The role of ecology, neutral processes and antagonistic coevolution in an apparent sexual arms race

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Abstract

Some of the strongest examples of a sexual 'arms race' come from observations of correlated evolution in sexually antagonistic traits among populations. However, it is unclear whether these cases truly represent sexually antagonistic coevolution; alternatively, ecological or neutral processes might also drive correlated evolution. To investigate these alternatives, we evaluated the contributions of intersex genetic correlations, ecological context, neutral genetic divergence, and sexual coevolution in the correlated evolution of antagonistic traits among populations of *Gerris incognitus* water striders. We could not detect intersex genetic correlations for these sexually antagonistic traits. Ecological variation was related to population variation in the key female antagonistic trait (spine length, a defense against males), as well as body size. Nevertheless, population covariation between sexually antagonistic traits remained substantial and significant even after accounting for all of these processes. Our results therefore provide strong evidence for a contemporary sexual arms race.

Sexually antagonistic selection is expected to drive sexually antagonistic coevolution (SAC) between male and female phenotypes (reviewed by Arnqvist & Rowe 2005; Rice & Gavrilets 2014). Despite this, empirical evidence for SAC is remarkably limited, in part because there are few cases for which sexually antagonistic traits have been well-characterized in both sexes (Perry & Rowe 2014). The strongest evidence for SAC comes from studies demonstrating the correlated divergence of sexually antagonistic traits (for example, male grasping and female anti-grasping morphology), where male and female traits covary among populations or species (Bergsten *et al.* 2001; Arnqvist & Rowe 2002a, b; Bergsten & Miller 2007; Rönn *et al.* 2007; Perry & Rowe 2012). Correlated evolution in male and female traits at the population level suggests rapid contemporary SAC at fine spatial scales, driven by sexually antagonistic selection acting on each sex in response to the antagonistic traits of the other sex (Arnqvist & Rowe 2005).

However, processes other than SAC could also contribute to the correlated evolution of sexual traits. For example, a sexually antagonistic trait in one sex might evolve through either natural selection (e.g., resulting from spatial ecological variation) or neutral divergence, with the trait of the other sex evolving in parallel if there is positive genetic covariance between the traits. Both of these alternatives can be rejected in the absence of an intersex genetic correlation between sexually antagonistic traits. Even in the absence of a shared genetic architecture, both natural selection and neutral evolution might result in a pattern of correlated trait evolution – for example, if either process generates population variation in one sex and the opposite sex then evolves in concert – in contrast to true coevolution. Indeed, the relative contribution of the ecological setting in shaping the evolution of sexually antagonistic traits is a key and open question in the field (Arnqvist &

Rowe 2005; Fricke *et al.* 2009; Arbuthnott *et al.* 2014; Perry & Rowe 2014; Anderson & Langerhans 2015).

To resolve these hypotheses, we explore the roles of ecological variation and neutral genetic evolution in the divergence of sexually antagonistic traits in a water strider, *Gerris incognitus*. Water striders are a model for sexual conflict (Rowe *et al.* 1994). In *Gerris* spp., the correlated exaggeration of sexually antagonistic traits between males and females has been identified within and among species (Arnqvist & Rowe 2002a, b; Gagnon & Turgeon 2011; Perry & Rowe 2012). The hypotheses we have outlined are particularly relevant for the correlated evolution of antagonistic traits among gerrid populations for several reasons. First, ecological variation is known to shape natural and sexual selection acting on sexually antagonistic traits in water striders. This occurs, for example, through increased predation risk and decreased female foraging efficiency during mating and pre-mating struggles (Rowe *et al.* 1994), and through female mating resistance – and hence selection on male grasping traits – varying with local resource abundance (Ortigosa & Rowe 2002). Second, a signature of recently shared genetic history might be more detectable at the population level than at the species level, where rapid SAC might erase evidence of phenotypic similarity through shared ancestry (e.g., Lande 1981; Gavrilets 2000). Finally, the key antagonistic phenotypes that show correlated evolution in *Gerris* spp. are male grasping and female anti-grasping structures that derive from homologous abdominal segments (the genital and pregenital segments), making an intersex genetic correlation between these traits plausible. In fact, positive intersex genetic correlations in pregenital traits have been reported in other water striders (Fairbairn 2007).

Here, we test predictions from the three hypotheses using 16 populations of *G. incognitus* that have been characterized for sexually antagonistic morphology and behaviour (Perry & Rowe 2012). We begin by evaluating whether the genetic basis of known antagonistic traits – female abdominal spines and male grasping pregenitals – is shared or sex-specific, by testing for a sex-specific relationship between offspring and maternal traits. Next, we characterize population genetic structure using neutral genetic markers. We then evaluate the contributions of ecological variation, coancestry, and spatial autocorrelation to population divergence in sexually antagonistic traits and body size. Finally, we ask whether there is variation remaining in the elaboration of female sexually antagonistic traits that is explained by male sexually antagonistic traits, after accounting for effects of ecological variation, coancestry and spatial autocorrelation.

We find no evidence for a positive intersex genetic correlation for male and female antagonistic traits, indicating that a genetic correlation cannot explain their correlated evolution. We find that divergence in female spines and body size can be partly explained by ecological variation. Accounting for these processes, we find that the correlated evolution between male and female sexually antagonistic traits remains strong, supporting an ongoing role for SAC in a contemporary sexual arms race.

Materials and Methods

Study system

Gerris incognitus (Hemiptera: Gerridae) is a small (length ≤ 1 cm) insect inhabiting still water bodies, with one generation per year and adults overwintering. It occurs in wingless and

114 winged forms, with winged individuals able to disperse. All individuals used in this study
115 were wingless; we collected few winged individuals overall (6.7%). We previously
116 investigated divergence in sexual morphology and behaviour in *G. incognitus* collected from
117 19 Canadian locations, including 17 in British Columbia (B.C.) and 2 in Nova Scotia (N.S.;
118 Perry & Rowe 2012). To maximise genetic isolation, we selected locations separated by both
119 dispersal barriers (e.g., rivers, ocean channels or mountain ranges, where possible) and
120 distances (10 to 445 km) exceeding the usual dispersal distance for winged *Gerris* spp.
121 (several hundred metres; Callahan 1974; Butler 1987). We sampled a single discrete water
122 body (a pond or bog) at several points for a representative sample at each site (53-182
123 individuals per site). In the present study, we focus on B.C. (Table 1) due to the spatial
124 discontinuity between B.C. and N.S. We excluded one site sampled in the previous study
125 (Princeton, B.C.) that had too few individuals for genetic analysis.

