Report

Current Biology

Genomic Evidence that Sexual Selection Impedes Adaptation to a Novel Environment

Highlights

- Some alleles respond to either natural or sexual selection in the same direction
- Evolutionary trajectories can reverse when both natural and sexual selection act
- This may be due to sexual conflict from male-induced female harm during courtship
- Details of the mating system can cause sexual selection to hamper adaptation

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In Brief

Sexual selection's contribution to adaptation is unclear. Chenoweth et al. reveal a class of allele beneficial to both sexual and nonsexual fitness in isolation. However, when both natural and sexual selection occur, these same alleles appear costly. Mating assays suggest that this arises from preferential male harm of intrinsically high-fitness females.

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Report

Genomic Evidence that Sexual Selection Impedes Adaptation to a Novel Environment

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SUMMARY

Sexual selection is widely appreciated for generating remarkable phenotypic diversity [1, 2], but its contribution to adaptation and the purging of deleterious mutations is unresolved [3]. To provide insight into the impact of sexual selection on naturally segregating polymorphisms across the genome, we previously evolved 12 populations of *Drosophila serrata* in a novel environment employing a factorial manipulation of the opportunities for natural and sexual selection [4]. Here, we genotype more than 1,400 SNPs in the evolved populations and reveal that sexual selection affected many of the same genomic regions as natural selection, aligning with it as often as opposing it. Intriguingly, more than half of the 80 SNPs showing treatment effects revealed an interaction between natural and sexual selection. For these SNPs, while sexual selection alone often caused a change in allele frequency in the same direction as natural selection alone, when natural and sexual selection occurred together, changes in allele frequency were greatly reduced or even reversed. This suggests an antagonism between natural and sexual selection arising from male-induced harm to females [5]. Behavioral experiments showed that males preferentially courted and mated with high-fitness females, and that the harm associated with this increased male attention eliminated the female fitness advantage. During our experiment, females carrying otherwise adaptive alleles may therefore have disproportionally suffered male-induced harm due to their increased sexual attractiveness. These results suggest that a class of otherwise adaptive mutations may not contribute to adaptation when mating systems involve sexual conflict and male mate preferences.

RESULTS AND DISCUSSION

Darwinian selection can arise both from variation in nonsexual fitness (i.e., viability and fecundity) and from variation in sexual fitness (i.e., reproductive success), with the latter being known as sexual selection. Although selection arising from variation in nonsexual fitness (hereafter "natural selection" for simplicity) will often improve the fit of organisms to their environments, the effects of sexual selection on adaptation are less clear. If healthier and more vigorous individuals tend to have higher reproductive success, then alleles increasing nonsexual fitness will also tend to increase sexual fitness, resulting in the alignment of natural and sexual selection [1, 3, 6]. Much of the genome likely contributes to an individual's health [6, 7], suggesting genome-wide sexual selection favoring alleles of high nonsexual fitness [3]. Alternatively, sexual selection may reduce nonsexual fitness through the evolution of costly secondary sexual traits and mate preferences [5, 8–11] and, because it is sex specific, can also generate sexual conflict [12–14].

Results of experimental studies of the alignment of natural and sexual selection are mixed. Several experiments have manipulated the opportunity for sexual selection and then measured the consequences for population mean fitness or components thereof, beginning either from standing genetic variance [4, 15–24] or from novel mutational variance [25–27]. This approach integrates genetic effects across much of the genome, meaning the net effect on fitness may be dominated by sets of segregating alleles with opposing effects on sexual versus nonsexual fitness, obscuring underlying patterns at individual loci. It is therefore unclear how to interpret the results of many of these studies that suggest a net cost of sexual selection. An alternative approach assays the effects of individual deleterious mutations on male sexual fitness [3, 28–33]. Although the effects of individual alleles are known in this case, inference is restricted to one or a few loci and tends to concern alleles with fitness effects that are likely unrepresentative of novel deleterious mutations or those fixed during adaptation. In addition, in both of the above approaches, measuring total fitness in the environment in which the populations have evolved is an empirical challenge.

