Genetic Constraints and the Evolution of Display Trait Sexual Dimorphism by Natural and Sexual Selection

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ABSTRACT: The evolution of sexual dimorphism involves an interaction between sex-specific selection and a breakdown of genetic constraints that arise because the two sexes share a genome. We examined genetic constraints and the effect of sex-specific selection on a suite of sexually dimorphic display traits in *Drosophila serrata*. Sexual dimorphism varied among nine natural populations covering a substantial portion of the species range. Quantitative genetic analyses showed that intersexual genetic correlations were high because of autosomal genetic variance but that the inclusion of X-linked effects reduced genetic correlations substantially, indicating that sex linkage may be an important mechanism by which intersexual genetic constraints are reduced in this species. We then explored the potential for both natural and sexual selection to influence these traits, using a 12-generation laboratory experiment in which we altered the opportunities for each process as flies adapted to a novel environment. Sexual dimorphism evolved, with natural selection reducing sexual dimorphism, whereas sexual selection tended to increase it overall. To this extent, our results are consistent with the hypothesis that sexual selection favors evolutionary divergence of the sexes. However, sex-specific responses to natural and sexual selection contrasted with the classic model because sexual selection affected females rather than males.

Keywords: Drosophila serrata, cuticular hydrocarbons, X chromosome, sex linkage, experimental evolution, intralocus sexual conflict.

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Sexual dimorphism is a pervasive pattern in nature. The sexes often differ in size, shape, and the degree to which sexual display traits are developed, with the last comprising a substantial component of biological diversity (Darwin 1871; Andersson 1994). Ultimately, the degree to which sexual dimorphism evolves reflects a historical interaction between sexually antagonistic selection and constraints arising from a genome that is predominantly shared between the two sexes (Lande 1980). Sexually antagonistic selection on shared traits generates intralocus sexual conflict, in which the adaptive evolution of one sex is impeded by that of the other (Rice 1984; Parker and Partridge 1998; Rice and Chippindale 2001). The evolution of sexual dimorphism is the ultimate means by which this conflict is resolved. Despite classic theoretical treatments and much recent interest, substantial empirical work remains if we wish to gain a comprehensive understanding of the evolution of sexual dimorphism, including the sources of sexually antagonistic selection (Hedrick and Temeles 1989) and how genetic constraints between the sexes are overcome (Lynch and Walsh 1998).

The evolution of sexual dimorphism requires that the genetic control of a shared trait be at least partially independent between the sexes such that the intersexual genetic correlation is less than 1 (Robertson 1959; Lande 1980). Strong and positive intersexual genetic correlations constrain the evolution of sexual dimorphism because a response to selection in one sex produces a parallel, correlated response in the other. Sustained sexually antagonistic selection favors mutations that allow male and female traits to approach their sex-specific selective optima, providing a resolution to intralocus sexual conflict (Lande 1980, 1987).

There are four broad mechanisms by which genetic constraints on the evolution of sexual dimorphism can be overcome: (1) the evolution of sex linkage, in which loci are relocated to the sex chromosomes (Fisher 1931; Rice 1984); (2) sex-specific epistasis, such as the evolution of sex-biased modifiers to the expression of loci with sexually antagonistic effects; (3) duplication followed by sex-limited expression of autosomal loci (Rhen 2000; Rice and

Chippindale 2002); and (4) the evolution of parent-of-origin effects via epigenetic processes such as genomic imprinting (Day and Bonduriansky 2004). The relative importance of each remains unclear, however, in large part because of a lack of data. Thus, empirical tests that can determine the relative roles of these mechanisms are required (Lynch and Walsh 1998; Bonduriansky and Rowe 2005; Fairbairn and Roff 2006).

As for selection, Darwin (1871) originally proposed two broad hypotheses for the source of sexually antagonistic selection that favors the evolution of sexual dimorphism. First, sex-specific natural selection may arise as a consequence of ecological processes such as intersexual resource competition. In this case, the resulting dimorphism permits ecological niche partitioning between the sexes, which alleviates the competition (Shine 1989). Second, sex-specific sexual selection may occur, in which different trait values maximize reproductive success in males and females. This is often hypothesized to occur because sexual selection is strong in one sex but weak or absent in the other (Price 1984; Preziosi and Fairbairn 1996). The classic quantitative genetic model for the evolution of sexual dimorphism is an example of this latter hypothesis: natural selection is assumed to act similarly on display traits in both sexes, whereas sexual selection acts only on males (Lande 1980).

These two hypotheses are not mutually exclusive, however, and there is evidence to suggest that both may be important in the evolution of display trait sexual dimorphism (Heinsohn et al. 2005). If natural and sexual selection often have sex-specific optima, a consideration of the combined effects of both processes may be critical to understanding the evolution of sexual dimorphism. To date, we lack direct experimental tests for how natural and sexual selection interact in affecting the evolution of display trait sexual dimorphism. Such data are important, however, because relying solely on correlational studies (i.e., selection gradients) may be problematic. For example, sex-specific selective optima may vary over life-history stages (Schluter et al. 1991), and contemporary selection gradients do not necessarily reflect the processes involved in creating a specific sexual dimorphism. Experimental evolution is one way to obtain such data.

Here we investigate the evolution of display trait sexual dimorphism in the fruit fly *Drosophila serrata*. *Drosophila serrata* has a well-characterized mate and species recognition system (Blows and Allan 1998; Higgie et al. 2000), in which both males and females discriminate among potential mates via display traits consisting of a suite of homologous, but sexually dimorphic, nonvolatile contact pheromones composed of cuticular hydrocarbons (CHCs; Chenoweth and Blows 2003). These CHCs have previously been shown to experience sexually antagonistic sexual selection under laboratory conditions (Chenoweth and Blows 2005), and sexual dimorphism has evolved during adaptation to novel laboratory environments (Rundle et al. 2005). However, how independent genetic control to permit such evolution has been achieved and the roles of natural and sexual selection in it have not been explored.