126 We used field-collected *G. incognitus* to rear a filial generation in a common garden
127 laboratory setting. To reduce potential maternal effects, we held females under standard
128 conditions (groups of 8 in aerated pools, 2400 cm^2) and provided frozen fruit flies and
129 crickets daily, ad libitum, for 9 to 21 days before collecting eggs. To collect eggs, we housed
130 10 females from each of 13 sites (omitting Bl, Ph and QI for logistic reasons; Table 1)
131 individually in cups containing water and styrofoam strips as an oviposition substrate, and
132 provided food as above. Strips were transferred to individual buckets every 2-3 days.
133 Emerging offspring were provided with fruit flies daily, ad libitum. Offspring were housed
134 individually from the third instar. Water was refreshed every 2-3 days. We preserved
135 offspring in 70% ethanol 2 days after their final moult.

From field-collected individuals, we genotyped 12 males and 12 females per location using nine polymorphic microsatellites (Perry & Rowe 2011). We extracted DNA from leg muscles (Gentra Puregene Tissue protocol; Qiagen, Valencia, USA) and assessed DNA quality by 1% agarose gel electrophoresis and by spectrophotometric absorbance. We used labelled reverse primers and dye-tagged oligos to allow pooling for genotyping (Schuelke 2000). Microsatellites were amplified in 12.5 µl volumes containing 1X ThermoPol buffer (New England BioLabs, Beverly, USA), 0.2 mM of each dNTP, 0.5 units Taq DNA polymerase, 3.0 pmoles of the forward primer and oligo, 1.0 pmoles of reverse primer, and ~100 ng of DNA. Amplification products were genotyped on an ABI3730xL Analyzer (Applied Biosystems, USA) at the Centre for Applied Genomics (Toronto, Canada). Alleles were called by visual inspection of electropherogram peaks by a single observer. To minimize scoring error, we re-genotyped samples with low-frequency alleles or stutter bands.

Measurements of morphology

To investigate the inheritance of sexual morphology and body size, we digitally photographed offspring and mothers from the lateral aspect, including a size standard. We placed 18 landmarks on the images along the dorsal and ventral body surfaces using tpsDig (version 2.12, Rohlf 2006 ; Arnqvist & Rowe 2002b; Perry & Rowe 2012). We used the landmarks to calculate centroid body size (the square root of summed squared distances of landmarks from the centroid) and the length of sexual armaments: the male pregenital segment, which houses the grasping genitalia, and the female's anti-grasping connexival spine (examined in previous studies; Arnqvist & Rowe 2002b; Perry & Rowe 2012).

Analyses

Mother-offspring regressions

To test whether male and female traits are positively genetically correlated, we regressed mean son or daughter traits (weighted by offspring number; N = 1-12) on maternal traits. We obtained adult offspring from 88 of 130 females (3-9 families per site). For a few specimens, we were able to measure sexual traits but not body size (e.g., due to a missing head). We had 73 and 69 families for daughter and son body size, and 76 and 70 families for daughter spines and son pregenitals, respectively.

Genetic structure among sites

We first evaluated whether we could detect genetic structure, before asking whether estimates of population coancestry could explain sexual trait divergence. Microsatellite variation is sometimes more strongly driven by mutation rates than by demographic processes. When this is the case, microsatellites are not useful for demographic inference. The correlation coefficient between F_{ST} and expected heterozygosity (H_S) can be used to identify such cases (Wang 2015). We found no negative correlation between *Hs* and F_{ST} (R_{GH} = -0.17, P = 0.28; 1000 permutations), indicating that our microsatellite data were suitable for demographic inference. We explored genetic structure with isolation-by-distance plots and a scaled principal components analysis (PCA). The above analyses were implemented in R (R Development Core Team 2008) with the *adegenet* (Jombart 2008), *ade4* (Dray & Dufour 2007), and *hierfstat* (Goudet 2005) packages.

Testing the roles of ecological variation, coancestry and spatial structure

We aimed to investigate how shared ancestry and ecological variation relate to population divergence in female spine size and body size (results were similar for male phenotypes;

Tables S1, S2). To do this, we evaluated models of these traits (population mean trait values for field-collected adults) that included measures of coancestry, ecological variation, and other spatially-structured variation, or additive combinations of these factors. We had no a priori reason to expect non-additive interactions and therefore did not include interaction terms to avoid unwarranted model complexity. To evaluate models, we used an information-theoretic approach based on Akaike's information criterion corrected for finite sample sizes (AICc; Akaike 1973; Sugiura 1978; Hurvich & Tsai 1989; Burnham & Anderson 2002). The minimum AICc value indicates the best supported model given the trade-off between fit to the data and model complexity. We included an intercept-only model in each set of competing models as a baseline for comparison (Burnham *et al.* 2011). For each model we recorded AICc, Δi (the difference between model *i*'s AICc and minimum AICc), the evidence ratio (the factor by which the best supported model is better than model *i*), and R^2 to facilitate evaluating the contribution of each additional variable. To avoid overly complex models, we excluded models from further consideration when they were more complicated versions of simpler models with lower AICc (i.e., when the more complex model contained all factors of the simpler model plus at least one additional factor; Burnham & Anderson 2002; Richards 2008). We conducted the analyses in JMP v. 12.1 (SAS Institute, North Carolina).

As a measure of coancestry, we calculated site-specific ancestry coefficients from the microsatellite data following Buckleton and colleagues' (2016) modification of Weir and Hill's (2002) population-specific F_{ST} estimator (Goudet 2005). This modified approach produces an unweighted estimator, which performs better than Weir and Hill's weighted estimator (Buckleton *et al.* 2016). When calculated in this way, assuming a drift model, this parameter

is proportional to the time since a set of populations diverged (Weir & Hill 2002; Buckleton *et al.* 2016).

