countered by stabilizing selection for species mate recognition (McPeek et al. 2008, 2009; Arbuthnott et al. 2010), particularly when SATs are involved in premating interactions as in water striders. Such stabilizing selection may result in rapid divergence in SATs after speciation events—producing a pattern of SAC across species—but very little divergence between speciation events, producing little correlated evolution among populations.

To address this gap, among-population coevolutionary studies are required. Here, we present a comparative population study of SATs and mating behaviors, using a water strider (*Gerris incognitus*) that has particularly exaggerated SATs (Arnqvist and Rowe 2002b). This species has previously been subjected to behavioral, functional, and selection studies (Arnqvist and Rowe 1995; Arnqvist et al. 1997; Arnqvist and Thornhill 1998; Rowe and Arnqvist 2002), and thus the function of, and selection on, its sexual traits is well understood. Its mating behavior is similar to that of other *Gerris* spp. (Rowe and Arnqvist 2002): males lunge at females they encounter and attempt to get into the mating position, in which males are atop females with their bodies aligned. If males achieve this position, a vigorous and lengthy struggle often ensues, during which males grasp females with their forelegs and genital segments and females perform back flips across the water surface in an apparent attempt to dislodge males. *Gerris incognitus* females possess traits that help them resist male mating attempts: they have enlarged and upturned connexival spines above their genitalia, which are angled downward, making it easier to repel copulation attempts by males (Rowe et al. 1994; Arnqvist 1997; Arnqvist and Rowe 2002b). Male genital segments are elongated and shaped for grasping females. During premating struggles and subsequent mating, the inflatable genitalia emerge from the pregenital segment that houses them and grasp the female's genital segments.

We focus on variation in morphological traits expected to play a role in antagonistic mating interactions: male and female genital structures, female connexival spines, and sexual size dimorphism (SSD) (Andersen 1993; Arnqvist and Rowe 1995; Arnqvist and Thornhill 1998; Arnqvist and Rowe 2002b). Because SAC is expected to generate rapid phenotypic change (Arnqvist and Rowe 2005; Chapman 2006; Parker 2006; Snook et al. 2009), and genital and nongenital structures in water striders tend to harbor additive genetic variation (Arnqvist and Thornhill 1998) and show local adaptation at small geographic scales (Fairbairn and Preziosi 1994; Fairbairn 2005), we expect significant evolutionary divergence in these traits among populations. To the extent that SAC is a dominant process in the evolution of these traits, we expect these traits to be positively correlated between the sexes across populations. In contrast, correlated evolution may be obscured if ecological variation drives trait evolution (independently of trait coevolution) or if selection for species recognition dominates.

We also determined whether the covariance in mating behaviors among populations is consistent with the hypothesis that ecological variation in optimal female mating rate dominates mating behavior, versus the hypothesis that variation in relative armaments dominates mating behavior (Rowe and Arnqvist 2002). In the first case, an increase in the optimal mating rate for females results in female struggling for shorter durations and consequently mating more frequently, a pattern seen in studies that manipulate optimal female mating rate in water striders (reviewed in Rowe et al. 1994; Rowe and Arnqvist 2002). In the second case, females struggle to avoid costly matings; when males have a relative advantage in armament exaggeration, they can withstand longer struggles, leading to a pattern of longer struggles associated with higher mating frequency, a pattern reported in an interspecific study of *Gerris* (Arnqvist and Rowe 2002a).

Methods

SPECIES

Gerris incognitus is a small (body length $\leq$ 1 cm) Nearctic species restricted to the west and east perimeters of North America (Gagnon and Turgeon 2010). Like its congeners, *G. incognitus* inhabits temporary and permanent still water bodies and scavenges for dead and struggling arthropods on the water surface (Callahan 1974).

POPULATION SAMPLING

Our goal was to sample natural variation in morphology and mating behavior in populations of *G. incognitus*. We collected water striders from 19 locations (Table A1). Populations within British Columbia, Canada, occurred along a 384 km north-south gradient and 488 km east-west gradient, while the two populations from Nova Scotia, Canada, were separated by 248 km. We keyed individuals to species (following Scudder 1971; Andersen 1993) and deposited voucher specimens at the Royal Ontario Museum (Toronto, Canada). Populations were sampled when the adults collected were of the overwintered reproductive generation (Callahan 1974; Spence 1983); we observed reproductive activity in all populations following collection (J. C. Perry and L. Rowe, unpubl. data). Upon collection, water striders were shipped on ice (to minimize activity) to the laboratory (Toronto, Canada) for behavioral observations (see below). Upon arrival, water striders were held at 4°C for no more than two days and then held for two days at room temperature in aerated pools at a 1:1 sex ratio with abundant food (fruit flies and crickets) and styrofoam strips as resting surfaces. The sexes were separated 24 h before the behavioral observation period, with males held in buckets containing several cm of water (three to four males per bucket) and females held in aerated pools (eight females per pool), and again provided with styrofoam resting surfaces and abundant food. We conducted behavioural observations on each population within seven days of collection from the field. We did not use individuals that appeared damaged (e.g., missing leg segments).

BEHAVIORAL OBSERVATIONS

We conducted behavioral observations on water striders held in bins (surface area 2400 cm^2) containing water to a depth of 2–3 cm, with two females and six males per bin. To initiate the observations, we marked each female's pronotum with a paint spot and released her into a bin. Females were allowed 15 min to acclimatize before males were introduced simultaneously. Upon male introduction, we immediately began recording sexual behavior using EthoLog software (version 2.2.5; Ottini 1999). We observed bins for 1 h and recorded the occurrence and duration of each stage of sexual interactions. Each interaction began with a harassment event, in which males lunge at females and may grapple with females in an apparent attempt to grasp them, typically causing females to move away. Harassment events could end in separation or progress to a struggle in which males were atop females and their bodies aligned in the premating position, typically with vigorous female resistance. Struggle events could end with separation or proceed to mating; mating could be distinguished from struggles when the male's genital segments were bent around the female's genital aperture and the female resumed skating normally on the water surface while carrying the male. Mating could progress to a postmating guarding period, in which the male remained on top of the female with the genitals detached, a postmating struggle, or separation without guarding or struggling.

We conducted a second set of observations aimed at measuring copulation duration, as the initial observation period was too brief for this purpose. Following the initial observations, the sexes were kept separately (males in bins at 10 males per bin, females held individually) and fed well for at least seven days. To conduct the observations, females were transferred individually to pails (surface area 707 cm^2) filled to approximately 10 cm depth of water, provided with abundant food (frozen *Drosophila*), and left undisturbed for 1 h. We then removed the food and placed a single male with each female. The pairs were observed continuously for 3 h and we recorded the duration of copulation. These second observations could not be conducted for two populations because the males were no longer available (Table A1).