Our study combines large-scale quantitative genetic analyses of natural populations with laboratory experimental evolution to provide a comprehensive analysis of the evolution of display trait sexual dimorphism in both natural and experimental populations. First, we characterize genetically based variation in CHC sexual dimorphism among nine natural populations along the east coast of Australia. Second, we employ quantitative genetic analyses to examine the genetic basis of sexually dimorphic CHCs in each of these nine populations and use a mixed-model approach to distinguish between the contribution of sex linkage and sex-modified expression of autosomal loci in lowering intersexual genetic correlations. Finally, we use laboratory experimental evolution to characterize how selection affects both male and female CHCs, independently manipulating the opportunities for both natural and sexual selection during adaptation to a novel laboratory environment.

Methods

Geographical Variation in Sexual Dimorphism

We sampled *Drosophila serrata* from nine natural populations spanning approximately 15° of latitude (1,450 km) in eastern Australia (fig. 1). Populations were founded from an average of 20 wild-caught females and were maintained as mass-bred populations at an average census size of 200 individuals for four generations to remove common environmental and maternal effects before quantitative genetic analysis. For each population, F4 flies were used to establish a paternal half-sib quantitative genetic breeding design consisting of between 40 and 54 sires per population, each mated randomly to three virgin females. Two virgin male and female offspring per full-sib family were assayed for CHC expression.

Nine homologous CHCs were analyzed using 4-day-old virgin flies (male and female) and employing standard gas chromatography methods: Z,Z-5,9-$C_{24:2}$, Z,Z-5,9-$C_{25:2}$, Z-9-$C_{25:1}$, Z-9-$C_{26:1}$, 2-Me-C_{26}, Z,Z-5,9-$C_{27:2}$, 2-Me-C_{28}, Z,Z-5,9-$C_{29:2}$, and 2-Me-C_{30} (Blows and Allan 1998; Howard et al. 2003). In some instances, the two dienes—Z,Z-5,9-$C_{24:2}$ and Z,Z-5,9-$C_{25:2}$—could not be resolved on gas chromatographs, and so these individuals were omitted from further analysis. On average, 237 individuals of each sex were assayed for CHC expression in each population, resulting in a total sample of 4,265 flies.

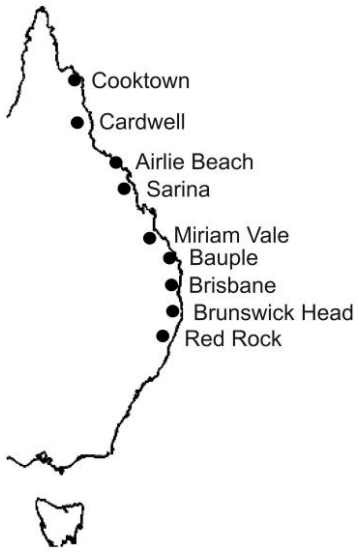

Figure 1: Locations of nine natural populations sampled over 15° of latitude (1,450 km) in eastern Australia: Cooktown (15°28′S, 145°15′E), Cardwell (18°16′S, 146°01′E), Airlie Beach (20°17′S, 148°41′E), Sarina (21°35′S, 149°11′E), Miriam Vale (24°24′S, 151°26′E), Bauple (25°49′S, 152°34′E), Brisbane (27°34′S, 152°59′E), Brunswick Head (28°32′S, 153°33′E), and Red Rock (29°59′S, 153°13′E).

The entire CHC phenotype of a fly is essentially a composition of individual hydrocarbons, the statistical analysis of which raises two issues. First, technical error associated with quantifying the total abundance of each hydrocarbon of an individual fly can be large when using gas chromatography, necessitating the removal of this error by expressing the abundance of each hydrocarbon as a proportion of the total hydrocarbons extracted. This removes among-fly variation in total CHC content. Second, the removal of this component of experimental error using proportions generates traits that are poorly suited to multivariate analysis. This is because the unit-sum constraint imposed by proportions increases multicollinearity among traits, which, in turn, leads to singular covariance matrices (Atchison 1986). The solution to this problem is to calculate logcontrasts of the original proportions (Atchison 1986; Blows et al. 2004). The area under the nine chromatograph peaks was integrated and transformed into eight logcontrast values for subsequent statistical analyses using Z-9-$C_{26:1}$ as the common divisor:

$$\text{logcontrast CHC}_n = \log_{10}\frac{\text{prop(CHC}_n)}{\text{prop(Z-9-C}_{26:1})}. \quad (1)$$

Note that the results of subsequent multivariate statistical analyses performed using these logcontrast trait values do not vary with the choice of divisor (Atchison 1986).

We tested for geographical variation in CHC sexual dimorphism among these nine natural populations, using univariate ANOVAs. For each of the eight logcontrast CHCs, we fitted the mixed model

$$c_{ijk} = \mu + s_i + p_j + s_i p_j + e_{ijk}, \quad (2)$$

where c_{ijk} is the CHC logcontrast value of the kth individual, s_i is the effect of the ith sex, p_j is the effect of the jth population, and $s_i p_j$ is the interaction between the effects of sex and population. Sex was fitted as a fixed effect, whereas population was random.

Intersexual Genetic Correlations

The X chromosome represents a significant proportion of the genome in *Drosophila* (e.g., 18% of euchromatin in *Drosophila melanogaster*; Fitzpatrick 2004). The standard analysis for paternal half-sib breeding designs, however, does not include the effects of X-linked additive genetic covariance between the sexes in estimates of the intersexual genetic correlation, because males are the heterogametic sex. Therefore, we estimated the intersexual genetic correlation for each CHC in two ways: as a result of additive autosomal effects only and as a result of the combined influence of the autosomes and the X chromosome, using the mixed-linear-model method developed by Cowley et al. (1986) and Cowley and Atchley (1988).