To model ecological variation, we sought to identify the best-supported ecological model based on 12 ecological variables that are important in either mediating costs of water strider sexual interactions or regulating growth and reproduction in these semiaquatic exothermic animals (Table 2). We carried forward the best-supported model for each trait into a comparison among ecology, coancestry and spatial models. We used AICc to evaluate models including each variable alone, a baseline intercept-only model for comparison, a model that combined variables that were a better fit than the baseline model, and a model including principal components summary of variables that were a better fit than baseline, separately for spine height and body size. Data for temperature, season length, winter severity, depth of snow as protection from winter cold, and precipitation were accessed from Environment Canada's Historical Climate Data online database. We lacked water acidity data for two sites (La and QI; Table 1). We imputed values for these sites based on spatial structure in water acidity variation, from a regression of pH values on spatial variables (dbMEMs, described below; $R^2 = 0.50$; Legendre & Legendre 2012). The imputed values fell within the range of observed values (6.8 and 6.4; range 6.0 – 7.0). The results were similar when we did not include imputed values (Table S3).

To model spatially-structured variation not captured by our other variables, we used distance-based Moran's eigenvector maps (dbMEMs) as surrogate explanatory variables that summarize spatially-structured variation (Borcard & Legendre 2002; Dray *et al.* 2006; Legendre & Legendre 2012). dbMEMs can be included as predictors in regression analyses to quantify variation – in female phenotypes in this case – across spatial scales. Recent

simulations found that this approach outperforms other commonly used spatial modelling approaches (e.g., the Mantel test; Legendre *et al.* 2015). dbMEMs summarize spatial structure by drawing eigenvectors through a distance matrix built from the spatial configuration of sites. dbMEMs are called Moran's eigenvector maps because the eigenvalues equal Moran's I coefficients of a neighbour network (the distance matrix) multiplied by a constant (Dray *et al.* 2006). To construct the neighbour network we considered all sites within the minimum distance that kept the network connected as linked. We found two significant and positive dbMEMs summarizing positive spatial autocorrelation. These dbMEMs reflect latitudinal variation (latitude and dbMEM1: $r = 0.91$; dbMEM2: $r = 0.41$). Although dbMEMs are orthogonal and therefore not correlated with each other, they are likely to be correlated with spatial variation in F_{ST} and ecological variables and in fact were correlated with these variables (Table S4).

Evaluating residual sexual covariation

We asked whether the sexual covariation we previously found in female spines and male pregenitals or male and female body size (Perry & Rowe 2012) remained detectable after accounting for variation explained by coancestry, ecological variation and spatial autocorrelation. To do this, we calculated residual male and female trait values from separate multiple regressions including all factors – F_{ST}, the best-supported ecological model and dbMEMs – to provide a conservative test by accounting for maximal variation. We tested the linear relationship between these residual male and female values. We explored additional models of sexual covariation, which yielded similar results (Table S5).

Results

A sex-specific genetic basis for sexually antagonistic traits

We tested whether shared genetic variation between males and females could explain the positive covariation between their traits. We found that sons' and daughters' sexually antagonistic traits – pregenitals and spines, respectively, both derived from abdominal segment 7 – had distinct relationships with their mothers' spine length. Daughter spine length was positively correlated with maternal spine length, but son pregenital length was not (Fig. 1a; daughters: $\beta = 0.206 \pm 0.093$ S.E., adjusted $R^2 = 0.048$, number of families = 79, $t_{74} = 2.22$, $P = 0.03$; sons: $\beta = 0.083 \pm 0.089$ S.E., adjusted $R^2 = -0.002$, number of families = 75, $t_{73} = 0.93$, $P = 0.36$). This pattern remained even when we excluded any between-site genetic variance and examined only the mean relationship between mother and offspring traits within sampling locations (mean slope: daughters, 0.323 ± 0.077; 95% C.I.: 0.154, 0.491; sons, mean slope: 0.029 ± 0.140; 95% C.I.: -0.275, 0.334). Hence, we found no evidence for a shared genetic basis for spine length in females and pregenital length in males.

We found mixed results for a shared genetic basis for body size between the sexes. Son and daughter body sizes were positively correlated with maternal body size across families, with a similar strength of relationship in both offspring sexes, supporting a shared genetic basis (Fig. 1b; daughters: $\beta = 0.299 \pm 0.117$ S.E., adjusted $R^2 = 0.069$, number of families = 76, $t_{74} = 2.56$, $P = 0.01$; sons: $\beta = 0.336 \pm 0.138$ S.E., adjusted $R^2 = 0.066$, number of families = 71, $t_{69} = 2.44$, $P = 0.02$). However, we found no significant relationship between offspring and maternal body size when averaging across sampling locations (mean slope: daughters, -0.157

± 0.321; 95% C.I.: -0.855, 0.542; sons: -0.027 ± 0.481; 95% C.I.: -1.074, 1.021). We note that this second test excludes genetic variation among sampling locations, which we know are genetically differentiated (see below); hence, in this sense it has lower power to detect genetic correlations than does the analysis of all families together.

Sites show spatial genetic structure

We detected substantial genetic diversity within and among sites, with an average of 16.2 alleles (range 4-30) across the nine loci and observed heterozygosity from 0.26 to 0.52 (Table 1). We detected spatial genetic structure from both the isolation-by-distance (Mantel r = 0.86, P = 0.001) and PCA plots (Fig. 2). There was a clear pattern of isolation-by-distance (Fig. 2a), suggesting a north to south cline in allele frequencies (Fig. 2b). The considerable overlap of individuals among sites suggests movement and gene flow along this cline (Fig. 2b).

Given that genetic variation and structure were detectable, we proceeded with the coancestry models below.

Ecological model selection

We evaluated the relationships between spine and body size and ecological variables expected to influence mating, growth and reproduction in water striders (Table 2). For spine height, five ecological variables – water acidity, temperature, season length, winter severity and snow protection from winter cold – were a better fit to the data than a baseline model that made no attempt to explain the data (Table 2). The best-supported model overall included two principal components summary measures of these 5 variables, with a model probability of 0.65 (i.e., of being the most parsimonious model in the set of models). For female body size, two ecological variables – water acidity and temperature – were better-

292 fitting than the baseline model (Table 2). The best-supported model overall included only
293 water acidity, with model probability 0.54. We carried forward the best-fitting models for
294 spine and body size to evaluate the relative contributions of ecological variation, coancestry
295 and spatial structure.