We did not collect behavioral data for the Princeton population due its small sample size (Table A1). Two populations showed precopulatory mating behaviors but did not exhibit copulation in our initial observations; however, mating was observed for both populations during the observations for copulation duration. Following the observations, all water striders were frozen and stored in 95% ethanol to preserve their morphology (Andersen 1993; Brennan 1993).

MORPHOLOGICAL MEASURES

We used a geometric morphometric approach to assess variation in water strider shape and size. To accomplish this, we captured digital images of pinned water striders from a lateral aspect. Each image included a ruler as a size standard. We placed 18 landmarks along the dorsal and ventral surfaces of the body in each image using tpsDig (version 2.12, Rohlf 2008; see Arnqvist and Rowe 2002b). Specimens were ordered haphazardly for landmarking. Sample sizes are given in Table A1.

STATISTICAL METHODS

Unless otherwise noted, analyses were performed using JMP 8.0 (2009, SAS Institute Inc., Cary, NC). For each parametric test, we applied transformations where appropriate to meet the test's assumptions or used the equivalent nonparametric test if no transformation was successful. Nonsignificant interactions were dropped from the final models.

Size variation

To assess variation among populations in body size, we used one-way analysis of variance (ANOVA) with the response variables of centroid body size (the square root of the summed squared distances of landmarks from the centroid), body length, and the centroid size of the genital segments. We calculated SSD following Lovich and Gibbons (1992) as female size divided by male size, less one, and tested for variation among populations in the extent of SSD. Population was a random effect in these and all other ANOVA models. We used reduced major axis regression to examine the allometry of SSD by testing whether the slope of the relationship between log-transformed male and female size differed from unity, using RMA software (version 1.17, Bohonak 2004).

Shape variation

To assess shape variation, we converted the landmark data (see above) to shape variables (including both the uniform and nonuniform components) and calculated a Procrustes superimposition using IMP CoordGen software (version 6h; Sheets 2001). Shape variables present a multivariate summary of an object's shape removing the effects of size, although allometric variation in shape may remain (Zelditch et al. 2004). The shape variables were scaled to a millimeter measure taken from the ruler included in each photograph. We first tested for variation among populations in whole body shape by a multivariate analysis of variance (MANOVA) on shape variables, and then examined the pattern of shape variation by canonical variates analysis (CVA), along with thin-plate spline (TPS) visualizations of shape variation and jackknifed assignment tests that assessed how well populations could be distinguished by shape (using IMP CVAGen software, version 6o; Sheets 2001). We examined the role of allometric variation in shape by repeating these analyses on shape variables corrected for allometric change in shape (by regressing partial warps and uniform components on centroid size using Regress version 6n and Standard version 6n software; Sheets 2001).

Sexual covariation

To examine the correlation between male and female size, we calculated Pearson correlation coefficients from the mean sex-specific size of each population.

We took two approaches to analyzing the population-level covariation in shape. First, we used a two-block partial least squares (2B-PLS) test (using tpsPLS software version 1.18; Rohlf 2008), which describes the covariation between two multivariate datasets (here, male and female shape) by determining linear combinations of variables that describe the greatest covariation (Rohlf and Corti 2000). We set α (a weighting parameter) to zero and included both the uniform and nonuniform shape variables (Zelditch et al. 2004). One can then use permutation tests to test whether the amount of covariation explained by these combinations of variables (i.e., PLS axes) is greater than expected by chance. We used TPS plots to visualize the pattern of covariation and to identify known antagonistic traits that appeared to covary strongly in this dataset. To examine whether the extent of covariation depended on allometric variation, we repeated the analyses on shape variables that had been corrected for allometry as above. Second, we tested the correlation between the lengths of male and female univariate traits (calculated from interlandmark distances; original and residual values) identified in the TPS plots from the 2B-PLS analysis. We used multiple regression to test whether male sexual trait sizes were more closely related to female sexual trait size or to female body size.

Mating behavior

All analyses of mating behavior were conducted on the mean value for each bin; the bin is thus the unit of replication (sample sizes given in Table A1). We assessed variation in seven mating behaviors by one-way ANOVA: the harassment index (the number of harassment events experienced by each female per minute each female was available, that is, not already engaged in an interaction with a male); the proportion of harassment events that lead to a struggle; struggle duration for struggles that did or did not lead to mating; the proportion of struggles that lead to mating; the mating rate (the number of matings per female per hour); and copulation duration (see Rowe and Arnqvist 2002).

Relationship between mating behavior and sexual morphology

To examine the relationship between mating behavior and sexual morphology, we conducted a principal components analysis (PCA) on the two univariate morphological traits (male pregenital segment length and female spine height) identified as sources of significant covariation among populations in the 2B-PLS analysis. The two axes from this PCA describe the major axis of covariation between the traits (PC1) and how populations deviate from this major axis (PC2; see Arnqvist and Rowe 2002a). We examined how these two multivariate measures of sexual morphology, along with SSD, influenced variation in mating behavior as summarized by a PCA of mating behavior. We then tested three hypotheses about how variation in mating morphology should influence specific stages of the premating interaction.

Results

SIZE VARIATION AND COVARIATION

Both sexes displayed significant variation in body size among populations (Fig. 1A; males: $F_{18,1005} = 44.3, P < 0.0001$, square-root transformed; females: $F_{18,799} = 31.2, P < 0.0001$). Body length also varied among populations in both sexes (males: $F_{18,1005} = 40.7, P < 0.0001$; females: $F_{18,799} = 32.6, P < 0.0001$, square-root transformed), indicating that body size differences were not simply due to variation in abdominal contents (e.g., food ingestion or female egg load). At least some of this size variation occurred in the genital segments (centroid size of male genital segments: Wilcoxon test, $\chi^2_{18} = 224.7, P < 0.0001$; females: $F_{18,799} = 21.1$, $P < 0.0001$, square-root transformed).

Across populations ($N = 19$), male and female body sizes were strongly and positively correlated (Fig. 1B; $r = 0.93$, $P < 0.0001$), as were male and female body length ($r = 0.92$, $P < 0.0001$) and the size of the genital segments ($r = 0.85$, $P < 0.0001$).

