Acknowledgements

We thank the authorities in Madagascar and the Seychelles for permission to conduct fieldwork. We thank J. Brown, T. Peterson, R. Prum, O. Rieppel, L. Trueb and E. Wiley for comments. C.J.R. and R.A.N. were supported by the National Geographic Society and the National Science Foundation, Washington.

Competing interests statement

The authors declare that they have no competing financial interests.

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Antagonistic coevolution between the sexes in a group of insects

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In coevolutionary 'arms races' between the sexes, the outcome of antagonistic interactions may remain at an evolutionary standstill. The advantage gained by one sex, with any evolutionary exaggeration of arms, is expected to be matched by analogous counteradaptations in the other sex[1,2]. This fundamental coevolutionary process may thus be hidden from the evolutionist's eye[3,4], and no natural examples are known. We have studied the effects of male and female armament (clasping and anti-clasping morphologies) on the outcome of antagonistic mating interactions in 15 species of water strider, using a combination of experimental and phylogenetic comparative methods. Here we present, by assessing the independent effects of both species-specific level of arms escalation and small imbalances in the amounts of arms between the sexes within species, the consequences of a sexual arms race. Evolutionary change in the balance of armament between males and females, but not in the species-specific level of escalation, has resulted in evolutionary change in the outcome of sexually antagonistic interactions such as mating rate.

Evolutionary conflicts of interests between the sexes are ubiquitous[1–4]. Such conflict is predicted to fuel sexually antagonistic coevolution[1,2], during which adaptations in one sex, which are harmful for individuals of the other sex, select for counteradaptations in the other sex to mitigate costs imposed by such adaptations. The resulting coevolution between the sexes is now recognized to be a central process of evolution, with the potential to shape various interactions between the sexes[2,5,6] and their gametes[7–8], as well as diversification[9], speciation and extinction rates[10–12]. At the core of this coevolutionary interaction is an arms race between the sexes that can include periods of both escalation and de-escalation of arms[13–15].

Theory suggests, however, that the outcome of antagonistic male–female interactions should remain relatively unchanged during an arms race because the build-up of arms in one sex may be balanced by a build-up in the other (Fig. 1a, 1–2). The consequences of such arms races on sexual interactions may thus be undetectable, which makes sexually antagonistic coevolution inherently difficult to show[1–4]. Perhaps for this reason, we have no direct empirical evidence for a primary role of arms races in the evolution of sexual interactions in natural systems.

Despite an expectation of some evolutionary balance in the level of arms between the sexes, one sex may at least temporarily evolve a greater quantity of arms relative to the other (refs 13–15; and Fig. 1a, 3–4). In such cases, the evolutionary consequences of the arms race for interactions between the sexes may be exposed. For example, in an arms race in which it benefits males but not females to mate several times[13], one may expect relatively high rates of mating in species where the advantage has shifted toward males (Fig. 1a, 4). The converse would be expected for species in which the advantage has shifted toward females (Fig 1a, 3). Thus, by using tests that are based on phylogeny[16,17], one might uncover sexually antagonistic adaptations by analysing the effects on the change in sexual interactions caused by evolutionary change in the relative levels of arms between the sexes.

We have studied the coevolution of relative armament of the sexes and the outcome of sexually antagonistic interactions in 15 congeneric water strider species. Water striders (Heteroptera; Gerridae) are a group of semi-aquatic insects, which have become a model system in which to study sexually antagonistic coevolution. Several experimental in-depth studies, carried out on several different species and using different approaches (reviewed in refs 18, 19), have shown that there is intense and overt sexual conflict over mating rate that results from a strong asymmetry between the sexes in the relative costs and benefits of mating. Matings are preceded typically by a violent pre-mating struggle, in which females try to dislodge harassing males to avoid superfluous and costly mating[18,19].

The ability of males to withstand these struggles is related to various morphological grasping adaptations, such as exaggeration of prolonged clasping genitalia and a more flattened abdomen[20,21], which allow males to grasp females more firmly. Females' ability to resist males is related to distinct morphological counteradaptations, such as prolongation of the female abdominal spines and the degree of downward tilting of the abdominal tip[20,22], which makes it more difficult for males to grasp females. We have shown elsewhere that the level of these arms in females coevolves closely with those in males within water striders[20]. Thus, species can be ordinated along a

Table 1 Effects of morphological armament on the outcome of sexually antagonistic interactions