296 *The contributions of ecological variation, coancestry, and spatial structure to phenotypic*
297 *variation*

298 We evaluated models of female trait variation that included ecological variation (following
299 ecological model selection, Table 2), coancestry (population-specific local F_{ST}), and spatial
300 structure (summarized by 2 dbMEM variables). We included spatial structure to explore
301 spatially-structured variation not captured by ecological variables or F_{ST}. We included in each
302 set of models the intercept-only model as a baseline for comparison.

303 For population divergence in female spines, the ecology model was best supported, with a
304 model probability of 0.77 indicating fairly strong support (Table 3). The coancestry model
305 performed better than the baseline model, but had a probability of 0.06; the ecology model
306 was 13 times better supported (Table 3). The spatial structure model was weakly supported,
307 providing no evidence that other spatially-structured variation – not accounted for by
308 ecological variables or F_{ST} – was related to spine variation (Table 3). Other models that
309 combined factors had higher AICc values than simpler models and were therefore not
310 considered further (Richards 2008).

311 For female body size, the water acidity model (i.e., the best-supported ecological model)
312 received strongest support (probability 0.49; Table 3).

313 Taken together, these results indicate that population variation in spine and body size is
314 consistent with similarity due to shared ecological variation. Our conclusions are similar
315 when male traits are used as response variables (Tables S1, S2). Below, we ask whether
316 population covariation in female spines and male pregenitals, and in female and male body
317 size, remains when controlling for all of these potential sources of variation.

318 *Evaluating residual sexual covariation*

319 As expected based on previous study of these populations (Perry and Rowe 2012), both male
320 pregenitals and female spines, and male and female body size, are positively correlated
321 among populations (Fig. 3a, c; pregenitals and spines, $r = 0.85$, $\beta \pm$ S.E. $= 0.797 \pm 0.133$, $F_{1,14} =$
322 36.1, $P < 0.0001$; body size, $r = 0.95$, $\beta \pm$ S.E. $= 1.163 \pm 0.107$, $F_{1,14} = 117.8$, $P < 0.0001$). We
323 tested whether these positive correlations remained after accounting for variation explained
324 by ecological variation, coancestry, and spatially-autocorrelated processes, by taking the
325 residuals of male and female traits from a multiple regression including the best-supported
326 ecological model for spine size or body size, F_{ST}, and dbMEMs, including all variables to
327 provide the most conservative test.

328 We found that a strong positive correlation remained when accounting for these sources of
329 variation (Fig. 3b, d), for both sexually antagonistic morphology and body size (residual
330 pregenitals and spines, $r = 0.87$, $\beta \pm$ S.E. $= 0.542 \pm 0.084$, $F_{1,14} = 42.1$, $P < 0.0001$; body size, r
331 $= 0.94$, $\beta \pm$ S.E. $= 0.973 \pm 0.097$, $F_{1,14} = 99.8$, $P < 0.0001$). These conclusions were unchanged
332 when we used residual trait values from multiple regressions that included alternative
333 combinations of ecological variables (Table S5).

334

Discussion

We evaluated predictions of three non-exclusive hypotheses for the correlated divergence of sexually antagonistic traits among water strider populations: genetically correlated evolution, a single-sex evolutionary chase where one sex diverges (through varying natural selection among environments or neutral processes) and the other sex follows as a response to selection, and sexually antagonistic coevolution. We found no evidence for a genetic correlation in sexually antagonistic traits, but found that ecological variation is related to phenotypic divergence in female spines and body size. However, female spines and male pregenitals remained strongly and positively correlated with each other after accounting for spatial-ecological and genetic variation, supporting a role for sexually antagonistic coevolution in driving current and ongoing population phenotypic divergence.

A genetic correlation does not explain correlated phenotypic divergence

Correlated divergence in male and female sexually antagonistic morphology among populations might arise through a shared genetic architecture. This hypothesis is of special interest in *Gerris* spp. because male and female antagonistic traits derive from the same segments or those in close proximity (genital and pregenital segments) and because positive intersex genetic correlations in some genital traits occur in another water strider (Fairbairn 2007). Our data do not support this hypothesis. We found no evidence for a positive intersex genetic correlation between antagonistic traits, with daughters' spines – but not sons' pregenital segments – positively regressing on maternal spine length. This result indicates that the genetic variation detected that is associated with daughters' spines is not also associated with sons' pregenitals. We found mixed evidence for a shared genetic basis for

body size, where it is expected based on studies of other water striders (Preziosi & Roff 1998; Fairbairn 2007), with both daughters' and son' body sizes showing positive relationships of similar magnitude with maternal body size. The absence of a detectable relationship when averaged across sampling locations might reflect the underpowered nature of this test, which does not incorporate genetic differentiation among sites (Fig. 2). Our results do not exclude the possibility of sire effects or maternal effects not eliminated by housing mothers in common conditions.

The contributions of ecological variation, coancestry, and spatial structure to phenotypic variation

For female spine divergence, we found strongest support for a model based on summarized ecological variation (PC1 and PC2), with only weak evidence for a model based on spatial genetic structure (Table 3). The synthetic PC1 variable represents an axis of warmth (Table 2), reflecting more frequent warm days and a longer reproductive season, whereas PC2 represents decreasing water acidity (i.e., increasing pH). Hence, warmer and less acidic locations produce females with smaller spines. It is currently unclear why this should be so, and identifying whether and how these variables influence the degree of sexual conflict within a population is a promising area for future work. We propose that it will be of interest to test the hypothesis that benign environments mean reduced sexual conflict in which smaller spines are favoured.

Although we detected relationships between spine divergence and these variables, we did not detect relationships with other variables expected to influence spine evolution. Previous work has established ecological factors that affect the economics of mating in *Gerris* spp.:

379 reductions in optimal female mating rate (e.g., due to decreased food availability or
380 increased predation risk) cause increased female re-mating resistance, resulting in selection
381 favouring enlarged male grasping traits (Rowe & Arnqvist 2002). In contrast to these findings
382 within populations, we did not detect an effect of predator presence on spine variation
383 among populations, nor a relationship between spine size and variables correlated with
384 predator abundance or food availability (canopy cover, emergent vegetation, water acidity,
385 and the presence of other water striders; Table 2). The absence of effects relating to
386 predators or food might mean that their effects are slight relative to other factors driving
387 population divergence in spines, such as those described by PC1 and PC2. Alternatively, the
388 measures we use here undoubtedly have error associated with them, such that future
389 studies might identify food or predation effects with more precise measures.