Furthermore, we detected variation in the extent of SSD among populations, indicated by a significant sex × population interaction (Fig. 1A; $F_{18,1804} = 1.7, P = 0.04$; main effects: population: $F_{18,1804} = 71.0, P < 0.0001$; sex: $F_{1,1804} = 7634.4$, $P < 0.00005$; log-transformed). This variation was not due to an allometric relationship between SSD and body size: there was no detectable correlation between SSD and mean body size among populations ($r = -0.01, \beta = -0.00 \pm 0.01, P = 0.96$), and the slope of the relationship between male and female body size was not statistically different from 1 (Fig. 1B; log-transformed, reduced major axis regression: $R^2 = 0.86, \beta = 0.92 \pm 0.08$, 95% CI for slope: 0.75, 1.09). These patterns also occurred when we used body length rather than centroid size: there was no correlation between SSD and body length ($r = 0.09, \beta = 0.00 \pm 0.01, P = 0.72$) and the slope of the relationship between male and female length was not different from 1 ($R^2 = 0.85, \beta = 0.92 \pm 0.09$, 95% CI for slope: 0.73, 1.10).

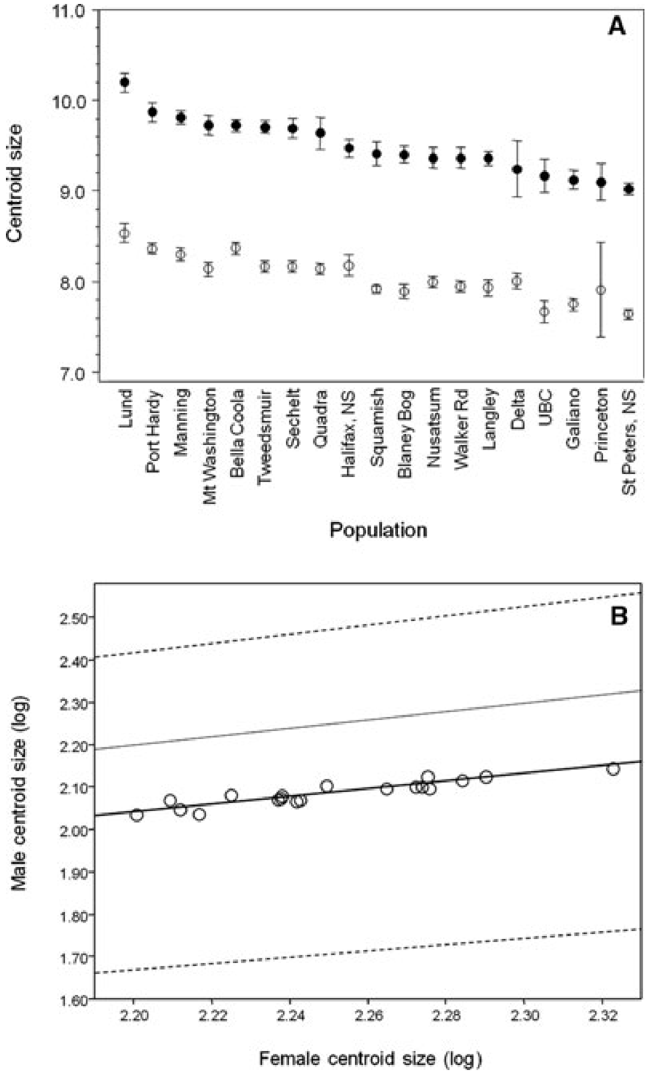

Figure 1. **Size variation among populations. (A) Mean (± 95% CI) body (centroid) size for females and males within each population (solid and open circles, respectively). Two populations were collected from Nova Scotia (indicated by NS); the remainder were collected from British Columbia, Canada. (B) Male and female sizes are positively correlated among populations (see text). The slope of the reduced major axis regression line (thick line; 95% CI given as dashed lines) was close to 1 (1:1 line given as a thin line), indicating that the degree of sexual size dimorphism does not change with mean body size (see text).**

SHAPE VARIATION

Body shape varied across populations for both sexes (MANOVA: males: Wilks' $\lambda = 0.03, P < 0.00005$; females: Wilks' $\lambda = 0.02$, $P < 0.00005$).

For males, the greatest population-level variation in shape (as indicated by the first canonical variates [CV] axis) occurred in the

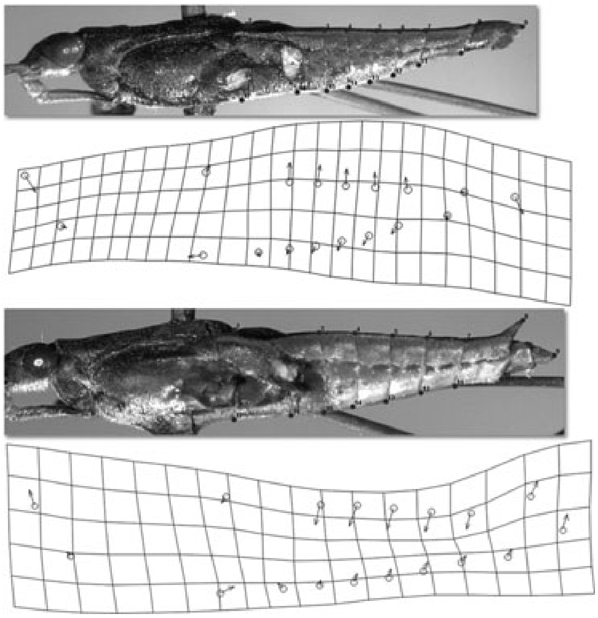

Figure 2. **The total shape deformation for males (upper image and thin-plate spline grid) and females (lower image and grid) implied by the first axis from a canonical variates analysis of shape variation among populations (CVA; P < 0.01; scaling factor = 0.1). Each shape deformation is paired with a reference image of a male or female *G. incognitus* specimen showing the landmarks corresponding to those on the grid. The vectors on each landmark indicate the direction and magnitude of shape variation among populations, with larger vectors and stronger curvature of the gridlines indicating larger changes in shape.**

thickness of the abdomen and the angle of the genital tip (Fig. 2), whereas the second CV axis also described a change in abdominal thickness as well as in the length of the pregenital segment (not shown). Overall, populations could be partially distinguished on the basis of male shape: an assignment test correctly assigned 545 of 1024 males (53%). The two Nova Scotia populations were most distinct in shape, having the highest correct assignment rates (Halifax, 81%; St Peters, 87%), whereas the Mount Washington population was least distinct (21%). Despite the disjunct distribution (Table A1) and distinct shape of the Nova Scotia populations, both mitochondrial and nuclear sequence analyses support their classification with the western group as a single species (Gagnon and Turgeon 2010). We tested for allometric variation in male shape and found that while male shape did change with size, the magnitude of allometric change was small and the results for male shape variation were similar when the data were corrected for allometry (see Supporting Information).