	Duration of pre-mating struggles			Male struggle success			Female mating activity			Female mating rate		
	β	t	P	β	t	P	β	t	P	β	t	P
Male persistence*	12.52	3.20	0.004	0.38	2.59	0.012	0.35	2.50	0.014	0.33	1.93	0.039
Female resistance*	–13.34	3.51	0.002	–0.47	3.28	0.003	–0.38	2.80	0.008	–0.35	2.09	0.029
Absolute level of arms (PC1)	0.83	0.87	0.399	0.06	1.71	0.113	0.02	0.73	0.482	0.02	0.40	0.693
Relative armament of the sexes (PC2)*	–14.83	3.13	0.004	–0.53	3.15	0.004	–0.45	2.83	0.007	–0.41	2.06	0.031

The effects of absolute and relative morphological armament on the outcome of sexually antagonistic interactions are shown. Multiple regression analyses, using behaviour as the dependent variable and either male persistence and female resistance, or PC1 and PC2, as independent variables. Regression models were based on phylogenetically independent contrasts ($n = 14$) and thus were forced through the origin[17]. Residuals did not differ significantly from normality (Kolmogorov–Smirnov tests, $P \geq 0.107$ for the first and $P \geq 0.101$ for the second group of models). Statistical power analyses of these models showed that our inability to detect any effects of absolute level of arms is not likely to be due to a lack of power. The combined probability of committing four type II errors was $\beta = 0.129$, assuming that absolute level of arms accounts for 10% of the variance in the dependent variable.

* P values reported test partial regression coefficients under directional null hypotheses (see Methods) and thus are one-tailed.

coevolutionary trajectory within a two-dimensional (2D) morphological space. These dimensions describe the degree of exaggeration in male arms that enhance persistence and in female arms that enhance resistance (Fig. 1b).

Such a trajectory is a predicted result of the coevolution of male and female arms[13–15]. We determined whether the coevolution of male and female morphologies along and/or away from this trajectory leads to evolutionary changes in the outcome of antagonistic mating interactions. If the coevolutionary trajectory reflects a balance of arms, then evolutionary deviation away from the line should affect these interactions, whereas evolutionary movement along the line should have little effect. Specifically, we expect that as females become more armed relative to males, their ability to resist will be elevated and the duration and success of antagonistic interactions (that is, pre-mating struggles) will therefore decline, leading to reduced superfluous and costly mating among females.

We analysed the effects of evolution of morphological armament in the sexes on evolution of four behavioural traits: duration of pre-mating struggles, male struggle success, female mating activity, and female mating rate (Methods). As predicted by theory, coevolutionary change of the absolute level of arms escalation was in no case related to the outcome of sexually antagonistic interactions, whereas changes in the relative armament of the two sexes was associated strongly with evolutionary alterations in the outcome of sexually antagonistic interactions (see Table 1).

Collectively, these results show that the average coevolutionary trajectory that we have identified describes an evolutionary path along which levels of arms in the two sexes is to a great extent balanced. Had coevolution proceeded precisely along this path, the effects of the arms race on mating interactions would have been obscured. But species evolve off this path to points where one sex gains relative advantage over the other, and it is these deviations that provide insight into the consequences of this arms race. Species thus differ considerably both in the escalation of arms and in relative armament of the sexes. Theory suggests that such variation can be caused by several different factors, such as differences in armament costs, environmental variation, or intraspecific variation in armament levels[13–15].

A closer inspection of the analyses (see Table 1) yielded four important insights. First, significant effects of relative, but not absolute, levels of armament were found consistently across all measures of the outcome of antagonistic interactions, which suggests that the sexual arms race propels evolution of the general mating system in this group of insects[30]. Second, our results were not affected by whether we used phylogenetically independent contrast or species-level data (Supplementary Information), which indicates that the degree of phylogenetic inertia is very low in our data[23]. The phylogenetic autocorrelations[16,24], which range from −1 to +1, observed at the species level were also generally very low (scaled Moran's I (ref. 24) for male persistence, −0.544; for female resistance, 0.278; range for the four behavioural variables, −0.03 to 0.393; $P > 0.110$ in all cases), confirming that phenotypic similarity across species is at most only weakly related to shared ancestry. Such a weak relationship between phylogenetic similarity and phenotypic similarity implies that the rate of character evolution is relatively high and non-directional[18,23,24]. Our results thus illustrate the dynamic nature of sexually antagonistic coevolution[13–15], with periods of escalation and de-escalation[20].

Third, evolutionary changes in the relative armament of the sexes accounted for a large proportion of the variation in evolution of interactions between males and females. Correlation analyses, using phylogenetically independent contrasts, showed that relative armament of the sexes alone (Table 1, PC2) explained 48, 41, 40 and 26% of the variance in our four behavioural variables, respectively. Finally, the sign of the relationship between relative armament of the sexes and the behavioural variables consistently corresponds to those predicted by *a priori* hypotheses. As females evolve a relative advantage in arms over males, they are able to dislodge harassing males more rapidly and more successfully in pre-mating struggles. Consequently, females evolve to mate less frequently and spend less time in superfluous matings.