Another approach for testing alignment is to track the frequency of individual alleles across generations when the opportunity for sexual selection is manipulated, allowing fitness components to be integrated by the evolutionary process itself. This has been applied for a handful of deleterious alleles [34, 35], revealing effects that vary among mutations. Given such variability, obtaining a general picture of the genome-wide consequences of sexual selection for adaptation via a single-mutation approach will be difficult. Improved inference may be achieved by genotyping multiple (as opposed to single) variants and focusing on naturally occurring genetic variation as opposed to mutations with conspicuous phenotypic effects that may affect sexual fitness directly. Doing this within the context of contrasting

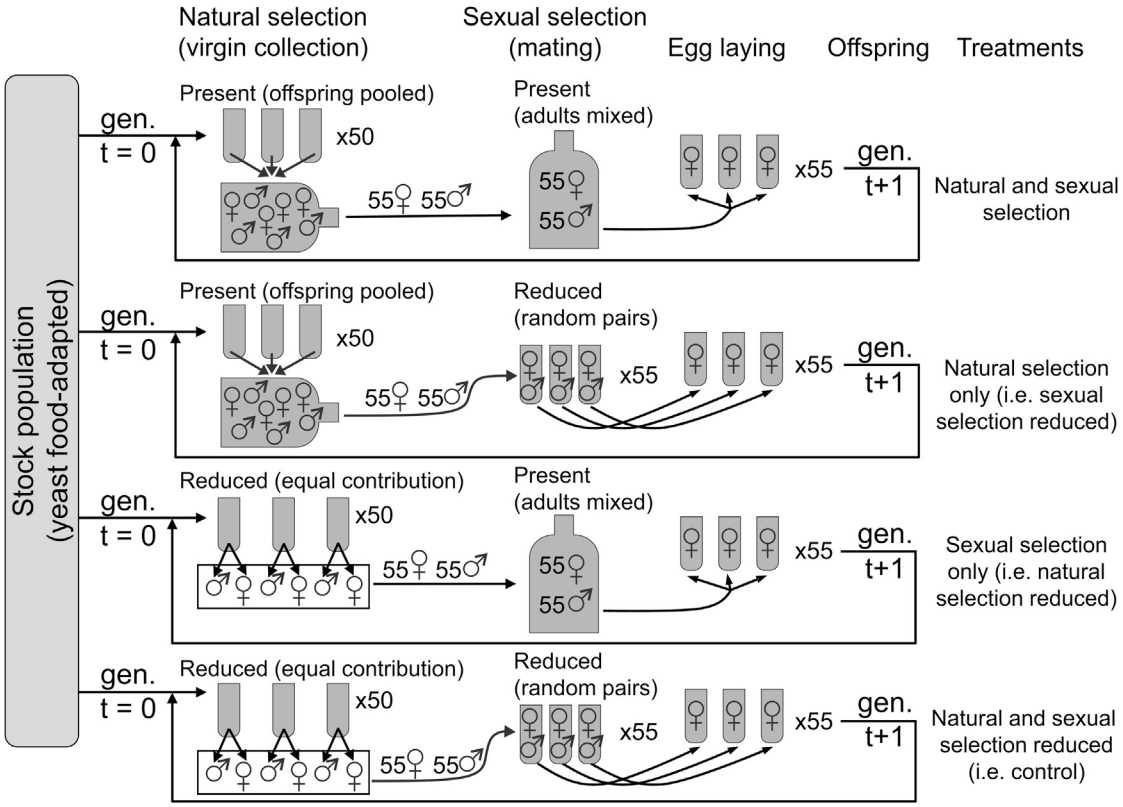

Figure 1. Design of the Evolution Experiment

Twelve populations were derived from a common ancestor and were propagated on a novel larval corn food for 13 generations under one of four treatment combinations that independently manipulated the opportunities for natural and sexual selection (one of three populations is depicted for each treatment combination). Natural selection was manipulated during virgin collection every generation either by selecting adults from among the pooled offspring of all vials (natural selection present) or by collecting a single male-female pair from every vial (natural selection reduced). Sexual selection was manipulated during the subsequent mating phase either by pooling the collected offspring within a single bottle for mating for several days (sexual selection present) or by randomly assigning them as single male-female pairs to separate vials (sexual selection reduced). After the mating period, females were placed individually in vials for egg laying and then discarded. Fifty vials with offspring were randomly selected for virgin collection in the next generation.