For females, the greatest change in shape among populations was in the size of the spine and thickness of the abdomen (Fig. 2). As with males, the assignment test for females was partially successful: 455 of 818 females (56%) were correctly assigned, with the two Nova Scotia populations being most distinct (correct assignment: Halifax 79%; St Peters: 80%) and the Squamish population least distinct (17%). Female shape changed significantly, albeit only slightly, with female size, but correcting for this allometric variation had little influence on the results (see Supporting Information).

SEXUAL COVARIATION IN SHAPE

Multivariate analyses

We excluded one outlier population (Lund; Grubb's test, $P < 0.05$) from the multivariate 2B-PLS analysis because PLS is sensitive to large outliers. We detected strong covariation in male and female multivariate shape across populations: the first two axes from the 2B-PLS analysis captured 94.8% of the covariance (PLS1: 71.5%; PLS2: 23.3%), significantly more than expected by chance (permutation test: $P = 0.002$). Furthermore, male and female scores were strongly correlated on both axes (Fig. 3; axis 1: $r = 0.94$, $P = 0.001$; axis 2: $r = 0.89$, $P = 0.001$).

Some of this shape covariation was associated with male and female sexual armaments: higher scores on PLS axis 1 represented populations with both relatively large female spines and large male pregenital segments (Fig. 3C). In contrast, there was little detectable change in the size of male external genitalia along the first axis of covariance, and no evidence for a change in the orientation of the female genital tip (Fig. 3C). This result contrasts with the covariation detected in these structures at the species level (Arnqvist and Rowe 2002b). Although there was allometric variation in shape among populations, these results were similar when we performed the PLS analysis on corrected shape variables (for which there were no outlier populations; Fig. S2).

Univariate analyses

Visual inspection of the shape covariation detected by the 2B-PLS analyses indicated that some covariation in body shape was associated with the height of the female spine and length of the male pregenital segment (Fig. 3C). We found that these traits in particular were strongly and positively correlated among populations, in both trait lengths ($r = 0.86$, $P < 0.0001$; Fig. 4A) and residual values from a regression of trait length on body size ($r = 0.62$, $P = 0.004$; Fig. 4B).

We next used multiple regression to assess whether male pregenital segment size is more closely correlated with female spine height or female body size. This analysis showed that male pregenitals are closely associated with female spines in particular ($\beta = 0.58 \pm 0.17$, $P = 0.003$) rather than female size in general ($\beta = 0.04 \pm 0.02$, $P = 0.10$; entire model: $R^2 = 0.78$, $F_{2,16} = 28.8$, $P < 0.0001$).

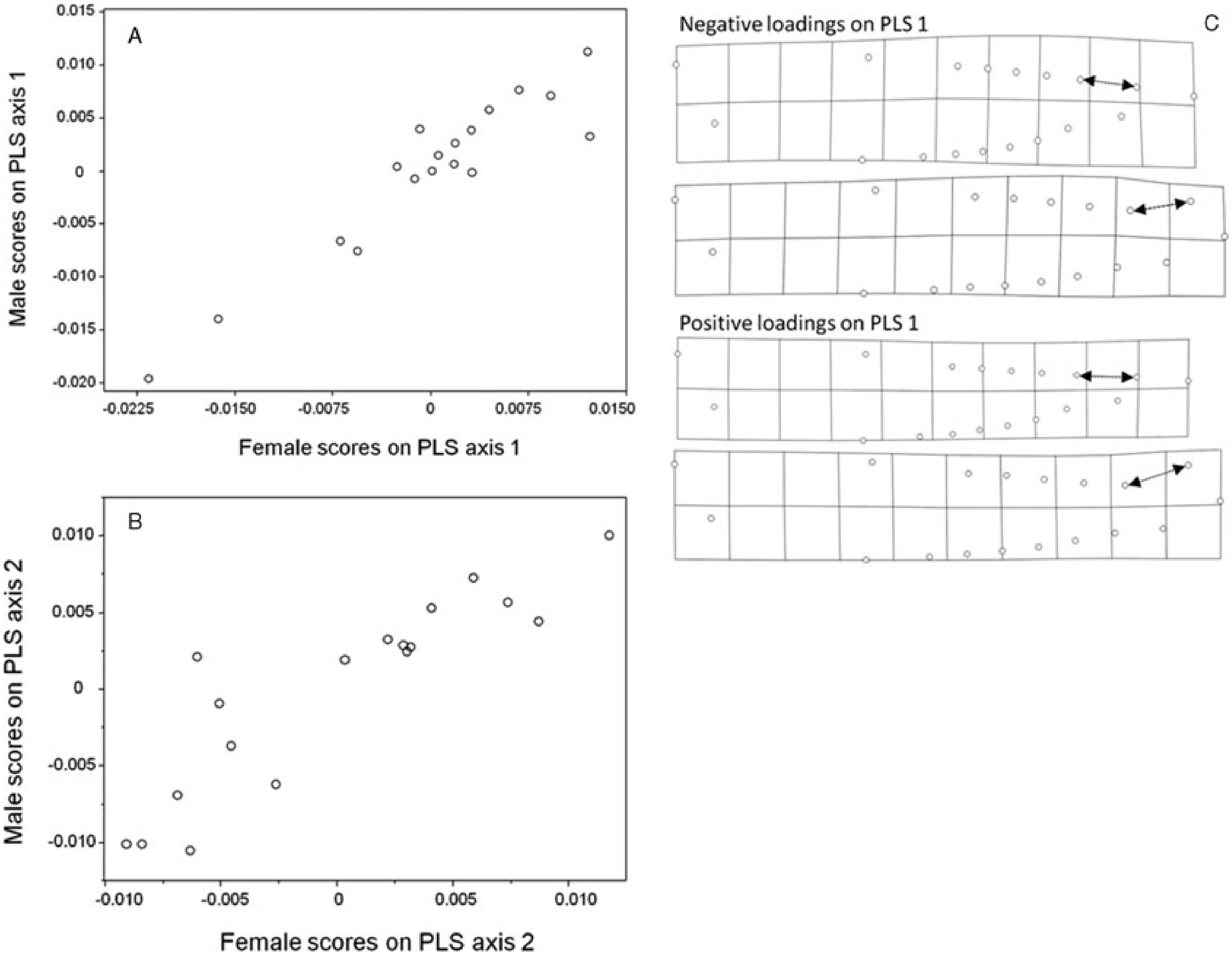

Figure 3. Results from a two-block partial least squares (2B-PLS) analysis of the covariation in male and female shape. Male and female scores were strongly correlated on both PLS axis 1 (A) and 2 (B). (C) Thin-plate spline depictions of the shape deformation implied by negative or positive scores on PLS axis 1. The landmarks correspond to those in Figure 2. The upper and lower images in each pair represent male and female shape, respectively. Arrows indicate the male pregenital segment (dorsal side) and female spine (dorsum to spine tip).