Rather than using information from sire-level terms exclusively, this method allows extraction of X-linked additive genetic variance within the sexes and covariance between them from other levels of relatedness within a half-sib breeding design. There are two caveats about using this approach. First, it assumes that dominance does not contribute substantially to the traits under consideration. Simulations have shown that the presence of dominance will bias estimates of the intersexual genetic correlations downward but that this bias is minimal when heritabilities are moderate to high (>0.6; Cowley and Atchley 1988). The average narrow-sense heritabilities for the majority of traits considered here fall within this moderate-to-high range (app. A in the online edition of the *American Naturalist*), suggesting that dominance should have a negligible effect on our estimates.

Second, a direct statistical test for differences between the two genetic correlation estimates (i.e., including and excluding X-linked effects) within a single population is not currently available using this method (Cowley et al. 1986). Although resampling may appear an attractive solution to this issue, it suffers from the problem that intersexual genetic correlations estimates are bounded statistics (W. Atchley, personal communication). We therefore took the alternate approach of estimating both genetic

correlations separately in each of nine independent populations, allowing an estimate of a specieswide error and permitting statistical comparison between estimates using standard nonparametric techniques.

For each CHC, we tested whether inclusion of the X-linked additive genetic effects lowered the intersexual genetic correlation, using nonparametric Wilcoxon signed-rank tests because the differences between the two estimates were not normally distributed (Shapiro-Wilk: $W = 0.547$, $P < .001$). Populations were treated as independent estimates of intersexual genetic correlations in these analyses. Variance and covariance components between the sexes were estimated using restricted maximum likelihood, with negative X-linked and autosomal variance components set to 0 (Mezey and Houle 2005). Full methodological details of this procedure are given in appendix B in the online edition of the *American Naturalist*, and further examples of its implementation can be found in Chenoweth and Blows (2003) and Mezey and Houle (2005).

Experimental Evolution

We investigated the sex-specific contribution of natural and sexual selection to the evolution of CHC sexual dimorphism, using an experimental-evolution approach. Dietary composition has been shown to affect CHC biosynthesis in many insects (Liang and Silverman 2000; Buczkowski et al. 2005; Rojas et al. 2005), including *Drosophila* (Etges et al. 2006). When *D. serrata* that have adapted to standard yeast-based larval food are allowed to adapt to a novel corn-based larval food that differs in amino acid content, male and female CHCs evolve independently toward new optima (Rundle et al. 2005). We took advantage of this evolution of CHC sexual dimorphism in a novel environment to conduct a more sophisticated experiment that manipulated the opportunities for both natural and sexual selection, allowing us to determine the independent and combined contribution of each process to the evolution of sexual dimorphism in a laboratory population. The experimental manipulation is outlined below; additional details can be found in Rundle et al. (2006)

A stock population of *D. serrata* was created by mixing laboratory populations originally collected from six sites along the east coast of Australia that encompass the latitudinal range sampled for the previous quantitative genetic analyses. These populations had been held in the laboratory under constant conditions (25°C; 12L : 12D photoperiod) on standard yeast-based larval food for four discrete generations before mixing them to generate the stock population. This mixing of populations was performed to increase genetic variance in the stock, thereby increasing the probability of observing a response to selection during experimental evolution; it is a valid approach when inferences are not concerned with specific patterns in nature. Once created, the stock population was maintained under the same constant conditions and on the same yeast-based larval food at a large population size (16 half-pint stock bottles) for 21 discrete generations before the start of the experiment. At the start of the experiment, 12 replicate populations were derived from the stock and maintained on a novel corn-based food medium. See Rundle et al. (2005) for medium recipes.

Populations were assigned in a two-way factorial design to one of four experimental treatments that varied in the opportunity for natural and sexual selection (Blows 2002); three populations experienced both natural and sexual selection (N + S), three experienced reduced natural selection with sexual selection present (S), three experienced natural selection with reduced sexual selection (N), and three experienced reduced natural and reduced sexual selection (control).

Every generation, 55 virgin males and 55 virgin females were collected from each population and then mated. How this was done determined the natural and sexual selection treatments. Natural selection was permitted by pooling the offspring from all females within a population and then randomly selecting 55 males and 55 females to form the next generation, making a female's expected contribution to the next generation proportional to her productivity. The opportunity for natural selection was greatly reduced by equalizing each female's contribution to the next generation. Once collected, virgin flies from each population spent 3–6 days in a mating treatment that varied the opportunity for sexual selection. The time spent in the mating treatments was always consistent among treatments within a generation but varied among generations because of logistical constraints. Sexual selection was permitted by mixing all flies from one population in a bottle and allowing them to choose mates. Sexual selection was removed by randomly assigning individual females with a single mate in a vial. After mating, males were discarded and females were transferred singly (without anesthesia) to vials for 24 h of egg laying, after which they were discarded. This produced low-density vials (average 22 offspring/vial using emergence data from Rundle et al. 2006) in which developmental time was minimized and adult emergence was highly coordinated.

After 12 generations of experimental evolution, CHCs were extracted from 20 virgin males and 20 virgin females from each population. All flies were assayed under identical experimental conditions, allowing among-population and among-treatment differences to be interpreted as genetic differences. The presence of the internal control treatment (in which the opportunities for both natural and

sexual selection were greatly reduced) allowed us to analyze CHCs on the corn medium as opposed to raising all lines for a further two generations on the ancestral yeast medium.