evolutionary manipulations of natural and sexual selection may also help identify loci that would otherwise go undetected because the varied pleiotropic effects on sexual and nonsexual fitness of different alleles may counter one another and are therefore obscured in measurements of total fitness. Although genomic approaches have been recently used to study the consequences of selection in (semi)natural settings [36, 37], no studies have yet been able to partition the independent and combined contributions of natural and sexual selection during adaptation.

We took advantage of an evolution experiment in *Drosophila serrata* to characterize the link between natural and sexual selection across the genome during the early stages of adaptation to a new environment. Using a two-way factorial design involving 12 replicate populations that were all raised on a novel larval food, the opportunities for natural and sexual selection were independently manipulated to create three populations that experienced both natural and sexual selection, three in which only natural selection was reduced, three in which only sexual selection was reduced, and three in which both natural and sexual selection were reduced (Figure 1). The phenotypic responses to these manipulations have been well characterized [38, 39], including a 52% increase in population mean fitness after 16 generations when natural selection was unimpeded, no detectable effect of sexual selection on adaptation, and evidence consistent with ongoing sexual conflict in the form of male-induced harm [4]. To provide a genome-wide portrait of the evolutionary response to these treatments after 13 generations of evolution, we used DNA pool-based restriction site-associated DNA (RAD) sequencing [40], treating populations as replicates.

RAD-seq identified 1,460 bi-allelic SNPs across the 12 experimental populations. A multivariate analysis of genome-wide differentiation using all 1,460 SNPs revealed a main effect of natural ($F_{1,8}$ = 1.1198, p = 0.045) but not sexual ($F_{1,8}$ = 1.04, p = 0.242) selection, and a marginally non-significant interaction ($F_{1,8}$ = 1.071, p = 0.146), consistent with the phenotypic pattern observed for changes in mean fitness in these populations [4]. Although these analyses demonstrate an effect of natural selection over genetic drift in shaping differentiation among our experimental populations, such a summary measure of many loci could obscure opposing effects between natural and sexual selection at individual loci that could effectively cancel each other. Therefore, to examine more closely the treatment effects on individual regions of the genome, we