The results of our analyses provide empirical evidence for a coevolutionary arms race between the sexes and for its consequences for the antagonistic interactions associated with reproduction[13]. Our comparative analytical strategy allowed us to confirm that sexually antagonistic coevolution may be hidden by continuous

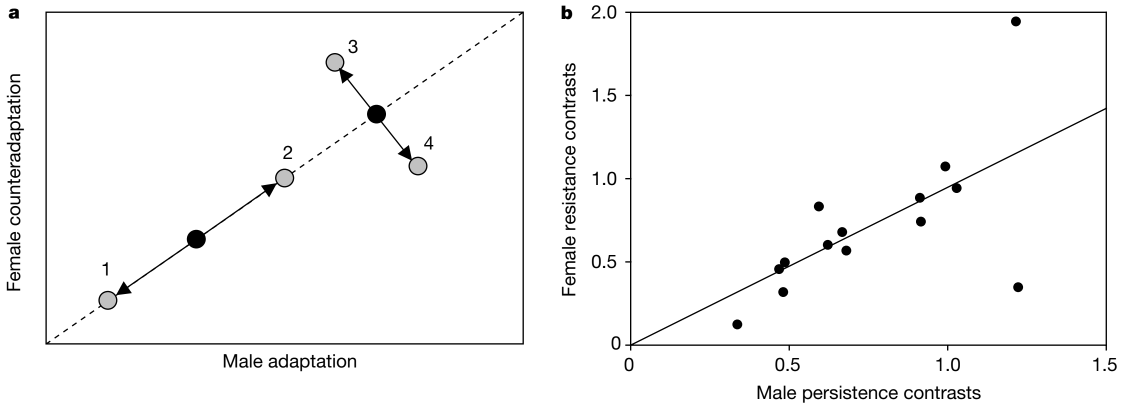

Figure 1 Coevolution of arms in males and females. **a**, Sexually antagonistic coevolution is expected to be hidden, because adaptations in one sex should be balanced by counteradaptation in the other. Mutual and matched de-escalation (1) or escalation (2) of armament in the two sexes should not therefore affect the outcome of antagonistic interactions. But when this arms race is not balanced perfectly, coevolution will lead to either females (3) or males (4) gaining a relative advantage in conflict. **b**, Water striders engage in pre-mating struggles. Species can be ordinated in a 2D space constructed by sexually antagonistic morphological adaptations in males and females that mediate this conflict[20], as identified by a two-block partial least-squares analysis[29] of male and female body shape, in which increases in magnitude represent increases in armament levels in both sexes. Shown are phylogenetically independent contrasts[16,17] of such male persistence and female resistance (solid line represents linear regression forced through the origin; test of H_0: $\beta = 0$, $t = 8.55$, $P < 0.001$; $r = 0.92$). Male persistence represents a multivariate measure of male expression of morphologies associated with male ability to secure a firm grip of females during pre-mating struggles. Female resistance represents a multivariate measure of female expression of morphologies that aid in mate rejection during pre-mating struggles. These 'arms' include, for example, elongation of grasping genitalia in males and abdominal spines in females that function to hinder grasping by males. The correlated evolution between male persistence and female resistance involves different traits in the two sexes, and is not caused by a common correlated evolution with body size (that is, size allometry[20]).

adaptation and counteradaptation[1–3]. It also shows how the evolutionary consequences of sexual conflict can be nevertheless determined, by analysing the effects of small imbalances in male and female adaptations that may occur during an arms race. Finally, our study illustrates the power of combining quantifications of adaptation and counteradaptation in both sexes with independent measures of the resulting interactions across populations or species. This comparative strategy will help us to understand the elusive but potent process of sexually antagonistic coevolution. □

Methods

We collected 15 congeneric water strider species (*Gerris* spp.) from various sites in Europe and North America during their peak reproductive season, and transported them to the laboratory for the experiments described below. These individuals were also subjected to morphological analyses. A list of species and detailed descriptions of the collection sites and morphometric methods is provided elsewhere[20].