Treatment effects were tested using MANOVA and are summarized by the linear model

$$\mathbf{c}_{ijkl} = \boldsymbol{\mu} + \mathbf{s}_i + \mathbf{t}_j + \mathbf{s}_i\mathbf{t}_j + \mathbf{p}_{k(j)} + \mathbf{s}_i\mathbf{p}_{k(j)} + \mathbf{e}_{ijkl}, \quad (3)$$

where $\boldsymbol{\mu}$ is a vector of trait means, $\mathbf{c}_{ijkl}$ is an observation vector for the eight CHCs, $\mathbf{s}_i$ is the effect if the ith sex, $\mathbf{t}_j$ represents the four treatments (control, N, S, N + S), $\mathbf{s}_i\mathbf{t}_j$ is an interaction vector between sex and treatment effects, $\mathbf{p}_{k(j)}$ is the effect of population nested within treatment, $\mathbf{s}_i\mathbf{p}_{k(j)}$ is an interaction vector between sex and population nested within treatment, and $\mathbf{e}_{ijkl}$ is unexplained error. Note that in this partially nested split-plot design, in which sex and treatment are fixed effects and population within treatment is a random effect, the interaction between sex and treatment, $\mathbf{s}_i\mathbf{t}_j$, which is of interest when interpreting the evolution of sexual dimorphism, is tested for significance using $\mathbf{s}_i\mathbf{p}_{k(j)}$ as the F ratio denominator (Quinn and Keough 2002; Rencher 2002).

The interaction between sex and treatment tests only that specific component of the evolutionary response that is sex dependent. However, because we also wished to visualize the evolution of sexual dimorphism in the context of the total evolutionary change of the two sexes in the novel environment (sex-specific evolution as well as evolution that was consistent between the sexes), we plotted the first two discriminant functions from a canonical discriminant analysis in which the effects of sex and treatment were collapsed into a single factor containing eight groups: two sexes for each of the four treatments. We tested the responses of males and females to natural and sexual selection separately on these two vectors of evolutionary divergence, using post hoc t-tests among treatments of interest and correcting for multiple comparisons (Rice 1989). An alternative approach to visualizing the evolution of sexual dimorphism would have been to plot the two canonical variates of the $\mathbf{s}_i\mathbf{p}_{k(j)}$ term from model (3). Doing so, however, would display only the sex-specific components of CHC evolution and would therefore not permit a direct comparison of this sex-specific evolution with any CHC evolution that occurred in a consistent fashion in both males and females.

Results

Natural Variation in Sexual Dimorphism

Drosophila serrata CHCs exhibited substantial variation in sexual dimorphism among the nine natural populations collected along the Australian east cost, with significant sex × population interaction terms for all eight logcontrast CHCs (table 1). There was also variation among CHCs in the relationship between dimorphism and latitude (fig. 2). For example, Z,Z-5,9-$C_{25:2}$, Z,Z-5,9-$C_{27:2}$, and Z,Z-5,9-$C_{29:2}$ varied in a clinal fashion, with sexual dimorphism decreasing in the northern (more tropical) populations. In contrast, sexual dimorphism in the methylalkanes (2-Me-C_{26}, 2-Me-C_{28}, and 2-Me-C_{30}) appeared less correlated with latitude, and the shortest hydrocarbon, Z,Z-5,9-$C_{24:2}$,, fluctuated among populations between being sexual dimorphic and sexually monomorphic.

Genetic Correlations between the Sexes

Autosomal-only intersexual genetic correlations for the eight sexually dimorphic CHCs were generally high and positive (fig. 3), indicating that alleles segregating at autosomal loci tend to have similar phenotypic affects in the two sexes. Nevertheless, trait correlations were significantly less than 1 in all eight cases (fig. 3), suggesting at least some degree of independent genetic control at autosomal loci. When X-linked loci were included in these estimates, however, intersexual genetic correlations decreased for every trait (fig. 3), and for some, the reduction was substantial (e.g., 67% for Z,Z-5,9-$C_{27:2}$ and 71% for Z,Z-5,9-$C_{29:2}$). This reduction was significant for four of the longer-chained hydrocarbons (fig. 3; Wilcoxon signed-rank tests: Z,Z-5,9-$C_{27:2}$ $Z = -2.24$, $P = .013$; 2-Me-C_{28} $Z = -1.68$, $P = .047$; Z,Z-5,9-$C_{29:2}$ $Z = -2.38$, $P = .009$; 2-Me-C_{30} $Z = -1.69$, $P = .046$).

The additive genetic covariance between the sexes is a critical component of intersexual genetic correlations because its sign indicates the net direction of allelic effects according to sex. We estimated the additive genetic covariance between the sexes as a consequence of both autosomal and X-linked loci. Negative covariance between the sexes was far more common in X-linked covariance terms than in autosomal covariance terms. Of the 45 trait-

Table 1: F ratio statistics and significance of sex × population interaction terms for each logcontrast cuticular hydrocarbon (CHC) analyzed using the mixed linear model in equation (2)

Logcontrast CHC	Sex × population F (df = 8, 4,247)	P
Z,Z-5,9-$C_{24:2}$	2.948	.003
Z,Z-5,9-$C_{25:2}$	6.825	<.001
Z-9-$C_{25:1}$	3.849	<.001
2-Me-C_{26}	8.302	<.001
Z,Z-5,9-$C_{27:2}$	21.155	<.001
2-Me-C_{28}	8.444	<.001
Z,Z-5,9-$C_{29:2}$	23.390	<.001
2-Me-C_{30}	9.961	<.001