390 For female body size divergence, we likewise found strongest support for ecological models.
391 The best-supported model was that of water acidity, with weaker support for a model
392 including the number of warm days. The former result is intriguing. Water acidity affects a
393 wide range of variables in aquatic ecosystems (Table 2), such that disentangling processes
394 responsible for divergence in *G. incognitus* body size is a challenge for future work. The
395 latter result is consistent with observed geographic clines where body size decreases with
396 latitude in other water striders (Blanckenhorn & Fairbairn 1995; Brennan & Fairbairn 1995).
397 Body size is likely subject to both natural and sexual selection in water striders (Brennan &
398 Fairbairn 1995; Arnqvist *et al.* 1996; Rowe & Arnqvist 1996; Arnqvist *et al.* 1997; Ferguson &
399 Fairbairn 2000; Preziosi & Fairbairn 2000; Fairbairn 2007; Perry & Rowe 2012). We did not
400 detect a relationship between body size variation and other previously reported variables,
401 including habitat stability (Fairbairn 1984). As with spine size, this might reflect the

dominance of other processes in population divergence compared with within-population selection.

The degree to which ecological variation sets the stage for sexual conflict and sexual arms races is a major open question. Although some theory predicts that sexually antagonistic traits should diverge in the absence of ecological variation (Lande 1981; Gavrilets 2000; Hayashi *et al.* 2007; Kimura & Ihara 2009), other models have emphasized the role of ecological variation in the outcome of sexual arms races (Härdling & Bergsten 2006; Kimura & Ihara 2009). Recent studies have supported a role for ecological variation in population-level sexually antagonistic evolution in laboratory (*Drosophila melanogaster* fruit flies; Arbuthnott *et al.* 2014) and field settings (Bahamas mosquitofish, *Gambusia hubbsi*; Anderson & Langerhans 2015). Our results therefore add to a growing body of evidence suggesting the potential for ecological influence of sexual arms races across systems.

A detectable signature of sexually antagonistic coevolution

We found that the population-level correlation between female anti-grasping spines and male pregenital segments – housing the grasping genitalia – holds after accounting for trait divergence that could be explained by ecological, genetic, and spatially-structured variation. As noted above, it is not possible to measure all ecological variation or to measure ecological and genetic variation without measurement error. It is therefore necessarily true that our models could not have accounted for all trait divergence associated with these variables. However, two factors contribute to our test for the signature of SAC being conservative. First, although ecological, genetic and spatial variation are difficult to disentangle, we control for as much variation related to all factors as possible. Second, shared ancestry is likely to be

correlated with both neutral divergence and adaptive divergence driven by SAC (i.e., the ghost of SAC past), such that some signature of SAC is removed by controlling for coancestry. In this way, the test for SAC gives it the highest chance of failure. The result suggests that some selection on each sex appears to result from phenotypic variation in the opposite sex, and that selection on and coevolution between these traits is ongoing and rapid enough to generate detectable divergence among populations.

We detected similar covariation in body size between the sexes after accounting for ecological, genetic, and spatial variation. This result has several possible interpretations. On one level, our data suggesting a positive genetic correlation between male and female body size provides a potential explanation sexual covariation in size among populations. It is possible that sexual coevolution shapes sexual covariation in body size in addition to constraints imposed by intersex genetic correlations. Previous studies have shown that the difference in body size between the sexes (rather than variation within a sex) influences pre-mating struggles in *G. incognitus* (Perry & Rowe 2012) and related species (Fairbairn 1988; Sih & Krupa 1992; Arnqvist *et al.* 1996; Rowe & Arnqvist 1996; Arnqvist & Danielsson 1999; Ortigosa & Rowe 2003). Disentangling these hypotheses requires alternative empirical approaches, such as measures of sex-specific selection on body size in natural populations.

Conclusion

Our results support the longstanding hypothesis that sexually antagonistic coevolution generates the correlated sexually antagonistic morphology of male and female *G. incognitus* – a model system for interlocus sexual conflict – across populations. The data also support roles for ecological variation in shaping sexual morphology and body size. Important

directions for future work include further investigating the nature of ecological variation and characterizing the genetic variation underlying the phenotypic sexual arms race in this model system. It would also be fruitful to examine the roles of ecological variation, neutral evolution, and sexually antagonistic coevolution in the few other insect systems where the correlated evolution of sexual morphology has been demonstrated.

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Figure legends

Figure 1

Regressions of mean offspring phenotypes on maternal phenotypes for (a) the sexually antagonistic traits of spines in daughters (filled circles) and pregenital segments in sons (open circles) and (b) body (centroid) size in daughters and sons. Female spines are derived from abdominal segment 7, which in males is the pregenital segment housing the grasping genitalia.

Figure 2

Genetic structure among sites. (a) The relationship between genetic distance (Nei's *D*; Nei *et al.* 1983; Takezaki & Nei 1996) and Euclidean geographic distance among sites (Mantel r = 0.86, P = 0.001). Hotter colours show the density of data points. (b) Site clustering based on the first two principal components (horizontal axis: PC1; vertical axis: PC2) describing genetic variation. Colours indicate the north to south cline grading from blue to red. Site labels are as in Table 1 and indicate the centroid of each site.

