

A shared developmental genetic basis for sexually antagonistic male and female adaptations in the toothed water strider

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Abstract

Sexual conflict can drive the divergence of male and female phenotypes and cross-species comparative analyses have documented patterns of correlated evolution of sex-specific traits that promote the evolutionary interests of the sexes. However, male–female coevolution can be highly dynamic, particularly if the male and female traits share an underlying genetic program. Here, we use water striders, a well-studied model system for sexually antagonistic coevolution, and ask whether sex-specific phenotypic adaptations covary across populations and whether they share a common developmental genetic basis. Using comparative analyses both at the population and species levels, we document an association between a derived male mate-grasping trait and a putative female anti-grasping counteradaptation in the toothed water strider *Gerris odontogaster*. Interestingly, in several populations where males have partly lost their derived grasping trait, females have also reduced their antigrasping adaptation. We used RNAi to show that these male and female traits are both linked to a common developmental genetic program involving Hox- and sex-determination genes, despite the fact that they are different structures on different abdominal segments. Our work illustrates the dynamic nature of sexually antagonistic coevolution and suggests that the pleiotropic nature of developmental genetic programs can blur the distinction between inter- and intralocus genetic conflict.

Keywords: sexual conflict, coevolution, evolution of sex, development

Lay Summary

The evolutionary interests of males and females often diverge, particularly over mating interactions. This coevolutionary pattern leads to the evolution of adaptations and counteradaptations that further the evolutionary interest of each sex. How the phenotypic divergence of the sexes emerges at small time scales and despite the shared genome is not fully understood. We address these questions in the toothed water strider, *Gerris odontogaster*, a classical model for the study of sexual conflict. By analyzing multiple populations and species, we uncover strongly correlated evolution between male abdominal teeth that act to grasp the female and a counteradaptation in the females consisting of a concealed proctiger that is the target of the male trait. We also show, through manipulating gene expression, that the male and the female traits share, at least partly, an underlying developmental genetic program containing both Hox- and sex-determination genes. This study paves the way to testing the extent of overlap between intra- and interlocus genetic conflict.

Introduction

Males and females share most of their genomes, yet their evolutionary interests often diverge which generates evolutionary conflict (Arnqvist & Rowe, 2005; Bonduriansky & Chenoweth, 2009; Parker, 2006). We distinguish between intralocus and interlocus sexual conflict (Schenkel et al., 2018). The former occurs when males and females have different optimal values for a shared

phenotypic trait, which is manifested at a genetic level as different alleles at a given locus being favored in males and females. Examples include height in humans (Stulp et al., 2012), age at maturity in salmon (Moble et al., 2021), and body size in beetles (Berger et al., 2016; Kaufmann et al., 2021). A symptomatic pattern of intralocus sexual conflict is a negative intersexual genetic correlation for fitness (Connallon & Matthews, 2019). In contrast,

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interlocus sexual conflict occurs when the optimal outcome of intersexual interactions differs in males and females. This can lead to the evolution of suites of sexually dimorphic traits encoded by different loci in males and females that affect a given outcome, through a process known as sexually antagonistic coevolution (Arnqvist & Rowe, 2005). Potential examples include the evolution of reproductive traits in seed beetles (Dougherty et al., 2017; Rönn et al., 2007), mollusks (Koene & Schulenburg, 2005), millipedes (Sloan et al., 2023), and guppies (Evans et al., 2011), as well as mate-grasping traits in diving beetles (Bergsten & Miller, 2007; Bergsten et al., 2001) and water striders (Arnqvist & Rowe, 2002a, b).

Although sexually antagonistic coevolution is thought to be a major driver of diversification and speciation (Arnqvist et al., 2000; Parker & Partridge, 1998; Rice et al., 2005), we actually know very little about its genetic basis (Pennell et al., 2016; Schenkel et al., 2018). This is unfortunate, because intra- and interlocus sexual conflict may not be entirely distinct if loci with effects on sex-specific traits involved in interlocus sexual conflict have pleiotropic effects with fitness consequences in the opposite sex (Geeta Arun et al., 2022; Mokkonen et al., 2016). If sexually dimorphic traits that are undergoing correlated evolution, to some degree, have a shared genetic basis, the genetic correlation between male and female traits could significantly affect the predicted dynamics of sexually antagonistic coevolution (Pennell et al., 2016).

In water striders (Gerridae), a monophyletic group of semiaquatic Heteroptera, conflict over mating is widespread and is known to drive escalation of both grasping traits in males and antigrasping traits in females (Arnqvist, 1997; Arnqvist & Rowe, 2002a, b). In this sexually antagonistic coevolution, males are favored to mate repeatedly while multiple mating can be superfluous and costly for females (Arnqvist & Rowe, 2005; Rowe, 1994). The outcome of male–female interactions is determined by premating struggles, during which the male strives to secure an anterior and a posterior grasp of the female, while the female tries to dislodge the male (Arnqvist & Rowe, 2005). Sexually antagonistic traits that affect the outcome of struggles include modifications of the appendages or other body parts into claspers in males, and the presence of spines, various integument projections, and modifications of the tip of the abdomen in females that make it more difficult for males to grasp females posteriorly during mating (Arnqvist, 1989, 1997; Arnqvist & Rowe, 2002b; Crumière et al., 2019; Rowe et al., 2006). Phenotypic manipulation and behavioral studies have demonstrated the function of male and female sexually antagonistic traits during premating interactions (Arnqvist, 1989; Arnqvist & Rowe, 1995; Crumière et al., 2019; Han & Jablonski, 2009; Khila et al., 2012; Ronkainen et al., 2005), and a few studies have identified important genes required for shaping these traits during development (Crumière & Khila, 2019; Khila et al., 2012).