VARIATION IN BEHAVIOR

In all populations, males made frequent attempts to mate with females. Overall, a female that was not already engaged with a male experienced an average of 0.80 ± 0.04 harassments per minute (see Table 1). The mating behavior we observed in the laboratory was similar to previous observations; for example, a field study of *G. buenoi* found that 15% of harassments lead to struggles and 22% of struggles resulted in copulation (Rowe 1992).

Six of the seven mating behaviors showed substantial population-level variation (Table 1). The exception was the duration of struggles that lead to mating; there were eight fewer observations available for this behavior because no mating occurred during our first observation set for two of the populations (see Methods) and mating was observed in only one bin for six populations. We used principal components (PC) to summarize variation in four of these behaviours (Table 1) that represented a considerable proportion of the overall variation in mating behaviour and for which we had a full dataset.

Two hypotheses may explain the function of female mating struggles. First, the ecological hypothesis proposes that females alter the intensity of struggles depending on their optimal mating rate, which may vary across populations (e.g., with predation risk and population density; reviewed by Rowe et al. 1994). This hypothesis predicts (1) a negative correlation across populations between mating rate and struggle duration (Rowe and Arnqvist 2002) and (2) that struggles that result in mating will be of shorter duration than those that do not. Second, the SAC hypothesis

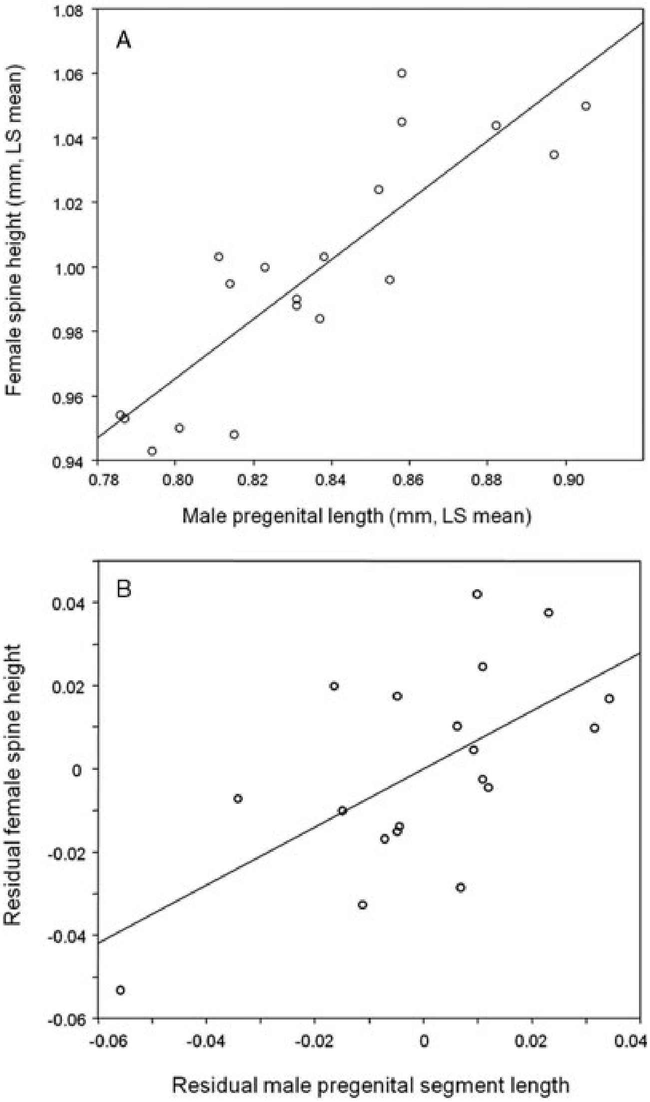

Figure 4. The population-level relationship between male pregenital segment length and female spine height, for both (A) trait lengths and (B) residual values from a regression of trait length on body size (A: $\beta = 0.92 \pm 0.13$, $N = 19$, $P < 0.0001$; B: $\beta = 0.70 \pm 0.21$, $P = 0.004$).

suggests that mating behaviour reflects the interests of the sex with a relative advantage in sexual armaments. When there is a male advantage, males will be better able to hold on to resisting females and consequently long struggles will be observed, and vice versa when there is a female advantage. This hypothesis thus predicts a positive correlation between mating rate and struggle duration (Rowe and Arnqvist 2002) and longer durations for struggles that result in mating compared to struggles that result in separation.

The data support both predictions of the SAC hypothesis. Struggle duration and mating rate were positively correlated ($r = 0.53, N = 18, P = 0.02; R^2 = 0.28, \beta = 0.005 \pm 0.002$). Furthermore, struggles that led to mating were substantially longer (by over sevenfold) than struggles that led to separation (means 90.5 sec ± 16.8 vs. 12.4 sec ± 2.6, respectively; $t = 5.1$, df = 15, $P = 0.0001$). This pattern held within each population: for each population, struggles that lead to mating were longer on average than struggles that lead to separation.

CONNECTING SEXUAL MORPHOLOGY TO MATING BEHAVIOR

Multivariate mating behavior

The above analyses identified two sexual traits—male pregenital segments and female spines—that were closely correlated among populations (Fig. 4). We also found that male and female size co-varied across populations and that the degree of SSD varied (Fig. 1). We next tested whether these aspects of morphology influenced mating behavior using multiple regression. We began with a PCA to summarize the relationship between male pregenital size and spine height, where PC1 scores reflected increased values for both male and female traits and PC2 scores reflected populations in which males or females had a relative advantage in sexual armaments (positive or negative scores, respectively). The multiple regression included PC1, PC2, and SSD, as well as the SSD × PC1 and SSD × PC2 interactions, as independent variables. We used a PC summary of mating behavior (described above) as the dependent variable (Table 1).