Figure 3

The positive relationships between male pregenital length and female spine length (a) and male and female centroid body size (c) among populations remain after accounting for variation related to coancestry, ecological measures, and spatial variation (b, d). Residual

643 trait values were taken from a multiple regression of male or female phenotype on measures
644 of ecological variation (the best supported ecological model; Table 2), coancestry
645 (population-specific F_{ST}), and spatially-structured variation (2 dbMEM variables).
646
647

Tables

Table 1

Characteristics of sampled sites, including location and geographic region (within British Columbia, BC), observed heterozygosity per locus (H_O; mean ± standard error) and number of alleles per locus (allelic richness, A; mean ± standard error)

site	acronym	latitude	longitude	region	H_O *	A *
Bella Coola	Bc	52.373398	−126.780081	north BC	0.26 ± 0.09	3.2 ± 0.5
Nusatsum	Nu	52.423151	−126.427574	north BC	0.38 ± 0.08	3.9 ± 0.5
Tweedsmuir	Tw	52.40577	−125.90435	north BC	0.28 ± 0.08	3.4 ± 0.5
Walker Rd	Wa	52.377171	−126.629705	north BC	0.27 ± 0.08	3.4 ± 0.6
Blaney Bog	Bl	49.264304	−122.601752	south BC	0.50 ± 0.10	7.6 ± 1.4
Delta	De	49.098529	−122.957225	south BC	0.48 ± 0.08	8.0 ± 1.3
Galiano Island	GI	48.948365	−123.502679	south BC	0.51 ± 0.07	6.8 ± 1.1
Langley	La	49.11087	−122.464441	south BC	0.38 ± 0.09	6.7 ± 1.3
Lund	Lu	49.982192	−124.758693	south BC	0.51 ± 0.06	5.3 ± 0.7
Manning	Ma	49.195615	−120.978355	south BC	0.44 ± 0.10	6.8 ± 1.4
Mt Washington	Mw	49.731366	−125.290822	south BC	0.48 ± 0.07	4.8 ± 0.9
Port Hardy	Ph	50.705794	−127.507372	south BC	0.43 ± 0.07	5.0 ± 0.5
Quadra Island	QI	50.209139	−125.283773	south BC	0.52 ± 0.09	5.7 ± 1.1
Sechelt	Se	49.511919	−123.750923	south BC	0.51 ± 0.08	5.7 ± 0.8
Squamish	Sq	49.75516	−123.134235	south BC	0.44 ± 0.10	4.8 ± 0.7
Vancouver	Va	49.253311	−123.244881	south BC	0.52 ± 0.10	6.6 ± 1.0

* N = 24 for each population

654 **Table 2**

655 The strength of evidence for ecological models of variation in female spine height or centroid body size

trait	model description	β*	S.E.	$AICc_i$	Δ_i	model probability†	evidence ratio†	R^2	adjusted R^2
spine	PC1 + PC2 for best supported factors‡			-67.9	0	0.65	1	0.59	0.52
height	water acidity§	-0.054	0.018	-64.9	3.0	0.14	5	0.38	0.33
	temperature§	-0.001	0.000	-63.7	4.2	0.08	8	0.33	0.28
	season length§	-0.001	0.000	-61.7	6.2	0.03	23	0.24	0.18
	winter severity§	0.002	0.001	-61.4	6.5	0.02	26	0.22	0.17
	snow protection from winter cold§	0.001	0.001	-60.9	7.0	0.02	33	0.20	0.14
	baseline (intercept only)	n/a	n/a	-60.4	7.5	0.02	43	0	0
	emergent vegetation index§	-0.008	0.006	-59.6	8.3	0.01	65	0.13	0.07
	altitude	0.000	0.000	-58.7	9.2	0.01	101	0.08	0.01
	presence of other water striders§	-0.016	0.016	-58.3	9.6	0.01	124	0.06	-0.01
	predator presence§	-0.014	0.018	-58.1	9.8	0.00	137	0.04	-0.02
	precipitation§	0.000	0.000	-58.0	9.9	0.00	144	0.04	-0.03
	water body area§	0.000	0.000	-57.4	10.5	0.00	194	0.00	-0.07
	canopy cover index§	0.000	0.007	-57.3	10.6	0.00	204	0.00	-0.07
	best supported factors combined§			-57.1	10.8	0.00	224	0.71	0.56
body size	water acidity§	-0.601	0.133	-1.0	0.0	0.54	1	0.59	0.56

PC1 for best supported factors‡	-0.183	0.046	0.7	1.7	0.23	2	0.54	0.47
best supported factors combined§			0.9	1.9	0.21	3	0.69	0.53
temperature§	-0.007	0.004	9.7	10.7	0.00	211	0.18	0.12
baseline (intercept only)	n/a	n/a	9.7	10.7	0.00	213	0	0
emergent vegetation index§	-0.077	0.051	10.4	11.4	0.00	299	0.14	0.08
altitude	0.000	0.000	11.0	12.0	0.00	403	0.11	0.05
predator presence§	-0.176	0.155	11.4	12.4	0.00	493	0.00	-0.07
water body area§	0.000	0.000	11.5	12.5	0.00	518	0.10	0.04
season length§	-0.003	0.003	11.9	12.9	0.00	633	0.06	-0.01
winter severity§	0.006	0.009	12.2	13.2	0.00	735	0.04	-0.03
snow protection from winter cold§	0.006	0.008	12.2	13.2	0.00	735	0.04	-0.03
presence of other water striders§	-0.094	0.148	12.3	13.3	0.00	773	0.03	-0.04
canopy cover index§	-0.022	0.059	12.6	13.6	0.00	898	0.01	-0.06
precipitation§	0.000	0.001	12.8	13.8	0.00	992	0.07	0.01

656 * For categorical factors (presence/absence of predators and other water striders), the difference in means is given comparing presence with
657 absence.

658 † Model probability gives the probability of each model within the set of models, given the data. The evidence ratio measures how many times
659 greater support is for the best supported model, compared with model *i*.

660 ‡ The principal components summarize variation in factors with AICc lower than baseline. For spine size, PC1 captures variation related to