Phylogenetic comparative analyses have revealed patterns of correlated evolution between the sexes under the influence of sexual conflict (Perry & Rowe, 2018). In water striders, Arnqvist and Rowe (2002a, b) showed that the primary correlated changes are manifested in the shape of genital segments in both sexes, which together determine the posterior grip during struggles, and variation in these sexually antagonistic traits correlates with variation in the economics of mating. Population-level analyses also revealed that ecological factors, such as food resources, predation, or population density, impact the patterns of antagonistic coevolution of the sexes by altering the costs and benefits of mating (e.g., Arnqvist, 1994; Perry & Rowe, 2012; Rowe, 1994). In theory, the dynamics of sexually antagonistic coevolution in terms

of escalation and de-escalation may thus be affected by both external and internal factors. First, environmental variation may act as a driver of sexually antagonistic coevolution and phenotypic divergence between males and females (Perry & Rowe, 2012; Rowe et al., 2018). Second, the evolution of sexually antagonistic adaptations will affect their relative efficacy, and this in itself can generate escalation and de-escalation (Parker, 1979; Rowe et al., 2005). Third, the genetics of interlocus sexual conflict can itself contribute to the dynamics of sexually antagonistic coevolution. In particular, between-sex pleiotropic constraints can act to generate cycles of escalation and de-escalation (Pennell et al., 2016).

It is therefore important to assess the genetic and developmental basis for sexually dimorphic traits involved in sexually antagonistic coevolution. Here, we focus on a classic water strider species in this context—the toothed pondskater *Gerris odontogaster*. We first use comparative analyses at the micro- and macro-scale, to assess whether and how a well-known male grasping trait (consisting of two extensions of the seventh abdominal tergite) is matched by a female counteradaptation. We study populations exhibiting significant variation, including the remarkable near loss of the male grasping trait. Using experimental manipulation of gene expression, we then link covariation in male and female traits to the role of a developmental genetic program containing both Hox- and sex-determination genes.

Materials and methods

Study species

The various populations of *G. odontogaster* used in this study, their geographic locations, and source are described in [Supplementary Table S1](#). Experimental populations of *G. odontogaster* from Sweden and *G. buenoi* from Canada were reared in water tanks in a room maintained at 28 °C with a relative humidity between 40% and 50%, under an artificial photoperiod of 14 hr of daylight. Animals were fed daily with frozen crickets. Females lay eggs on floating Styrofoam pieces that were removed weekly and put into new water tanks to let the eggs hatch.

Phenotyping

Males and females from each species and population were photographed using a microscope, and then measured using software associated with the microscope. For males, the length of the male abdominal processes (MAPs) and of the body were measured from a lateral view of the animal (see [Supplementary Figure S1](#)). For females, the visible part of the proctiger and the plate that recovers it were measured from a dorsal view (see [Supplementary Figure S2](#)).

Phylogenetic reconstruction and PGLS analysis

A transcriptomic-based phylogenetic reconstruction approach was used as described in [Armisen et al. \(2022\)](#). Transcriptomic data were generated using Illumina sequencing for the freshly collected populations of *G. odontogaster* (excluding museum samples) as well as for *G. buenoi*, *G. lacustris*, *G. incognitus*, and *G. marginatus*. Transcriptomes were assembled using Trinity ([Grabherr et al., 2011](#)) and used to define clusters of orthologous genes using OrthoFinder ([Emms & Kelly, 2019](#)). After testing for stationary, reversible, and homogeneous consistency ([Naser-Khdour et al., 2019](#)), a final list of 5,658 gene clusters was retained for phylogenetic reconstruction using IQ-TREE ([Nguyen et al., 2014](#)). To further test the relationship between the three *G. odontogaster* populations, an additional likelihood mapping analysis was performed to determine the phylogenetic relationship between the

three *G. odontogaster* populations (Supplementary Figure S3). To do so, we used IQ-TREE (option: -n 0 -lmap 10,000 -m TEST -bsam GENESITE) to test possible topologies between: outgroups (other *Gerris* species), *G. odontogaster* Swedish, *G. odontogaster* France, and *G. odontogaster* Italy (Supplementary Figure S3). Phylogenetic least-squares regression was performed with ape v. 5.8 and in R v 4.3.3 (R_Core_Team, 2024).

RNA interference against *Abd-B* and *dsx*

For this experiment, nymphs from the fourth and early fifth instars of the Swedish population (largest MAPs) were injected with double-stranded RNA (dsRNA) of *Abdominal-B* and *doublesex*, separately, to induce degradation of transcripts corresponding to these genes. The cloning of *doublesex* and *Abdominal-B* was done by PCR based on sequences of *G. odontogaster* that were extracted from a full transcriptome database hosted at IGFL Lyon (<https://gerromorpha.igfl.ens-lyon.fr/equipes/a-khila-developmental-genomics-and-evolution/>). Primers were designed based on these sequences using Primer-BLAST software. The cloning of *Abd-B* and *dsx* fragments was carried out using standard PCR and cloning techniques. Primers and gene sequences can be retrieved in GenBank under the following accession numbers: *Abdominal-B*: PQ283678, *doublesex*: PQ283677.

In vitro transcription of *dsx* dsRNA

Templates flanked by T7 promoters were synthesized by PCR using the primer sequences by Lynch and Desplan (Lynch & Desplan, 2006). A total of 1 µg of each T7 PCR template was used to synthesize double-stranded RNA of *dsx* and *Abd-B* (Lynch & Desplan, 2006). In total, 10 µl of 10× transcription buffer, 10 µl of 10 mM dNTPs, 1 µl of T7 RNA polymerase Plus (200 Units/µl), 1 µl of RNaseOUT, and a volume of nuclease-free water to obtain a final volume of 100 µl. The reaction was incubated at 37 °C overnight: 2 µl of DNase (1 Unit/µl) was added to the reaction, which was incubated at 37 °C for 10 min. Subsequently, the dsRNA preparation was purified using the RNeasy Mini Kit (Qiagen). Injection buffer (Rubin & Spradling, 1982) 10× was added (10% of total volume). Finally, the dsRNA solution was filtered with Syringe Driven Filter Unit Cat and was centrifuged at 5,000 rpm for 30 s.

Injection of dsRNA of *dsx* and *Abd-B* into nymphs of *G. odontogaster*

Individuals from the fourth instar to the early fifth instar of the Swedish population of *G. odontogaster* were used for this experiment due to the fact that the formation of MAPs begins 65 hr after the fifth molt. Both sexes were injected to test whether or not the genes had a sex-specific function. Animals were anesthetized with carbon dioxide for 15–20 s and then were injected between the sixth and the seventh abdominal segments with a 0.7 µg/µl dsRNA solution for *dsx* and 0.5 µg/µl for *Abd-B*. Each individual was injected with approximately 0.5 µl of this solution. Animals injected with the same volume of injection buffer 1× were used as negative controls to ensure that the trauma caused by injection did not induce a change of phenotype. Injections were delivered via quartz needles with filaments using a Zeiss Stereo Discovery V8 binocular magnifier. After injection, the animals were kept in separate water tanks and were fed on crickets twice a day until adulthood. After the injection of *doublesex*, males and females were kept separated in case the depletion of *dsx* transcripts had an effect on sex determination. When they became adults, individuals were sacrificed in ethanol, screened for phenotypes, and

photographed using a Keyence VHX 7000 microscope. The experiment was terminated when at least 10 male adults per condition survived and had a modified phenotype.