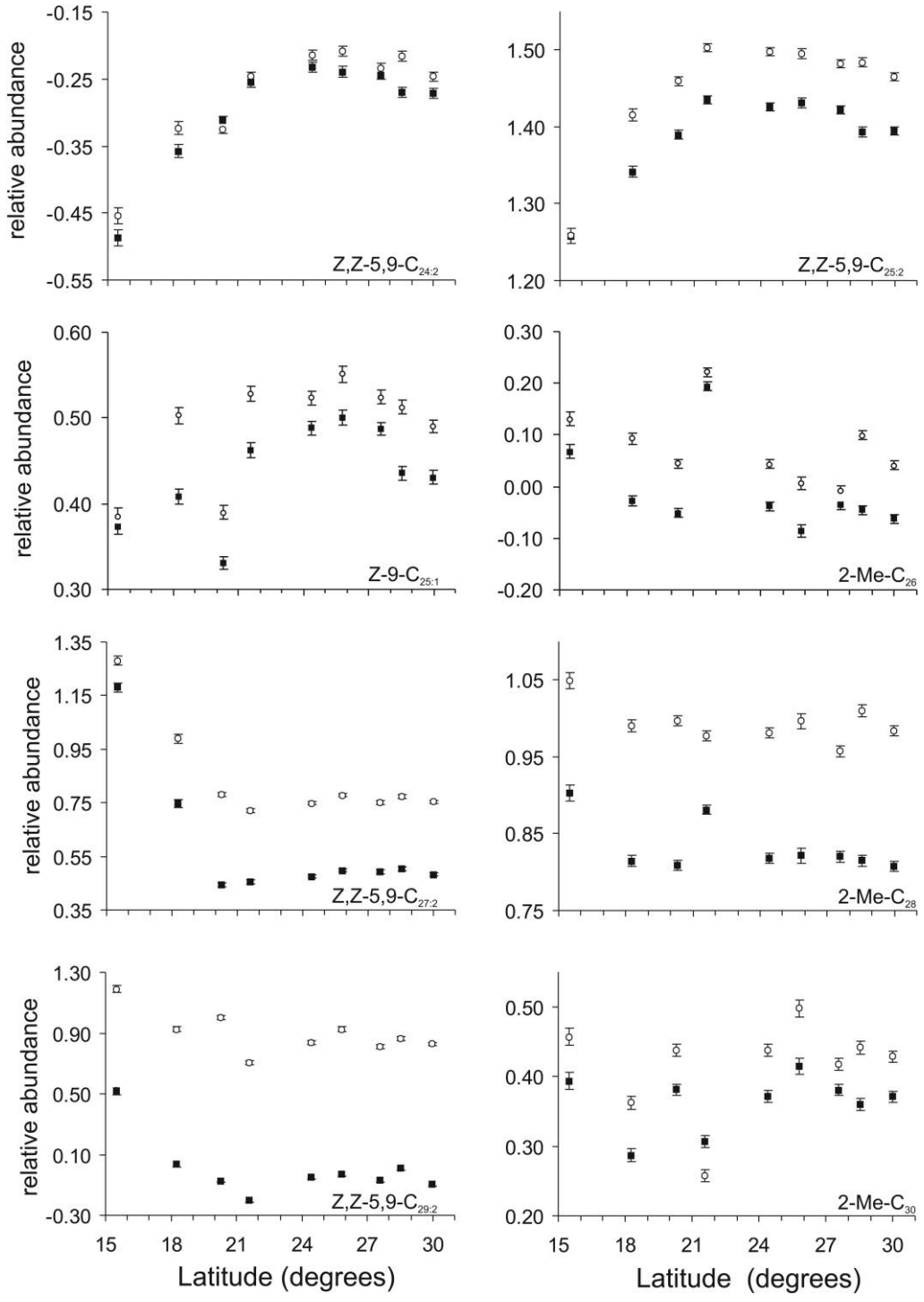

Figure 2: Geographical variation in sexual dimorphism for eight cuticular hydrocarbons (CHCs) in *Drosophila serrata*. Each panel shows mean ± 1 SE logcontrast CHC values for males (*squares*) and females (*circles*) from the nine natural populations. Populations appear from north to south; see figure 1 for locations. There were significant interactions between the effects of sex and population for all eight logcontrast CHCs in univariate ANOVAs (table 1).

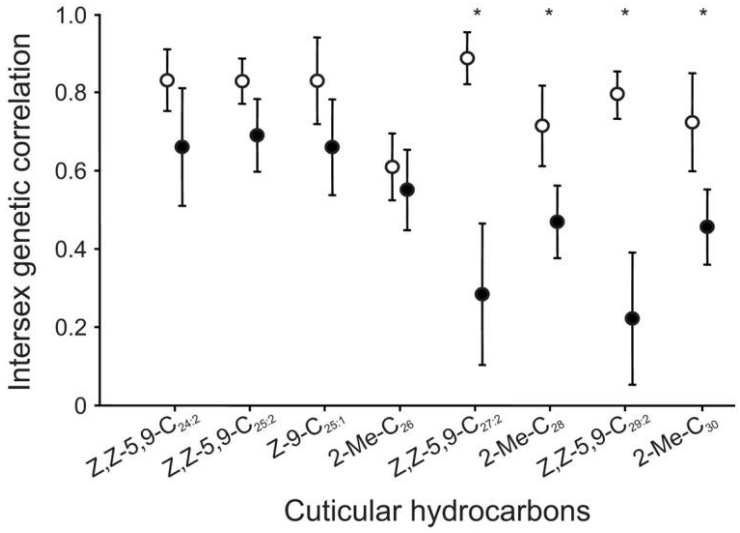

Figure 3: Autosomal-only (*open circles*) and autosomal-plus-X-linked (*filled circles*) intersexual genetic correlations for eight logcontrast cuticular hydrocarbons (CHCs) in the nine natural populations of *Drosophila serrata*. Values plotted are mean (±95% confidence intervals) genetic correlations for each CHC based on estimates from each of the nine populations. Traits marked with an asterisk are those for which genetic correlations that included both autosomal and X-linked genetic variance were significantly lower than autosomal-only correlations (Wilcoxon signed-rank test, $P < .05$).

population combinations in which the inclusion of X-linked loci reduced the intersexual genetic correlation, the decline was a consequence of negative covariance between the sexes at X-linked loci in 38 (84.4%). In contrast, covariance due to autosomal loci was positive in all cases except one, and the difference in sign between X-linked and autosomal covariance was highly significant (sign test: $P < .001$, $n = 45$), suggesting that the effect on CHCs of X-linked genetic effects was, on average, to increase sexual dimorphism.