The multiple regression showed that sexual morphology was correlated with mating behavior (Table 2). Across populations, the degree of imbalance in sexual armaments (PC2)—but not the absolute level of armaments (PC1)—was related to mating behavior. Furthermore, the effect of imbalances in armaments on mating behavior depended on the degree of SSD (Fig. 5). When SSD was strongly female biased, populations scored low for mating-related activity. When SSD was minimally female biased, the outcome for mating behavior was variable and strongly correlated with the degree of imbalance in sexual armaments: when that imbalance favored males, mating activity increased (Fig. 5).

Stages of the premating interaction

The above results showed that sexual morphology, and specifically imbalances in sexual armaments and body size, influenced mating behavior. We next tested three hypotheses examining how the degree of male advantage in sexual armaments influenced male performance at three stages of the premating interaction: (1) orientation in the precopulatory position upon attempting to grasp a female (the proportion of harassments that proceed to a struggle); (2) maintenance of a grasp on a resistant female (the duration of struggles); and (3) achievement of mating once in the struggle position (assessed by both the proportion of struggles that lead to mating and mating rate). We used multiple regression as described in the previous section.

Table 1. Population-level variation in mating behavior and eigenvectors on principal components axis 1for the four behaviors included in a PCA (eigenvalue: 2.7; 68.5% of the variation explained). Test statistics given in bold are significant at $P < 0.0001$.

Behavior	Statistic	Range	Score on PC1
Harassment index[1]	$\mathbf{F_{17,139} = 6.3}$	0.43, 1.39	
Proportion of harassments that resulted in struggles[2]	$\mathbf{F_{17,139} = 3.1}$	0.14, 0.42	0.51
Proportion of struggles that resulted in mating[3]	$\boldsymbol{\chi^2_{17} = 49.9}$	0.00, 0.24	0.49
Duration of struggles that did not result in mating (second)[4]	$\mathbf{F_{17,137} = 4.4}$	10.3, 31.1	0.43
Duration of struggles that resulted in mating (second)[5]	$F_{9,30} = 1.7$	17.4, 202.3	
Mating rate[3]	$\boldsymbol{\chi^2_{17} = 53.4}$	0.00, 1.17	0.56
Copulation duration (minute)[1]	$\mathbf{F_{15,200} = 5.1}$	14.8, 110.6	

[1] Log-transformed.

[2] Arcsine square root transformed and weighted by the number of harassment events.

[3] We employed a nonparametric Wilcoxon test because no transformation was successful

[4] Box–Cox transformed.

[5] Square-root transformed. $P = 0.12$.

Male ability to achieve the struggle position upon attempting to grasp a female depended more on the degree of SSD than on the absolute or relative level of sexual armaments (Table 2). Males were better able to achieve the struggle position in populations in which they were closer in size to females (i.e., a negative relationship with the degree of female bias in SSD; Table 2). There was also some evidence that the balance of sexual armaments played a role (a marginally significant PC2 × SSD interaction), in a manner similar to that for the multivariate summary of behavior (Fig. 5).

In contrast, SSD had no detectable influence on a male's ability to hold on to a struggling female. This aspect of mating

Table 2. Results from multiple regression models examining the effects of male and female sexual morphology and sexual size dimorphism (SSD; higher values indicate greater female size bias) on mating behavior. PC1 and PC2 scores are taken from a PCA on male pregenital segment length and female spine height; PC1 reflects absolute sizes of male and female traits, whereas PC2 reflects imbalances in sexual traits (higher values indicate a male advantage). R^2 values for each model are given with error d.f. ($N = 18$). Statistically significant covariates are given in bold.

Behavioral variable	Independent variable	Slope(± S.E.)	P-value
Multivariate summary of mating behavior	PC1	0.0 ± 0.2	0.99
(R^2=0.53, 13, P=0.03)	PC2	2.4 ± 0.9	**0.02**
	SSD	-64.3 ± 25.7	**0.03**
	PC2 × SSD	-204.1 ± 88.5	**0.04**
Proportion of harassments that result in struggles[1]	PC1	0.01 ± 0.01	0.27
(R^2=0.55, 13, P=0.02)	PC2	0.11 ± 0.05	0.05
	SSD	-4.77 ± 1.65	**0.01**
	PC2 × SSD	-12.23 ± 5.66	0.05
Duration of struggles[2]	PC1	0.04 ± 0.14	0.77
(R^2=0.46, 14, P=0.03)	PC2	1.67 ± 0.54	**0.008**
	SSD	-23.66 ± 15.6	0.15
Proportion of struggles that result in mating	PC1	-0.01 ± 0.01	0.12
(R^2=0.61, 13, P=0.01)	PC2	0.07 ± 0.03	**0.03**
	SSD	-2.13 ± 0.81	**0.02**
	PC2 × SSD	-7.57 ± 2.78	**0.02**
Mating rate	PC1	-0.04 ± 0.04	0.32
(R^2=0.60, 13, P=0.01)	PC2	0.41 ± 0.15	**0.02**
	SSD	-12.18 ± 4.25	**0.01**
	PC2 × SSD	-37.97 ± 14.63	**0.02**

[1] Arcsine-root transformed and weighted by the number of harassment events.

[2] Log-transformed. The PC2 × SSD interaction was dropped from the final model (23.84 ± 55.76, $P = 0.68$).

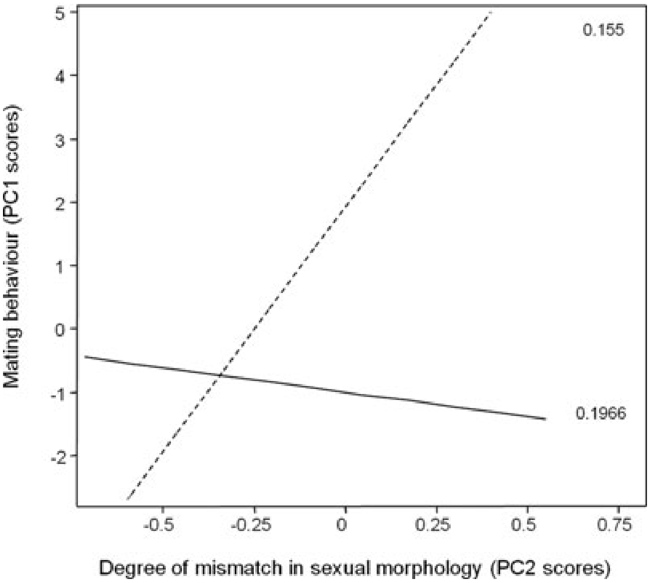

Figure 5. An interaction plot depicting how mating behavior—predicted PC scores (Table 1)—is related to the degree of mismatch in sexual armaments (PC2 of male pregenital length and female spine height; positive values indicate a relative male advantage) and sexual size dimorphism (SSD; larger values indicate larger relative female size). Prediction lines are give for the minimum (0.155) and maximum (0.197) values of SSD observed.

behavior was most strongly influenced by the imbalance in sexual armaments: as males gained an advantage in pregenital length compared to female spine height, struggle duration increased (Table 2).