Fluorescence staining of MAPs

Mid-fifth instar male nymphs were dissected and the tergite of the seventh abdominal segment with developing MAPs recovered. The tissue was fixed for 30 min in 4% paraformaldehyde, then washed five times in phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PTW). Tissues were blocked for 1 hr in an antigen-blocking solution containing 0.1% bovine serum albumin, 1% normal goat serum, 1× PBS, and 0.05% Tween-20. The tissues were then incubated with mouse acetylated alpha-tubulin antibody overnight at 4 °C. The tissues were washed five times, 10 min each, with PTW and incubated 2 hr with an antimouse antibody and phalloidin (actin dye). Tissues were washed five times, 10 min each, with PTW and then 5 min in 1× PBS containing 30% glycerol (in PBS). Tissues were incubated for 10 min in 1× PBS, 50% glycerol containing DAPI (4',6-diamidino-2-phenylindole). Finally, the tissues were mounted on slides in 8% glycerol. Images were captured using a Zeiss LSM 710 confocal microscope.

Data analyses

Statistical analyses were conducted using R (v 4.2.2). The ratio of MAPs size/body size was compared between the males of the different *G. odontogaster* populations. The effect of population on this ratio was tested through a one-way analysis of variance. The normal distribution and homoscedasticity of the residuals were tested, respectively, through a Shapiro–Wilk test and a Bartlett test, and a graphical inspection of residuals was performed. When a significant difference was detected a Tukey post-hoc test was used to determine which populations differed. The same procedure was used to determine the impact of population and/or species on the ratio of proctiger size/plate size between females.

Results

Correlated evolution of a male adaptation and a putative female counteradaptation

Males of *G. odontogaster* use their MAPs (Figure 1A) to grasp the proctiger of the female during premating struggles and MAPs play an important role in increasing the mating rate by providing posterior grasp (Arnqvist, 1989). Females with a proctiger that is harder to clasp by MAPs should lower potential fitness costs associated with superfluous matings and, therefore, be favored by selection. We here hypothesize that a concealed female proctiger represents an adaptation in females to counter the effect of MAPs. This tenet is based on (a) comparative analyses in this clade showing negatively correlated evolution between male clasping ability and the accessibility of the proctiger, which affects the mating rate (Arnqvist & Rowe, 2002b), (b) experimental manipulations showing that a less accessible tip of the abdomen makes it easier for females to dislodge males (Arnqvist & Rowe, 1995; Ronkanen et al., 2005), and (c) studies of other semiaquatic heteropterans showing that concealed genitalia represents a female counteradaptation (Han & Jablonski, 2009; Maroni et al., 2023). Populations of *G. odontogaster* differ considerably in the mean length of MAPs, even at a small geographic scale, which is partly related to local population density and predation (Arnqvist, 1992b, 1994). Remarkably in some populations, males have significantly reduced MAPs and only show rudiments of this structure (Figure 1A–D). This was first reported in Mongolia (Lansbury, 1966; Lundblad, 1934), and