Experimental Evolution of Sexual Dimorphism

Cuticular hydrocarbon sexual dimorphism evolved among the 12 experimental populations, with significant independent evolution of the sexes occurring in only 12 generations (MANOVA, sex × treatment interaction: Pillai's trace = 2.708, $F = 3.48$, df = 24, 9, $P = .028$). The first canonical variate from the canonical discriminant analysis (in which the effects of sex and treatment were collapsed into a single factor, thereby encompassing the total evolutionary response of the two sexes) represented 95% of the total phenotypic response to selection among the eight sex and treatment combinations, and the second canonical variate described a further 4% of this response. A single canonical variate dominating such a large proportion of the evolutionary response suggests that the group mean vectors in this analysis are predominantly collinear—that is, changes associated with adaptation to the novel corn-based environment occurred predominantly in a single linear combination of CHCs. The loadings for the first canonical variate were dominated by a strong contrast between two methylalkanes, 2-Me-C_{28} and 2-Me-C_{30} (table 2).

We tested for the sex-specific responses to natural and sexual selection on these two vectors of evolutionary divergence. In each case, the contribution of natural and sexual selection to the evolution of CHC sexual dimor-

Table 2: Standardized coefficients of the first and second discriminant functions from the canonical discriminant analysis describing differences in logcontrast cuticular hydrocarbons (CHCs) between treatments and sexes

	Standardized coefficients	
CHC	First canonical variate (95%)	Second canonical variate (4%)
Z,Z-5,9-$C_{24:2}$	.323	−.711
Z,Z-5,9-$C_{25:2}$	−.917	−.342
Z-9-$C_{25:1}$	.433	1.499
2-Me-C_{26}	2.494	6.019
Z,Z-5,9-$C_{27:2}$	−1.342	−2.460
2-Me-C_{28}	−6.951	−10.587
Z,Z-5,9-$C_{29:2}$	1.713	.460
2-Me-C_{30}	5.634	7.921

Note: Values in parentheses are the percent variance accounted for by each discriminate function.

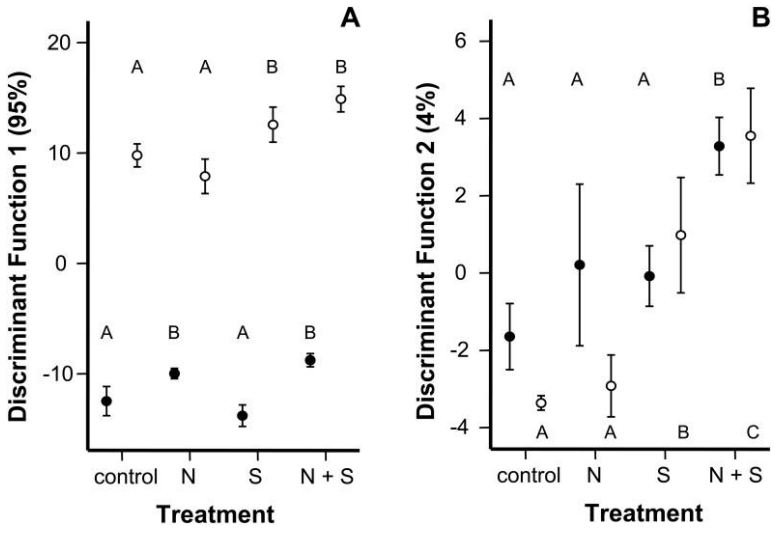

Figure 4: Cuticular hydrocarbon (CHC) evolution in response to independent manipulation of the opportunities for both natural and sexual selection in 12 replicate laboratory populations evolving for 12 generations in a novel corn-based larval medium. Vertical axes are the first (*A*) and second (*B*) discriminant functions from a canonical discriminant analysis that discriminated male and female CHCs according to selection treatment. Combined, these two discriminant functions represent 99% of the total response to selection in the eight CHCs. Open circles (females) and filled circles (males) indicate mean values ± 2 SE ($n = 3$) for each combination of treatment and sex. Horizontal axes indicate specific treatment (control, $N =$ natural selection permitted, $S =$ sexual selection permitted, $N + S =$ both natural and sexual selection permitted). Within each sex, treatments with different letters were significantly different ($P < .05$) in post hoc pairwise comparisons after sequential Bonferroni correction for multiple tests (Rice 1989).

phism differed dramatically between the sexes (fig. 4). For the first canonical variate, representing 95% of the selection response, the overall results suggest that a single, but different, evolutionary process dominated CHC evolution in each sex. In males, there was no significant response of CHCs to sexual selection alone (S), whereas the response was significant in the two treatments that permitted natural selection (N and N + S; fig. 4*A*). This response in males favored a combination of CHCs similar to that present in females, causing a reduction in sexual dimorphism. In contrast to that in males, there was no significant response of CHCs in females to the presence of natural selection alone (N; fig. 4*A*). However, CHCs did evolve significantly in the two treatments in which sexual selection was permitted (S and N + S). The resulting evolution was away from male mean values, thereby increasing sexual dimorphism. In neither sex was there any indication of the presence of an interaction between natural and sexual selection in CHC evolution. The overall pattern of evolution was that sexual selection tended to increase CHC sexual dimorphism, whereas natural selection tended to decrease it.