Finally, male ability to achieve matings—as measured by both the proportion of mating struggles that resulted in mating and the mating rate—was influenced by both SSD and PC2 (Table 2), in a manner similar to the response for the multivariate summary of mating behavior (Fig. 5).

Discussion

Our results support an interpopulation pattern of SAC that is congruent with both theory and the interspecific pattern known in *Gerris* water striders. Among populations, the degree of exaggeration in male and female sexual armaments co-varied positively, reminiscent of an arms race. The fact that we see this at the interpopulation level suggests that the arms race in this species is a current and ongoing process. Moreover, this interpopulation variation in sexual morphology was reflected in mating behavior: mating activity shifted toward the presumed male (high) or female (low) optimum depending on the relative advantage in sexual armaments characteristic of that population (matching the species-level pattern; Arnqvist and Rowe 2002a). We demonstrate that this effect is mediated by the population-specific value for SSD, a pattern not previously reported. Our results also suggest that SAC drives much of the divergence in sexual morphology, given that the statistical models presented here explain large proportions of the variation in these structures and these structures in turn affect mating behavior.

VARIATION IN SEXUAL MORPHOLOGY

Models of SAC and other forms of sexual selection predict the rapid evolution of sexual structures and several empirical studies support this expectation (reviews Arnqvist and Rowe 2005; Chapman 2006; Snook et al. 2009). Our data are congruent with these predictions and observations. We found that populations of *G. incognitus* are differentiated in size, SSD, and body shape (Figs. 1 and 2). Much of the shape variation was associated with the genital segments, as expected if SAC is an important driver of population divergence. Such variation can be produced by evolved differences among populations or by plastic responses to varying environmental conditions, and these alternatives are difficult to directly disentangle in our design. On the one hand, environment (e.g., food and rearing temperature) can have a strong effect on water strider size and SSD and to some extent shape (e.g., Blanckenhorn 1994; Arnqvist and Thornhill 1998; Fairbairn 2005). For this reason, we designed the analysis of our data to remove size effects, and the patterns in shape remain in the size-adjusted data. On the other hand, these same genital and nongenital traits have been shown to be moderately heritable in water striders, and exhibit local adaptation on smaller scales than studied here (Fairbairn and Preziosi 1994; Arnqvist and Thornhill 1998; Fairbairn 2005). Thus, sexual morphology, and the correlation between nonhomologous male and female traits, has the potential to evolve rapidly in this species.

SEXUAL COVARIANCE IN SIZE AND SHAPE

Theory for SAC predicts that SATs may coevolve in a correlated fashion (Chapman and Partridge 1996; Rice 1996; Arnqvist and Rowe 2002b). However, the predicted covariation among antagonistic traits may be masked by other selection pressures on these traits (Rowe and Day 2006). In spite of this potential masking, our results support the hypothesis that SAC shapes intraspecific variation in sexual morphology. Across populations, males and females co-varied in body shape and specifically in antagonistic traits: the male pregenital segment, which houses the grasping genitalia, and the female connexival spine, which acts as an anti-grasping trait. Our results support a close relationship between these two sexual traits in particular. The covariation in shape could not be attributed to a simple association with body size because both size-corrected shape and the size-adjusted univariate traits show covariation. Although other examples of intraspecific sexual coevolution in morphology are known, the function of and form of selection on the coevolving traits is typically not well understood in these systems (e.g., Bergsten et al. 2001; Pitnick et al. 2003; Beese et al. 2006), making it difficult to distinguish SAC from other forms of sexual coevolution.

The covariation we detected in sexual armaments is generally similar to the pattern observed across *Gerris* spp. (Arnqvist and Rowe 2002b). At the species level, females with enlarged spines and downward-turned genital tips occur with males with long genital and pregenital segments and flatter genital segments relative to the proximal abdomen. At the population level, the most obvious covariation occurred in male pregenital segments and female spines; there was no detectable covariation in the remaining traits that have been shown to covary among species (Fig. 3C). Covariation in some of these traits may have been too small to detect at the intraspecific level, or may not have occurred at all.

We found that the male pregenital segment appeared to be most relevant for sexual coevolution. The external genitalia and supporting musculature emerge from this segment and remain partially emerged when the genitalia are not in use (Andersen 1982); thus, the size of the pregenital segment should reflect genital size. Because the external genitalia are affixed to the pregenital segment by soft tissue, the degree to which they emerge from the pregenital segment is variable and this may have prevented us from detecting sexual covariation for the external genitalia. There have been conflicting reports about sexual selection on genital size in water striders. Both selection (Bertin and Fairbairn 2005) and functional studies (Fairbairn et al. 2003) in *A. remigis* suggest positive sexual selection on genital size. However, Arnqvist et al. (1997) detected sexual selection favoring reduced genitalia in *G. incognitus*, although the mechanism behind this result was not known and selection on pregenital size was not investigated. Our findings suggest that the pregenital segment may have a more important role in *G. incognitus* than previously thought. Larger pregenital segments provide a larger attachment site for the muscles that maneuver the external genitalia during premating struggles and allow a deep marginal notch for free rotation of the genitalia (Andersen 1982; Fairbairn et al. 2003).

COVARIANCE IN SHAPE AND BEHAVIOR

In support of these traits playing a mediating role in the outcome of antagonistic mating interactions, we found that interpopulation variation in mating behavior reflected variation in these sexual structures. The intraspecific pattern we observed showed similarities and differences from the species-level pattern (Arnqvist and Rowe 2002a). Similar to the interspecific pattern (Arnqvist and Rowe 2002a), we found that the absolute level of sexual armaments in a population was not related to mating behavior; rather, imbalances in armaments were influential. However, we also found that the effect of an imbalance in sexual armaments was mediated by SSD (Table 2, Fig. 5). When SSD was very female biased, females effectively shut down mating activity and imbalances in sexual armaments did not affect the outcome. In contrast, when SSD was minimally female biased, the outcome depended strongly on the imbalance in armaments, swinging toward higher mating rates when males had an advantage. This is a new observation and it suggests that general descriptions of sexual dimorphism (size plus shape) may obscure meaningful patterns in many systems.