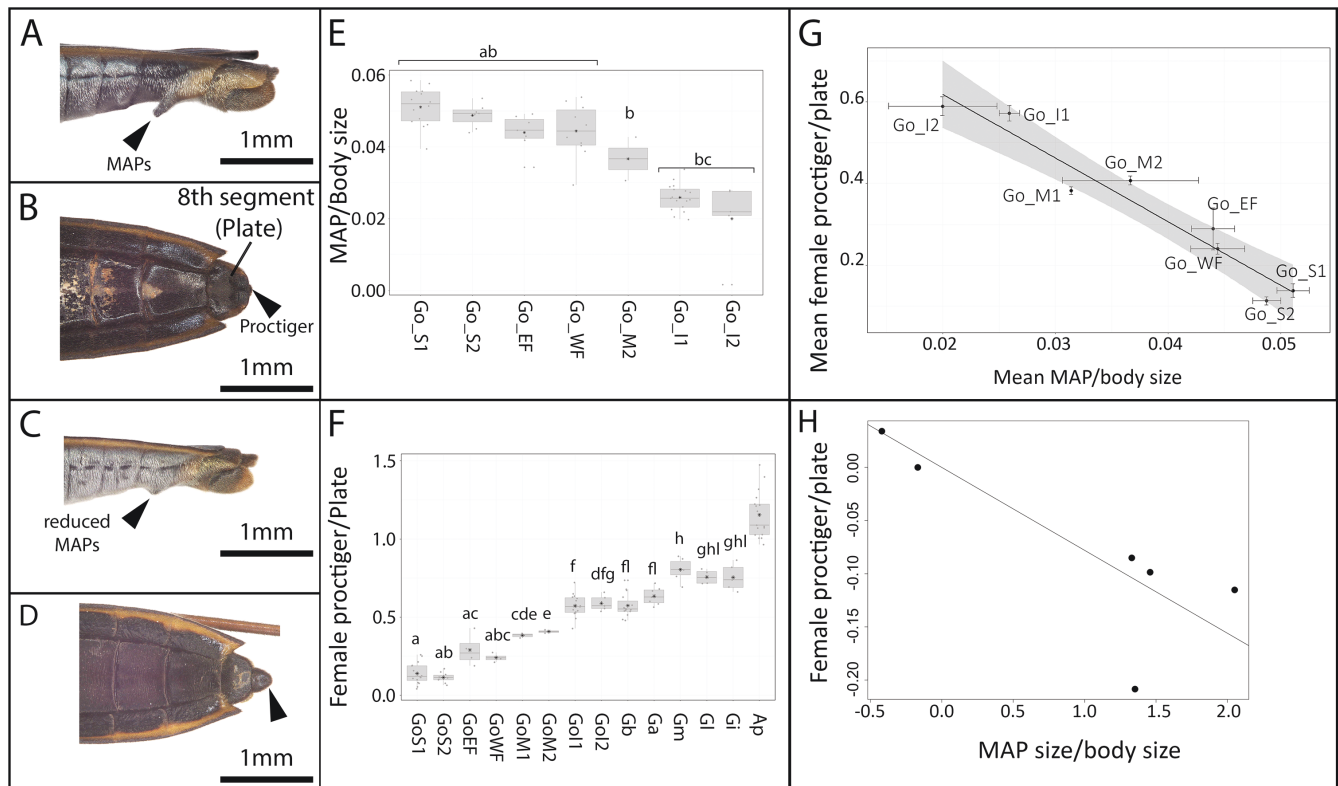


Figure 1. Phenotypic variation in the size of MAPs on the seventh abdominal segment and the state of proctiger (eighth abdominal segment) concealment in *G. odontogaster*. (A, B) A male and a female of a Swedish population. (C, D) A male and a female of an Italian population. (E) MAPs size across seven populations of *Gerris odontogaster*. Note that one Mongolian population is represented by a single male and therefore not included in MAPs analysis. Letters denote significant ($p < 0.05$) differences between populations in Tukey post-hoc tests. (F) Proctiger concealment across eight populations of *Gerris odontogaster* and other Gerridae (see [Supplementary Table S1](#) for description of populations). Go: *Gerris odontogaster*; S1 and 2 are two distinct populations from Sweden; EF: Eastern France; WF: Western France; I1 and 2: two distinct populations from Italy; M1 and M2 are two distinct populations from Mongolia. (G) Plot of the mean MAPs size and proctiger concealment in the eight populations of *G. odontogaster*. Error bars correspond to SE, line represents a linear regression and shaded area the 95% CI. (H) Phylogenetic generalized least square regression of proctiger concealment on MAPs size and phylogenetically independent contrast values across three populations of *G. odontogaster* (Sweden, France, and Italy) and four additional species of *Gerris*. Abbreviations: Gb: *Gerris buenoi*; Ga: *Gerris argentatus*; Gm: *Gerris marginatus*; Gi: *Gerris incognitus*; Gl: *Gerris lacustris*; Ap: *Aquarius pallidum*.

later found in northern Italy (Tamanini, 1979; Wagner, 1958) and Slovenia (Tamanini, 1979). Individuals from these populations were even given subspecific status as *G. odontogaster brevispinis* (Lundblad, 1934), although such populations are thought to represent local forms rather than a taxonomic unit (Andersen & Chen, 1993). To test our predictions that the proctiger in females is a counteradaptation to male MAPs, we phenotyped males and females from in total eight populations of *G. odontogaster* (Figure 1E and F, also see [Supplementary Table S1](#) for population origin; [Supplementary Table S2](#) for raw measurements). First, we phenotyped two Swedish *G. odontogaster* populations where males have fully formed and functional MAPs (Figure 1A and E). In these populations, the proctiger of the females is almost fully concealed under a dorsal plate consisting of a modified eighth abdominal segment (Figure 1B and F). Second, we phenotyped two Italian populations (a contemporary population collected in 2022 and a population collected in 1947) and two Mongolian populations where males have rudimentary MAPs (Figure 1C–F) (Lansbury, 1966; Lundblad, 1934). Here, we predicted that rudimentary MAPs should be associated with relaxed selection on the concealment of the females' proctiger. Consistent with this prediction, the females from the Italian populations have a proctiger that is about four times more exposed compared to *G. odontogaster* from Sweden (Figure 1F). The same result was seen in the Mongolian populations, as females have a proctiger that is some three times

more exposed than the Swedish populations (Figure 1F). Third, we phenotyped *G. odontogaster* from Western and Eastern France, where males have MAPs that are of intermediate size between the Swedish and the Italian populations (Figure 1E). Interestingly, females also exhibited an intermediate state of proctiger concealment (Figure 1F). For comparison, we also phenotyped a population of *G. buenoi*, a closely related species to *G. odontogaster*, where males lack MAPs altogether. Again, the females of *G. buenoi* have an exposed proctiger (Figure 1E and F). A closer inspection showed that the degree of proctiger concealment is primarily defined by the size of the eighth abdominal segment, which consists of a dorsal plate on top of the proctiger. In *G. odontogaster* from Sweden, this plate is significantly larger and covers the proctiger, whereas this segment is narrower in *G. odontogaster brevispinis* from Italy and in other *Gerris* species allowing the proctiger to remain exposed (Figure 1B–D). The intersexual correlation between the mean length of MAPs in males (the ratio between MAP length and body size) and average proctiger concealment in females (the ratio between proctiger protrusion and the length of the dorsal plate) across the eight *G. odontogaster* populations was $r = -0.92$ ($p < 0.001$), providing evidence for negatively correlated evolution across populations (Figure 1G).

Next, we wished to better place the above covariation between MAPs and the state of proctiger concealment across *G. odontogaster* populations in a phylogenetic context. A phylogenetic

reconstruction, based on the transcripts of 5,658 genes (Armisen et al., 2022), of a sample of *Gerris* spp along with three populations of *G. odontogaster* allowed us to assess the history of sexually antagonistic coevolution and to compensate for the potential effect of phylogenetic dependencies through the use of phylogenetic generalized least squares (PGLS) regression (Rohlf, 2001). The PGLS test of correlated evolution between the length of MAPs in males and proctiger concealment in females (Supplementary Table S3) revealed significant negative correlated evolution ($b = -0.078$, $SE_b = 0.016$, $p = 0.005$). The phylogenetic reconstruction also revealed that *G. odontogaster* from France and *G. odontogaster brevispinis* from Italy are sister populations and that *G. odontogaster* from Sweden is basal to both (Figure 2; Supplementary Figure S3). In addition, this reconstruction placed *G. buenoi* as a sister to all populations of *G. odontogaster* (Figure 2), confirming that the acquisition of MAPs is a derived state in *G. odontogaster*. Interestingly, this reconstruction also showed that the loss of MAPs in *G. odontogaster brevispinis* from the Italian population is a secondary event that followed their acquisition (Figure 2), providing evidence for a de-escalation of sexually antagonistic coevolution. The evolutionary pattern of proctiger concealment is consistent with the state of the MAPs, suggesting that this concealment might be costly and that the ancestral state of proctiger exposure is regained by females when MAPs are lost in males (Figures 1 and 2). These data further strengthen the suggestion that selection on proctiger state in females is related to MAPs and involved in sexually antagonistic coevolution.