Patterns were somewhat different for the second canonical variate, although this axis describes only 4% of the total selection response. Sexual dimorphism decreased in treatments that included sexual selection. The decrease was largely a function of female CHCs evolving to be closer to those of males. Male CHCs differed significantly from controls only in the N + S treatment, whereas female CHCs responded when sexual selection was present either by itself (S) or with natural selection (N + S; fig. 4*B*). In females, natural selection alone did not cause any significant CHC evolution.

Experimental manipulations of natural and/or sexual selection necessarily confound variation in effective population size (N_e) with the selection treatments. This is because for evolution by selection to occur, variance must exist in the contribution of an individual to subsequent generations (Santiago and Caballero 1995). Therefore, a pertinent question in such experiments is whether there is any evidence that variance among treatments in N_e could explain the patterns of evolution (Rice and Holland 2005). This does not appear to be the case in our situation. The N_e hypothesis predicts that the response to selection should be positively correlated with N_e and that within treatments, among-population variance should increase as N_e decreases. Neither pattern is present in our data: the response to selection was greatest in both males and females in the populations in which N_e should be lowest (i.e., populations experiencing both natural and sexual selection), and among-population variance in CHCs did not increase with decreasing N_e.

Discussion

Genetic Constraints between the Sexes

The level of sexual dimorphism expressed in homologous traits ultimately reflects a historical interaction between sexually antagonistic selection and genetic constraints imposed by a shared genome. We have shown that *Drosophila serrata* exhibits substantial among-population variation in display trait sexual dimorphism throughout much of its natural range. Geographical variation in sexually dimorphic CHCs has also been reported for one other species of *Drosophila*, *D. mojavensis*, in which eight of 21 CHCs exhibited geographical variation in sexual dimorphism (Etges and Ahrens 2001). Such natural variation in sexual dimorphism suggests that genetic constraints on the evolution of CHC sexual dimorphism may be relatively weak in these species.

We used nine half-sib quantitative genetic breeding experiments to assess the strength of genetic constraints between the sexes in *D. serrata*, estimating intersexual genetic correlations for each natural population. Our replicated estimates of intersexual genetic correlations were significantly less than 1, suggesting the presence of segregating genetic variation for independent evolution of the sexes. This result is consistent with theoretical expectations for sexually dimorphic traits (Robertson 1959; Lande 1980, 1987).

Although intersexual genetic correlation estimates do vary between species (Roff 1997), reports demonstrating low genetic correlations for sexually dimorphic traits are becoming increasingly common and now span a wide range of taxa, including plants (Ashman 2003; McDaniel 2005), birds (Møller 1993), and insects (Simmons and Ward 1991; Bonduriansky and Rowe 2005). Studies using alternative methodologies to quantitative genetics have also provided support for a breakdown of genetic constraints between the sexes. For example, sex-specific quantitative trait locus (QTL) effects have been detected in *Drosophila melanogaster* for sexually dimorphic quantitative traits such as life span (Nuzhdin et al. 1997; Wilson et al. 2006), abdominal pigmentation (Kopp et al. 2003), sensory-bristle number (Dilda and Mackay 2002), and CHCs (Foley et al. 2007). Sexual dimorphism has also been shown to respond to artificial sexual selection (Wilkinson 1993; Reeve and Fairbairn 1996), and sex × genotype interactions have been reported in analyses of genome-wide transcription in *D. melanogaster* (Jin et al. 2001).

Although the above studies suggest that the resolution of intralocus sexual conflict over shared phenotypic traits may be relatively common in nature, they must be reconciled with the fact that there is strong evidence for intralocus sexual conflict for adult fitness in a laboratory population of *D. melanogaster*. In this case, the intersexual genetic correlation for adult fitness is negative (Chippindale et al. 2001), and it has been shown using experimental evolution that when selection is removed from females, evolved fitness increases in males correspond to equal fitness decreases in females (Prasad et al. 2007). Thus, while resolution of conflict over specific shared phenotypic traits may be relatively common, at any one time there may be substantial sexually antagonistic genetic variance for fitness at a genome-wide scale. It remains to be seen whether the result reported for this long-adapted laboratory population of *D. melanogaster* is a common phenomenon in other taxa and in more variable environments.

How the breakdown of genetic constraints over shared phenotypic traits is achieved remains unclear. Our quantitative genetic analysis of X-chromosomal contribution to intersexual genetic correlations in *D. serrata* showed a clear pattern in this regard. For all eight traits, intersexual genetic correlation estimates that included X-linked genetic variance and X-linked intersexual covariance were lower than estimates due to autosomes only, with the reduction being significant in four cases. Our analysis is likely to be conservative because estimates of autosomal genetic correlations are biased downward in the presence X-linked genetic variance (Cowley and Atchley 1988; see also app. B). This replicated result, employing nine natural populations, confirms previous estimates derived from a single long-term laboratory population of *D. serrata* (Chenoweth and Blows 2003). A reduction of intersexual correlations due to X-linked effects was also observed in nine of 15 morphological traits (head, thorax, and leg traits) in a study in *D. melanogaster* (Cowley and Atchley 1988). In contrast, despite large amounts of X-linked genetic variance, intersexual genetic correlations for wing traits in *D. melanogaster* remained high in nine of 13 cases when analyzed using the methods presented here (Cowley et al. 1986). In *D. serrata*, of the four traits for which intersexual genetic correlations decreased significantly as a consequence of X linkage, three (the exception being Z,Z-5,9-C_{27}) have been shown to experience strong sexual selection under laboratory conditions (Hine et al. 2002; Chenoweth and Blows 2003).