Behavioural experiments

Interspecific behavioural variation in antagonistic mating interactions were quantified in two sets of replicated laboratory experiments conducted separately on each of the 15 species. Water striders were kept in aerated pools (water depth 3–6 cm) provided with Styrofoam strips at 20 ± 1 °C, and were fed frozen insects (fruit flies and crickets). We made every possible effort to standardize the biotic and abiotic protocol under which these large experiments were carried out. In the first set, eight individuals were introduced into each of 6–10 replicate experimental pools (40 × 60 cm) per species at a sex ratio of 3:1. By continuously observing all interactions (for 1 h), we measured the average duration of premating struggles that led to dislodgement of the male and the proportion of struggles in which females yielded in each replicate pool. The latter struggles led to mating and hence measure male struggle success.

In the second set of experiments, eight individually marked water striders were placed in each of 14–20 replicate experimental pools (40 × 60 cm) per species at an average sex ratio of 1:1 and were allowed to acclimatize to these conditions for a period of 24 h. We then measured female mating activity (the mean proportion of time spent mating) and female mating rate, by conducting spot checks at 10-min intervals in all replicates (total observation time per replicate: 9–25 h over two consecutive days).

All analyses presented were carried out using species averages across all replicates. Note that because both struggles and superfluous mating are costly for females[25,26], the evolution of reduced magnitudes of all four behavioural variables is clearly in the interest of females.

Statistical methods

We based all statistical inference described below on phylogenetically independent contrasts[17] of all variables, which we computed using the CONTRAST module of PHYLIP[27] and a phylogenetic hypothesis that is based on both molecular (820 base pairs (bp) from the mitochondrial COI gene and 515 bp from the nuclear EF-1α gene) and morphological (63 characters) data[28]. These contrasts account for bias that may be introduced by shared ancestry among our species, and thus allow direct comparative analyses of correlated evolution[16,17,23]. No branch lengths have been estimated for our phylogenetic hypothesis, so equal branch lengths were assumed[16,17]. In no case was the absolute magnitude of the contrasts correlated (Pearson product–moment correlations) significantly with the estimated standard deviation (ref. 17; $0.074 \le P \le 0.822$ in all cases). To test whether the outcome of antagonistic interactions between the sexes coevolves with the evolution of morphological arms in the two sexes, we analysed our data using two different approaches. First, we fitted male persistence and female resistance simultaneously as independent variables to each of the behavioural variables in a series of multiple regression models. Because each independent variable in a multiple regression model estimates effects that are independent of other independent variables included in the model, these models estimate the effects of both male persistence and female resistance, given that the other variable is held constant. In these models, we therefore predict that an increase in male persistence (that is, $\beta > 0$) and a decrease in female resistance (that is, $\beta < 0$) will both be associated with increases in our behavioural variables, because such changes both result in a relative male advantage in arms.

Second, we tested for the independent effects of (1) absolute level of arms, or the position along the average coevolutionary trajectory, and (2) relative armament of the sexes, or the degree to which the level of arms is mismatched in the two sexes (Fig. 1a), using the following procedure. To generate statistically independent measures of these two parameters, we first subjected the two correlated variables, male persistence and female resistance (see Fig. 1b), to a principal component analysis, which was based on the correlation matrix to ensure that variance in male persistence and female resistance was given equal weight, and which yielded two principal components (PCs). The first (PC1) was highly correlated with both male persistence ($r = 0.98$) and female resistance ($r = 0.98$), and thus provides an integrative measure of the absolute level of arms in males and females. The second (PC2) is by definition orthogonal to (that is, uncorrelated with) the first, and hence directly measures the degree of mismatch, or imbalance, in arms level between the two sexes: PC2 was negatively correlated with male persistence ($r = -0.20$) and positively with female resistance ($r = 0.20$).

We carried out a series of multiple regression analyses using PC1 and PC2 as independent variables, and behaviour as the dependent variable. Because a positive score on PC2 corresponds to a relative female advantage in arms (Fig. 1a, 3), we predict that there is a negative coevolutionary relationship between PC2 and our behavioural variables ($\beta < 0$). For comparative purposes only, we also carried out these analyses with species-level rather than contrast data (Supplementary Information).

Received 22 August; accepted 29 November 2001.

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Supplementary Information accompanies the paper on *Nature*'s website (http://www.nature.com).

Acknowledgements

This study was supported by the Swedish Natural Science Research Council, the Natural Sciences and Engineering Research Council of Canada, the Swedish Foundation for International Cooperation in Research and Higher Education, the Knut and Alice Wallenberg Foundation and the Magnus Bergvalls Stiftelse. We thank N. M. Andersen and J. Damgaard for phylogenetic information; J. Felsenstein and F. J. Rohlf for developing software; and T. Day, U. Friberg, D. Schluter and T. Tregenza for comments.

Competing interests statement

The authors declare that they have no competing financial interests.

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