There has been considerable study of sexual selection and the evolution of SSD in water striders. In *Aquarius remigis*, there is a pattern of hypoallometry for SSD, where males and females become more similar in size as mean size increases, and there is some evidence that this is related to sexual selection on size (e.g., Fairbairn and Preziosi 1994; Preziosi and Fairbairn 1996; Sih et al. 2002; Bertin and Fairbairn 2005; Fairbairn 2005). We could not detect this pattern of hypoallometry in *Gerris*, where instead SSD appeared independent of size. However, our results are consistent with earlier findings for the relationship between SSD and sexual selection. In *A. remigis* sexual selection on genital length tends to increase as SSD decreases; in other words, as males get closer in size to females, sexual selection on these grasping genitalia increases (Preziosi and Fairbairn 1996). Our data are consistent with this: across populations, as male size gets closer to female size, the importance of relative armaments in determining mating rate increases, thus increasing the potential for sexual selection to act (Fig. 5).

We found that different aspects of morphology affected different stages of premating interactions (Table 2), which are also a novel observation. The extent of SSD mattered most during the initial grappling phase, when males attempt to gain purchase on a struggling female. Imbalances in sexual armaments mattered more during struggles when males attempt to maintain their grip on resistant females. Finally, both SSD and imbalances in armaments influenced the ability of males to achieve matings once pairs are struggling.

SEXUALLY ANTAGONISTIC COEVOLUTION IN *G. GILLETTEI*

While the current study was under review, a study was published that explored similar questions of SAC and mating behavior among populations of a closely related species, *G. gillettei* (Gagnon and Turgeon 2011). This study reported evidence of correlated evolution between the sexes in potentially antagonistic sexual traits; however, in contrast to the current study and previous work (Rowe and Arnqvist 2002; Arnqvist and Rowe 2002b), the evolution of female spine size was not correlated to male genital traits and no relationship was detected between morphological variation and mating behavior. These differences may reflect the biology of the particular species, although both antagonistic traits and behaviors appear to be shared across *Gerris* (Rowe and Arnqvist 2002; Arnqvist and Rowe 2002b).

There were also substantial differences in study design. First, the study examined populations over a much smaller geographic scale compared to the current study, which may have meant more closely related populations and more similar ecological conditions resulting in less interpopulation divergence. It is noteworthy, though, that some correlated evolution in sexual morphology was detected on even a very small geographic scale. Second, an important limitation of the behavioral assays used in the study was the use of virgin females, which typically show little resistance to mating by displaying fewer and shorter struggles (e.g., Ortigosa and Rowe 2003). It is during these struggles that SATs are employed, and with very low levels of female resistance, it is perhaps not surprising that the study detected no relationship between SATs and mating behavior. Finally, our own results suggest that the relationship between sexual morphology and mating behavior is mediated by SSD, a possibility not explored by Gagnon and Turgeon (2011).

FUTURE DIRECTIONS

This study has shown that antagonistic coevolution in water strider sexual armaments occurs at the intraspecific level, demonstrating that the microevolutionary process of sexually antagonistic selection scales up to generate patterns at the population level that are similar to the interspecific level. These findings open new opportunities to address outstanding questions. First, our observation of an evolutionary trajectory at the interpopulation level provides the opportunity to explore how the ecological setting mediates SAC. For *G. incognitus*, ecological factors are likely to contribute to what drives populations to different points in the coevolutionary trajectory. Two factors that are sensitive to ecological setting in water striders (the extent of female resistance and natural selection on the antagonistic traits; Rowe et al. 1994; Arnqvist 1997b) will in theory affect dynamics (e.g., Parker 1979; Gavrilets et al. 2001; Härdling & Smith 2005; Rowe et al. 2005). Second, there has been considerable interest in how SAC can contribute to reproductive isolation (Arnqvist et al. 2000; Gavrilets 2000; Svensson et al. 2005). Our data show that populations of water striders have become differentiated in traits that function in premating struggles, and an extension of this process may lead to reproductive isolation. An exciting challenge is to understand the conditions under which this occurs, and how morphological divergence interacts with environmental variation to generate reproductive isolation.

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Appendix

Table A1. Collection sites. Sites identified with "NS" are in Nova Scotia, Canada; all others are in British Columbia, Canada. All collection dates are in 2008. *Gerris incognitus* has both winged and apterous morphs; we focused on the more abundant apterous individuals for the morphological analyses. We conducted behavioral observations on bins of two female and six male water striders (see text). We conducted separate observations of copulation duration, except for three populations (see text).

	Coordinates			No. of apterous *G. incognitus* measured			
Site	Latitude	Longitude	Date	Males	Females	No. of bins observed	No. of copulations observed
Bella Coola	52.373398	−126.780081	22 May	40	62	6	9
Blaney Bog	49.264304	−122.601752	24 April	49	51	8	-
Delta	49.098529	−122.957225	24 April	53	14	8	14
Galiano Island	48.948365	−123.502679	18 April	47	36	8	21
Halifax, NS	44.688557	−63.663572	6 May	16	33	3	27
Langley	49.11087	−122.464441	19 Apr	31	31	5	21
Lund	49.982192	−124.758693	18 May	45	30	6	8
Manning	49.195615	−120.978355	22 April	64	105	10	18
Mt Washington	49.731366	−125.290822	19 May	62	39	10	-
Nusatsum	52.423151	−126.427574	22 May	51	30	7	11
Port Hardy	50.705794	−127.507372	20 May	88	47	15	14
Princeton	49.368052	−120.571153	22 April	6	17	0	-
Quadra Island	50.209139	−125.283773	19 May	54	28	8	10
Sechelt	49.511919	−123.750923	17 May	65	51	10	9
Squamish	49.75516	−123.134235	23 April	146	36	18	12
St Peters, NS	45.659265	−60.812722	7 June	63	56	10	3
Tweedsmuir	52.40577	−125.90435	21 May	75	83	5	8
University of British Columbia campus	49.253311	−123.244881	17 April	24	29	12	23
Walker Rd	52.377171	−126.629705	21 May	45	41	8	9

Supporting Information

The following supporting information is available for this article:

Figure S1. Allometric variation in male (upper panel) and female (upper panel) body shape, depicted by thin-plate splines as the change from the smallest specimen to the largest.
Figure S2. Results from a two-block partial least squares (2B-PLS) analysis of the covariation in male and female shape, performed on shape variables corrected for allometric shape change.

Supporting Information may be found in the online version of this article.