Cellular and developmental genetic basis of MAPs

Water striders belong to the Heteroptera suborder with post-embryonic development consisting of five successive molts (Andersen, 1982) (Supplementary Figure S4). Phenotypic

differentiation of males and females can only be observed in the genital segments at the fourth and fifth nymphal instars (Supplementary Figure S4). The first signs of MAP specification appear at the fifth, and final, nymphal instar about 65 hr after molting from the fourth instar (Figure 3A and B). The developing MAPs can be observed through the cuticle of the fifth instar nymph after treatment with alcohol (Figure 3A), or by peeling the cuticle of the nymph and uncovering the differentiating preadult integument (Figure 3B). MAPs development starts as a pair of cell populations organized into concentric circles that prefigure their position in the future adult (Figure 3B and C). The lumen of the developing MAPs is particularly rich in cytoskeletal material as revealed by actin and acetylated alpha-tubulin staining (Figure 3D). By the end of the fifth nymphal instar, MAPs extend and can be readily observed in the preadult after removing the nymphal integument (Figure 3C). These cellular and developmental dynamics suggest that the development of MAPs involves cytoskeleton remodeling and morphogenetic processes.

Hox- and sex-determination genes shape MAPs during development

The development of MAPs is highly heritable (Arnqvist, 1989), but the genes involved are not known. We hypothesized, based on the state of knowledge about the role of Hox- and sex-determination genes in establishing differences along the body axis between males and females, that *Abdominal-B*, a posterior Hox gene, and *doublesex* will be required for the development of male and female traits. We therefore used the RNA interference technique to knock down the transcripts of these genes and test their roles in the development of MAPs and proctiger concealment (Supplementary Table S4). Control males from the Swedish population, injected with buffer, were indistinguishable from wild-type males and MAPs were present and fully developed on the seventh

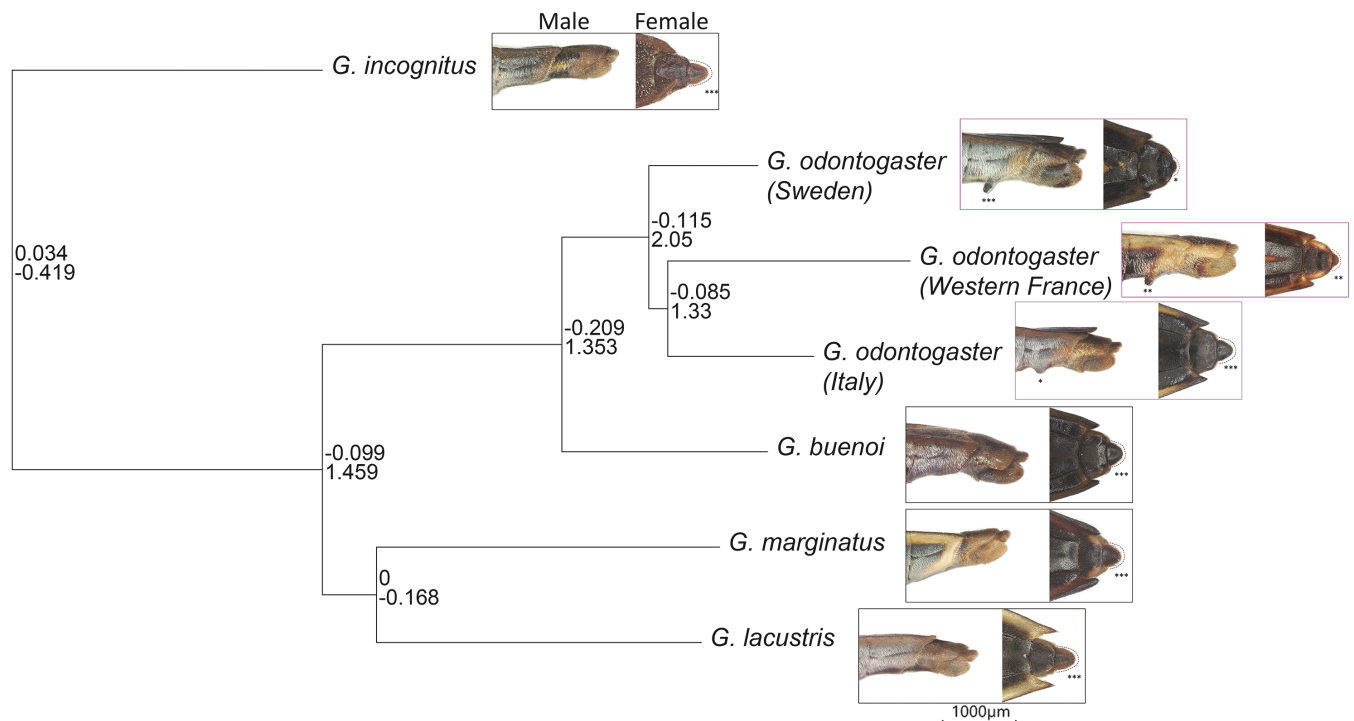


Figure 2. Phylogenetic relationships between three populations of European *G. odontogaster* and a sample of *Gerris* spp. Number of asterisks reflects the size of MAPs and the state of proctiger exposure. Phylogenetically independent contrast values for the male and female traits are indicated in each node.