When intersexual genetic correlations are measured using the method of Cowley and Atchley (1988), there are two means by which they can be reduced as a consequence of X-linkage: (1) traits may exhibit substantial X-linked genetic variance in both sexes but little X-linked covariance, or (2) the X-linked additive covariance between the sexes for the trait can be negative. The signs of additive intersexual genetic covariance components indicate the net direction of X-linked allelic effects according to sex. In *D. serrata*, when genetic correlations were reduced as a consequence of segregating X-linked factors, negative covariance between the sexes was far more common in X-linked

covariance terms than in autosomal covariance terms. Although X-linked effects have been reported for sexually dimorphic CHCs in other species of *Drosophila* (Foley et al. 2007; Liimatainen and Jallon 2007), in no case has segregating X-linked genetic variance been shown to lower intersexual genetic correlations in natural populations, as we observed here. Lower genetic correlations due to X linkage are consistent with theory (Rice 1984), comparative studies of reciprocal crosses (Reinhold 1998), and X-chromosome effects contributing to the evolution of increased sexual dimorphism during artificial selection (Wolfenbarger and Wilkinson 2001; Wilkinson et al. 2005). However, there is also evidence against disproportionate sex linkage of sexually selected traits (Fitzpatrick 2004), suggesting that more data are required.

Two observations suggest that the X chromosome may be at least unusual with respect to sex differences. First, X-linked effects contribute to a negative intersexual genetic correlation for fitness and a disproportionately large amount (97%) of the genome-wide sexually antagonistic fitness variation in *D. melanogaster* (Gibson et al. 2002). Our results for *D. serrata* appear to be inconsistent with this finding because negative intersexual genetic covariance at X-linked loci permits the evolution of CHC sexual dimorphism. Because CHCs are under sexually antagonistic selection (Chenoweth and Blows 2005), this should help resolve intralocus sexual conflict and thus contribute positively to the fitness of both sexes.

Second, studies of the genomic distribution of sexually dimorphic gene expression in both *Drosophila* (Parisi et al. 2003; Ranz et al. 2003) and worms (Reinke et al. 2000) report a general paucity of male-biased genes on the X chromosome. At face value, a "demasculinized" X chromosome appears contradictory to the idea that male sexually selected traits should be X-linked. Critically, however, the comparison of genome-wide patterns and specific phenotypic traits that are known to experience sexually antagonistic selection requires caution because the existence of sexually antagonistic selection on specific transcripts remains untested. Moreover, these studies do not identify the genomic location of polymorphisms regulating the expression of sex-biased genes, and therefore the possibility that X-linked loci regulate the expression of autosomal genes controlling sexually dimorphic traits (e.g., Chase et al. 2005) cannot be ruled out. Future work aimed at both linking transcription with sex-specific fitness and mapping QTLs for sex-biased gene expression will be helpful in this regard.

Sex-Specific Responses to Natural and Sexual Selection

While it is difficult to study the combined influences of sex-specific natural and sexual selection on display traits under field conditions in natural populations of flies, laboratory experimental evolution is a useful tool for investigating the combined influence of these two processes within a manipulative rather than a correlational inference framework. Our evolutionary manipulation allowed us to examine the contribution of natural and sexual selection to an observed change in sexual dimorphism and to also examine the sex-specific effects of these two processes. After 12 generations of experimental evolution, CHC sexual dimorphism evolved, with sex-specific evolution occurring on two linear combinations of CHCs. For both traits, when neither form of selection was constrained, changes in sexual dimorphism involved significant CHC evolution in both sexes. Rapid sex-specific CHC evolution suggests that the novel environment created new sex-specific phenotypic optima for CHCs in both sexes.

A qualitative difference between the sexes in the operation of sexual selection (present in males but absent from females) is regarded as the primary source of sex-specific selection that favors the evolution of sexually dimorphic display traits (Lande 1980). In our experiment, however, both natural and sexual selection affected the evolution of CHC sexual dimorphism in *D. serrata*. For 95% of the selection response (canonical variate 1; fig. 4*A*), sexual selection tended to increase sexual dimorphism, and natural selection tended to reduce it. In this regard, our results are consistent with the classic hypothesis that sexual selection favors an increase in sexual dimorphism, whereas natural selection acts similarly in each sex, therefore tending to decrease dimorphism (Darwin 1871; Lande 1980).

Our results differ from the classic model, however, in the sex targeted by sexual selection: a response to our manipulation of sexual selection was detected only in females. A response to sexual selection in females is consistent with a previous observation of sexual selection on CHCs as a consequence of male mate choice in a laboratory population of *D. serrata* (Chenoweth and Blows 2005). The lack of a response in males is inconsistent with previous observations of sexual selection on CHCs by female mate choice in laboratory populations. However, it has been demonstrated that genetic variance is very low for male CHCs in the direction of sexual selection in both laboratory and field populations of *D. serrata* (Blows et al. 2004; Hine et al. 2004). Therefore, the response of male CHCs to sexual selection might be expected to be small. Nevertheless, we do not know whether genetic variances for male sexually selected CHCs were equally low in the ancestral population considered here or whether the male sexual selection optima differed between our ancestral and novel environments.

Conclusions

We have demonstrated that genetic constraints on the evolution of sexual dimorphism in a suite of sexual display traits have been overcome in *D. serrata* to the extent that sexual dimorphism varies among natural populations and can evolve in a laboratory population under experimental conditions that alter sex-specific phenotypic optima. Quantitative genetic analyses suggest that segregating variation due to the X chromosome has contrasting phenotypic effects on CHCs in the two sexes, lowering intersexual genetic correlations overall. However, more-detailed QTL studies will be required to test this hypothesis more directly. Experimental evidence is consistent with the longstanding idea that sexual selection tends to increase display trait sexual dimorphism while natural selection may reduce it, although how these processes operate in each sex may be more complex than previously considered. Future studies will need to focus on how different genetic mechanisms can lower intersexual genetic correlations and how natural and sexual selection interact to generate sexually antagonistic selection on shared traits under natural conditions.

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