661 general warmth and summarizes 57.0% of variation, with water acidity loading weakly (0.080), temperature and season length loading
662 positively (0.503 and 0.487), and winter severity and snow protection from winter cold loading negatively (-0.516 and -0.487; β ± S.E.: -0.012 ±
663 0.006). PC2 summarizes 24.0% of variation and represents water acidity, with all variables loading positively (in the same order: 0.861, 0.299,
664 0.122, 0.303, 0.251; β ± S.E.: -0.014 ± 0.005). For body size, PC1 summarizes 66.9% of the variation, with both water acidity and temperature
665 loading positively (loadings 0.707 for both).
666 § Water acidity, measured at each site: shapes aquatic invertebrate community structure (Layer et al. 2013); higher acidity associated with
667 reduced water strider and predator density (Bendell & McNicol 1987; Karaouzas & Gritzalis 2006) and increased pollutants in water strider
668 bodies (Jardine et al. 2009). Temperature: mean number of days annually where temperature > 10°C, the growth threshold for *Gerris* spp.
669 (Spence *et al.* 1980). Season length: mean number of frost free days annually; associated with larger bodies in *Aquarius remigis* water striders
670 (Blanckenhorn & Fairbairn 1995). Winter severity: mean number of days annually in which temperature < -10°C, close to the lower lethal limit
671 of -15°C (Ditrich & Koštál 2011; <-15°C data unavailable); winter mortality is severe in water striders (Fairbairn 1986). Snow protection from
672 winter cold: mean depth of snow, December to February; protects overwintering adults against cold (Ditrich & Koštál 2011). Emergent
673 vegetation index: from 0 to 5 (0, none; 1, 0-20%; 2, 21-40%; 3, 41-60%; 4: 61-80%; 5: 81-100%); associated with *Gerris* spp. community
674 structure (Spence & Scudder 1980; Spence 1981); increased vegetation associated with higher macroinvertebrate diversity and abundance,
675 including predators of water striders (Battle & Golladay 2001). Presence of other water striders: other *Gerris* spp. or *Limnoporus* spp. sampled

676 at each site; potential competitors for food, which mediates body size in *Gerris* spp. (Spence 1986). Predator presence: detection of corixid
677 bugs, dytiscid or gyrinid beetles, water spiders, minnows, frogs and ducks, by three net samples or visual and audial inspection at each site
678 Spence 1986); predator presence increases mating costs (Rowe 1994) and body size in *Gerris* spp. (Spence 1986). Precipitation: mean
679 precipitation during the breeding season, April to July. Water body area: estimated surface area; reflects habitat stability; associated with
680 larger body size in *A. remigis* (Fairbairn 1984). Canopy cover index: as for emergent vegetation index; decreased cover associated with higher
681 diversity and abundance of macroinvertebrates, including water striders and their predators (Spence 1983; Binckley & Resetarits 2009; Plenzler
682 & Michaels 2015). Best supported factors combined: factors with AICc lower than baseline combined without interactions.

683 **Table 3**

684 The strength of evidence for models of population variation in female spine size or centroid

685 body size

trait	model description	factor(s)	AICc$_i$	Δ_i	model probability*	evidence ratio*	R^2	adjusted R^2
spine height	ecology	PC1 + PC2	-67.9	0	0.77	1	0.59	0.52
	ecology + coancestry	PC1 + PC2 + F_{ST}	-64.1	3.8	0.11	7	0.60	0.50
	coancestry	F_{ST}	-62.8	5.1	0.06	13	0.29	0.24
	ecology + spatial structure	PC1 +PC2 + dbMEMs	-60.7	7.2	0.02	37	0.65	0.52
	baseline	intercept only	-60.4	7.5	0.02	43	0	0
	spatial structure	dbMEMs	-59.9	8.0	0.01	55	0.32	0.22
	ecology + coancestry + spatial structure	PC1 + PC2 + F_{ST} + dbMEMs	-57.0	10.9	0.00	233	0.71	0.56
	coancestry + spatial structure	F_{ST} + dbMEMs	-55.5	12.4	0.00	493	0.32	0.15
body size	ecology	pH	-1.0	0	0.49	1	0.59	0.56

ecology + spatial structure	pH + dbMEMs	-0.1	0.9	0.31	2	0.73	0.66
ecology + coancestry	pH + F_{ST}	1.2	2.2	0.16	3	0.61	0.56
ecology + coancestry + spatial structure	pH + F_{ST} + dbMEMs	4.2	5.2	0.04	13	0.75	0.65
baseline	intercept only	9.7	10.7	0.00	211	0	0
coancestry	F_{ST}	12.2	13.2	0.00	735	0.04	-0.03
spatial structure	dbMEMs	14.9	15.9	0.00	2836	0.09	-0.05
coancestry + spatial structure	F_{ST} + dbMEMs	18.8	19.8	0.00	19930	0.12	-0.10

686 * Model probability gives the probability of each model within the set of models, given the
687 data. The evidence ratio measures how many times greater support is for the best supported
688 model, compared with model *i*

689

Figures

Figure 1

Regressions of mean offspring phenotypes on maternal phenotypes for (a) the sexually antagonistic traits of spines in daughters (filled circles) and pregenital segments in sons (open circles) and (b) body (centroid) size in daughters and sons. Female spines are derived from abdominal segment 7, which in males is the pregenital segment housing the grasping genitalia.