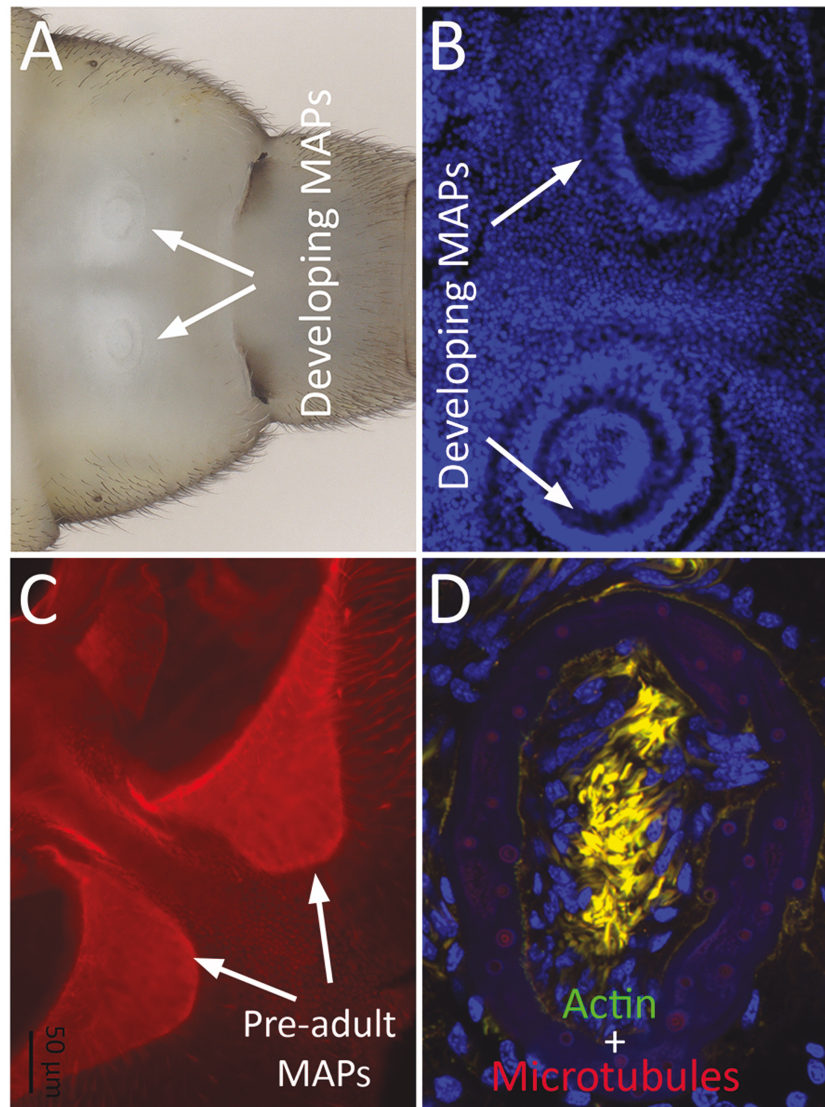


Figure 3. Development of MAPs. (A, B) The first signs of the development of MAPs appear at the middle of the fifth, and final, nymphal instar. (A) Two structures, prefiguring adult MAPs, can be visualized by imaging the nymphs in alcohol. (B) Epithelium of a preadult dissected out of the fifth instar nymph and stained with the nuclear marker DAPI. Developing MAPs consist of two sets of concentric cell populations which extend into full MAPs at the adult stage (C). (D) Actin and acetylated alpha-tubulin staining shows that the lumen of the developing MAPs is rich in cytoskeleton and microtubules. The yellow color results from the overlap of actin (green) and tubulin (red).

abdominal sternite ($n = 7$, Figure 4A and B; Supplementary Table S4). In wild-type and control males, the posterior part of this sternite had the shape of a trough that, we suggest, enables males to extend their genitalia in a more ventral angle in order to better reach females' genitalia during permuting struggles and mating (dashed outline in Figure 4B). A total of 15 fourth and early fifth instar males injected with dsRNA targeted against *Abdominal-B* and 13 males injected with dsRNA targeted against *dsx* reached adulthood. Knockdown of both *Abdominal-B* and *doublesex* resulted in the reduction or the complete loss of the MAPs (Figure 4C and E; see Supplementary Table S4 for quantification). In addition, the knockdown of both genes resulted in the disruption of the trough-shaped posterior part of the seventh abdominal sternite, which instead became more linear (dashed outline in Figure 4D and F). This overlap in the effect of *Abdominal-B* and *doublesex* suggests that the two genes interact within a network that shapes male abdominal processes.

***Abdominal-B*, but not *doublesex*, is necessary for concealing female's proctiger and shaping the genitalia**

Control-injected fourth and early fifth instar females were indistinguishable from wild-type females ($n = 7$, Figure 4G and H). A total of 11 females reached the adult stage after the injection of *Abdominal-B* double-stranded RNA. Interestingly, the eighth abdominal segment that covers the proctiger became narrower leaving the female structure exposed ($n = 11$, Figure 4I). This suggests that *Abdominal-B* is part of a genetic mechanism that shapes a female's putative counteradaptation through the concealment of the proctiger. The gonocoxae of wild-type females cover the ovipositor and the gonapophyses (Figure 4H). *Abdominal-B* knockdown resulted in a partial exposure of these structures ($n = 7$), and for two females they were completely exposed (Figure 4J). Adult females resulting from *dsx* knockdown had no clear morphological differences compared to controls ($n = 16$, Figure 4K and

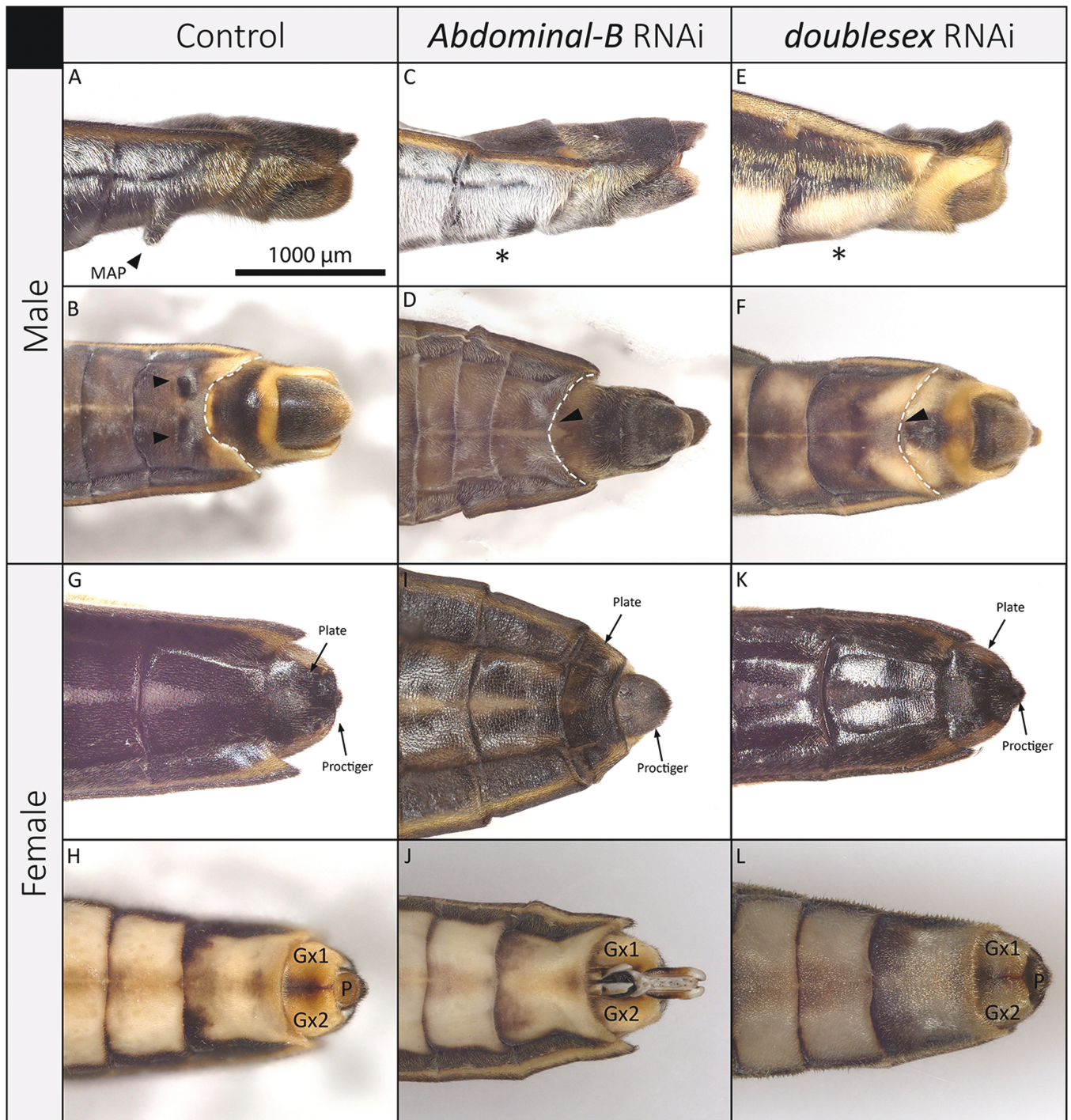


Figure 4. All images represent the Swedish population of *G. odontogaster*. Role of *Abdominal-B* and *doublesex* in the development of MAPs and proctiger concealment. (A) Lateral view of a control male showing fully extended MAPs. (B) Lateral view of a control male showing the trough shape of the seventh abdominal segment (dashed outline). (C, D) *Abd-B* RNAi and (E, F) *dsx* RNAi treatments result in the complete or near complete loss of MAPs and the disruption of the trough shape of the segment (dashed outlines in D and F). (G, H) Control females showing the state of proctiger concealment. (I) *Abd-B* RNAi results in the narrowing of the plate that conceals the proctiger, leaving it exposed. (J) Other female genital structures, such as the gonocoxae, are also affected. (K and L) *dsx* RNAi does not seem to alter the concealment of the proctiger or the genital segments.

L). The absence of any effects of *doublesex* knockdown in females suggests that this gene is not involved in the development of *G. odontogaster* female morphology during the final nymphal instar.

Discussion

We have demonstrated a tight negative correlation between a male sexually antagonistic trait (MAPs) and an apparent female

counteradaptation (proctiger concealment) across several populations of *G. odontogaster*. These populations diverged recently from the North American species *G. buenoi* (Armisen et al., 2022; Damgaard et al., 2014), which does not carry these traits. Moreover, our phylogenetic reconstruction showed that when MAPs are lost, the ancestral state of the proctiger (exposed) is regained. Importantly, we provide experimental evidence for a genetic link between these two coevolving traits. The Hox gene *Abdominal-B*

is involved in the development of both male and female sexually antagonistic traits, whereas the sex-determination gene *doublesex* is involved in the development of the male trait. Both genes are necessary for extending MAPs during development in males and their roles overlap significantly, whereas *Abdominal-B* is required for the concealment of the proctiger in the female. We first discuss the coevolutionary pattern of these two traits, and then their developmental biology and its implications for sexually antagonistic coevolution in this species.

Our analyses of the rapid coevolution of these sex-specific traits add to previous empirical examples of sexually antagonistic coevolution (Arnqvist & Rowe, 2002a; Bergsten & Miller, 2007; Dougherty et al., 2017; Koene & Schulenburg, 2005; Perry & Rowe, 2012; Rönn et al., 2007). Previous phylogenetic comparative analyses in the genus *Gerris* have revealed correlated evolution between sex-specific and sexually antagonistic genital and pre-genital traits and showed that this is associated with the evolution of male–female interactions (Arnqvist & Rowe, 2002a, b). Our data mirror these findings at a microevolutionary scale, operating within a single species (see also Perry & Rowe, 2012), and further suggest that episodes of concerted escalation and de-escalation of sexual armaments can proceed rapidly in both sexes. This is the first clear example of de-escalation in both sexes that we are aware of. We suggest that de-escalation is an expected outcome of sexually antagonistic coevolution. Parker (1979) showed that sexually antagonistic coevolution may result in unresolvable evolutionary chases with escalation and de-escalation whenever male and female traits carry investment costs. In fact, models of sexually antagonistic coevolution that include natural selection on the antagonistic traits demonstrate that even where equilibria exist, changes in natural selection on both the traits and the outcome of male–female interactions (i.e., mating) will shift those equilibria, causing both escalation and de-escalation (e.g., Pennell et al., 2016; Rowe et al., 2005).

Natural selection on these coevolving traits is likely occurring in *G. odontogaster*. In terms of MAPs, these are known to be costly to males in terms of complicating the molt between the fifth larval instar and adulthood (Arnqvist, 1994). This should increase the risk of cannibalism and predation during molting, and the length of MAPs is negatively correlated with population density and predator presence across populations (Arnqvist, 1994). In terms of proctiger concealment in females, we suggest that this may also come at a cost. Since the proctiger primarily functions during egg deposition, trade-offs between efficacy as a resistance morphology versus egg deposition may render a more concealed proctiger suboptimal in terms of attaching eggs efficiently on aquatic substrates.