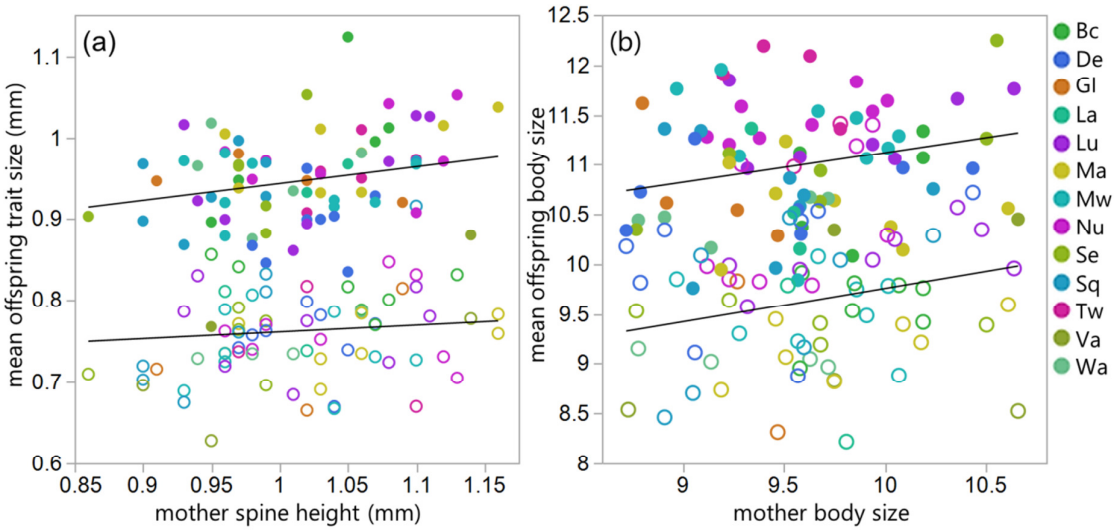
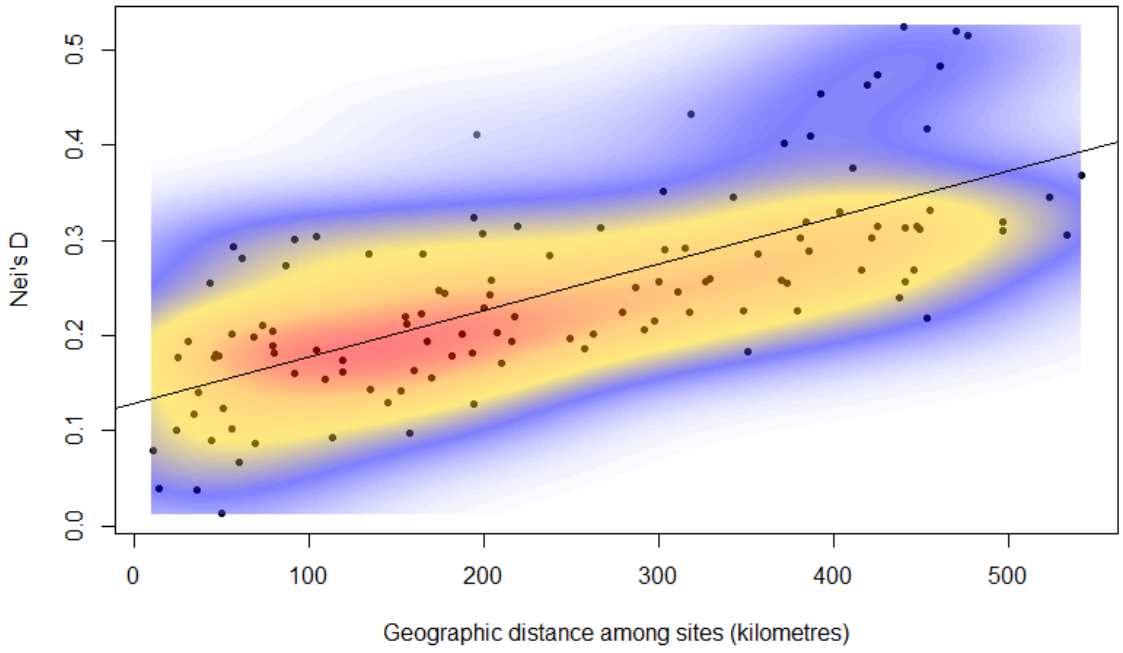


Figure 2

Genetic structure among sites. (a) The relationship between genetic distance (Nei's *D*; Nei *et al.* 1983; Takezaki & Nei 1996) and Euclidean geographic distance among sites (Mantel *r* = 0.86, P = 0.001). Hotter colours show the density of data points. (b) Site clustering based on the first two principal components (horizontal axis: PC1; vertical axis: PC2) describing genetic variation. Colours indicate the north to south cline grading from blue to red. Site labels are as in Table 1 and indicate the centroid of each site.

(a)



710 (b)

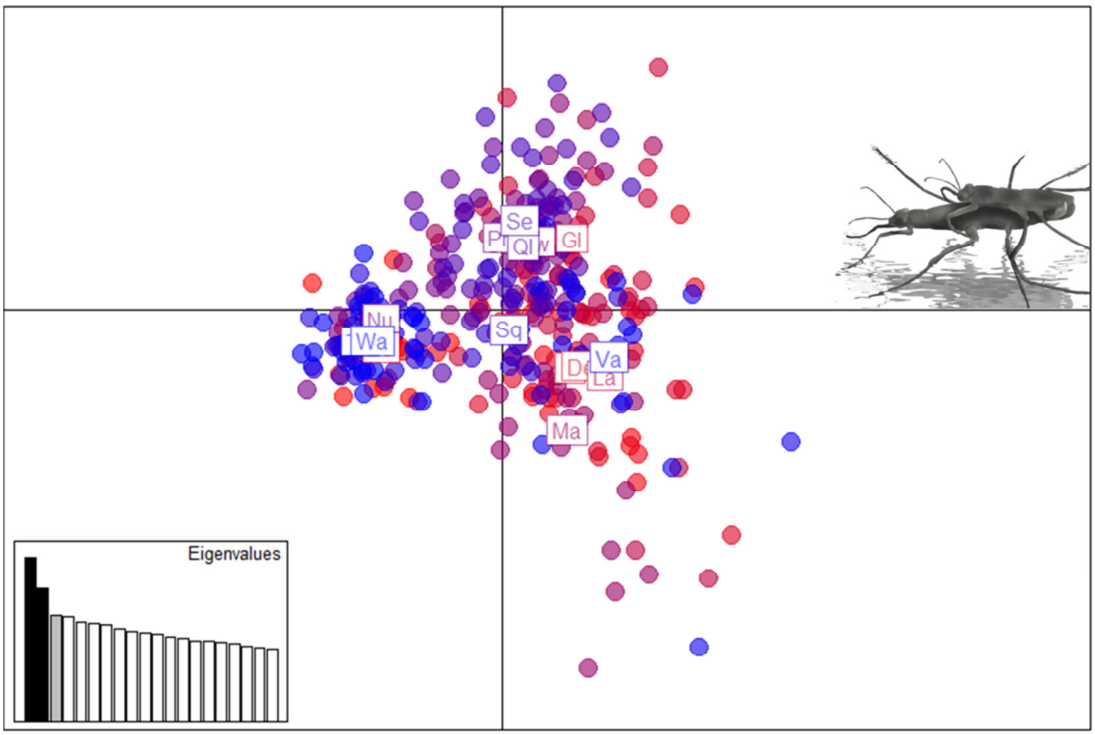


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Figure 3

The positive relationships between male pregenital length and female spine length (a) and male and female centroid body size (c) among populations remain after accounting for variation related to coancestry, ecological measures, and spatial variation (b, d). Residual trait values were taken from a multiple regression of male or female phenotype on measures of ecological variation (the best supported ecological model; Table 2), coancestry (population-specific FST), and spatially-structured variation (2 dbMEM variables).