Ecological impacts on the economics of sexually antagonistic interactions are well-studied in water striders. Ecological factors, such as food abundance, predation, sex ratio, and population density, are all known to affect the costs and benefits of sexually antagonistic traits in water striders (Arnqvist, 1994; Arnqvist & Rowe, 2005; Perry & Rowe, 2018; Rowe, 1994; Rowe et al., 1994). For example, food manipulation experiments in *Gerris* have shown that hungry females become more resistant to mating (Rowe, 1992) and this biases mating success toward persistent males (Ortigosa & Rowe, 2002). In addition, high population density and male-biased sex ratios are both associated with relaxed sexual selection for long MAPs in *G. odontogaster* (Arnqvist, 1992a, b). Since *G. odontogaster* has a wide Palearctic distribution, the dynamic pattern of escalation and de-escalation of MAPs and proctiger concealment is consistent with variation in the ecological setting across its range. It is possible that the intensity

of conflict varies across the wide geographic range of *G. odontogaster*, due to variation in the ecological setting of its populations. Future studies should examine whether and how the uncovered patterns of escalation and de-escalation of sexually antagonistic traits relate to variations in ecological factors.

Experimental manipulation of gene expression can provide information about the genetic programs shaping traits in both sexes, thus providing an important method to test whether correlated variation of male and female traits observed at the phenotypic level is linked to shared developmental genetic pathways. Depletion of two important developmental regulators, namely, *Abdominal-B* and *doublesex*, resulted in the loss or severe alteration of male and female traits. This result is consistent with similar findings in *Drosophila melanogaster* (Kopp et al., 2000) and in *Photuris* fireflies (Stansbury & Moczek, 2014). Our work shows that MAPs in *G. odontogaster* males are developmentally at least partially controlled by a network including these two proteins. In flies, *Abdominal-B* and *doublesex* interact to establish sexual dimorphism in abdominal pigmentation (Burtis, 2002; Kopp et al., 2000). *Abdominal-B* positively regulates the expression of *doublesex* and it is a one-way interaction (Wang & Yoder, 2012). Here, *doublesex* and another Hox protein (*sex comb reduced*) are known to regulate one another through a positive feedback regulatory loop (Tanaka et al., 2011). In our experiment, the roles of *Abdominal-B* and *doublesex* were similar, as knockdown of either gene in males resulted in the loss of MAPs and the alteration of the trough-shaped seventh abdominal tergite. This result indicates that the two genes interact and are part of a genetic network that shapes this male sexually antagonistic trait. The effects on two sex-specific male traits also show that these proteins have pleiotropic effects within males, which results in a genetic integration of MAPs and the shape of the seventh abdominal tergite. Perhaps most importantly, *Abdominal-B* RNAi also reduced the width of the dorsal plate leaving the proctiger exposed in females. This result demonstrates that this protein is required for the development of the coevolving male and female traits, a pleiotropic effect that should result in an intersexual genetic correlation between the traits, provided there is standing genetic variation in *Abdominal-B*. Several similar cases of other developmental regulators acting on male and female traits involved in reproduction have been previously reported. These include the role of the Hox gene *Sex combs-reduced* in the development of interacting but distinct male and female secondary sexual traits in the water strider *Rhagovelia antilleana* (Crumiere & Khila, 2019). In various beetles and milkweed bugs, distinct male and female genital traits are known to be shaped during development by overlapping genetic components (Aspiras et al., 2011; Macagno & Moczek, 2015), further supporting the conclusion that pleiotropy in the developmental genetic programs that shape sexual dimorphism in genital structures is widespread and may blur the distinction between intra- and interlocus sexual conflict.

Given that the male and female focal traits are different structures located on different segments, it would be easy to imagine them being underlain by independent loci. This is the common assumption of traits thought to be evolving independently and the use of interlocus coevolutionary models in efforts to understand coevolutionary diversification leans on this assumption (Arnqvist & Rowe, 2005). However, we have shown that *Abdominal-B* is necessary for the development of both of these traits. This result provides a rare empirical demonstration of a direct genetic link between intra- and interlocus sexual conflict (Mokkonen et al., 2016). If the observed evolutionary change in the two traits reflects either the evolution of *Abdominal-B* or its

expression, then there is the potential for intralocus sexual conflict to play a role in their evolutionary dynamics despite the fact that these traits are not shared between the sexes. The theory has shown that a genetic correlation between male and female traits can act to destabilize sexually antagonistic coevolution, especially when traits are costly (Hårdling & Bergsten, 2006; Hårdling & Karlsson, 2009). This is particularly true when the genetic correlation is caused by pleiotropy, blurring the distinction between intra- and interlocus sexual conflict (Pennell et al., 2016), when cyclic dynamics involving de-escalation in both sexes can occur. Alternatively, the evolution of both of these traits may reflect the evolution in the downstream targets of *Abdominal-B*, and those targets may be independent of one another. A related example of this is the loss of abdominal pigmentation in *Drosophila santomea*, where both the evolution of *Abdominal-B* and its targets have played a role (Glassford et al., 2015; Liu et al., 2019). Our identification of *Abdominal-B* as a necessary component of the developmental network(s) underlying these rapidly coevolving traits enables more targeted studies of the networks underlying their evolution and their degree of overlap. This role of pleiotropy should be taken into account when interpreting patterns of phenotypic coevolution of the sexes involving distinct but interacting traits. Some fraction of loci underlying an adaptation in one sex might simultaneously code for related adaptations in the other sex, and this process will affect the dynamics of sexually antagonistic coevolution. A direct approach to test the extent of overlap between intra- and interlocus sexual conflict is to identify the genetic variation underlying the coevolving male and female traits. The *Gerris* study system offers this opportunity through the high within-species variation, which provides the possibility of mapping the genetic basis of male and female traits.

Supplementary material

Supplementary material is available online at *Evolution Letters*.

Data and code availability

All sequences used in this work are available in GenBank under the following accession numbers: PQ283678 and PQ283677. Raw data, measurements, and the R code for PGLS analysis are available in the supplementary online information.

Author contributions

A.K. and D.A. designed the research, A.K., D.A., C.P., and G.A. secured samples from various populations, D.A., C.P., P.R., and A.H. conducted the analyses, A.K. and C.P. wrote the initial version of the article, and L.R., G.A., A.H., and D.A. refined the final version of the article.

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