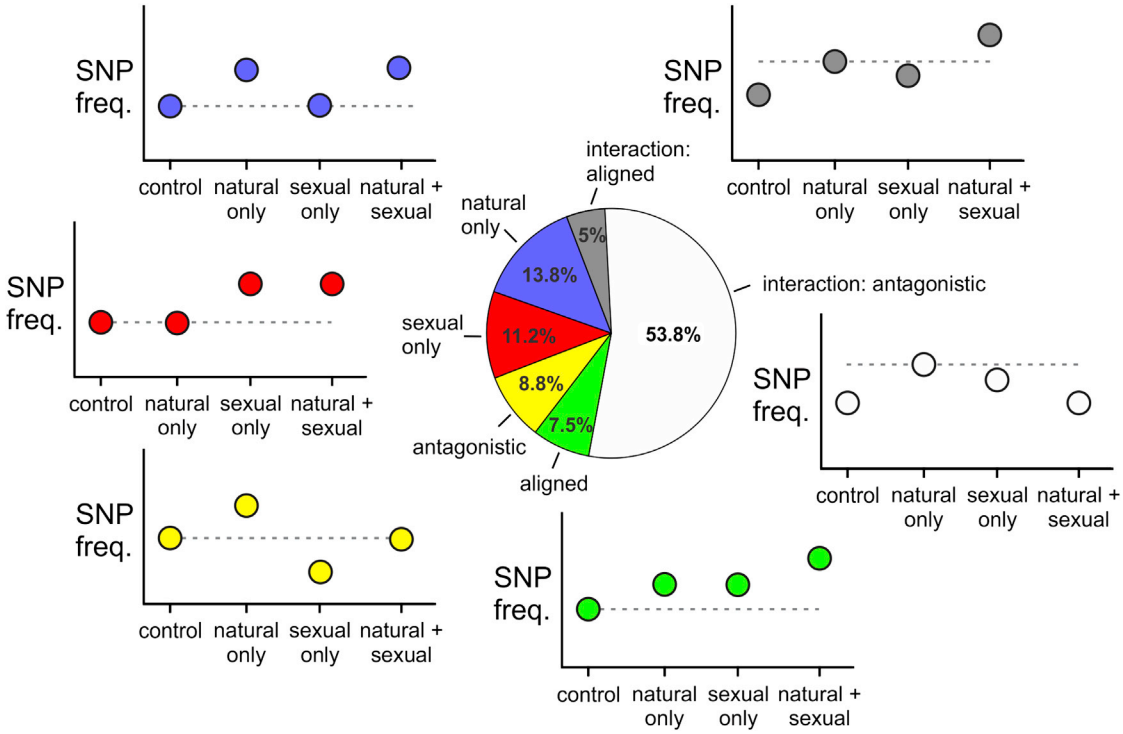

Figure 2. Classifying SNP-Level Responses to the Independent Manipulation of Natural and Sexual Selection

Plots depict population mean SNP frequency within each treatment for the different classifications and are illustrative only. Eighty SNPs showed evidence of selection-induced allele frequency change (overall model $q \leq 0.1$), treating populations as replicates. For 33 of these (colored wedges), the effects of natural and sexual selection were independent and were subdivided into those that responded to natural selection only (11 SNPs, blue), sexual selection only (9 SNPs, red), or both natural and sexual selection either antagonistically (7 SNPs, yellow) or concordantly ("aligned"; 6 SNPs, green), as shown by the corresponding plots. The remaining 47 SNPs (gray and white wedges) showed a significant interaction between natural and sexual selection. These were subdivided by comparing the response to natural selection alone with the response to both natural and sexual selection (i.e., the effect of adding sexual to natural selection). Relative to natural selection alone, the additional presence of sexual selection caused a further change in frequency in the same direction for only 4 SNPs (gray), whereas it caused a change in the opposite direction, and was therefore antagonistic, for the remaining 43 (white). For all classes not involved in an interaction, dashed horizontal lines refer to the average allele frequency in the control lines. For SNPs with a significant interaction, the dashed lines are set at the "natural selection only" treatment to highlight the effect of adding sexual to natural selection.

dissected the selection response, analyzing changes in frequency of each individual SNP.

Individual SNP Analyses Suggest an Antagonism between Natural and Sexual Selection

We fit a generalized linear mixed model to each SNP to test the effects of natural selection, sexual selection, and their interaction on allele frequency change. Populations were treated as replicates to account for the effects of genetic drift and other sources of among-population variance (see Supplemental Experimental Procedures). 80 SNPs had overall model significance (false discovery rate [FDR]-corrected $q \leq 0.1$), providing evidence of treatment-level allele frequency changes in the genomic regions tagged by these SNPs that exceeds that expected by chance alone (i.e., changes in allele frequency that were sufficiently parallel among populations within one or more treatments to infer selection; Table S2). We classified these SNPs according to the presence or absence of natural and sexual selection, as well as any interaction between them, focusing on whether their effects were aligned with or opposed to one another (Figure 2). Of the 80 SNPs with overall model significance, the majority (47 SNPs) showed a significant interaction ($p \leq 0.05$), indicating that the effect of sexual selection on allele frequency change depended on the presence or absence of natural selection. Of the 33 SNPs that did not show an interaction ($p > 0.05$), 11 were affected by natural selection alone, 9 were affected by sexual selection alone, and 13 responded in both treatments, indicating either pleiotropy or linkage with respect to sexual and nonsexual fitness. Among this latter class, there was a more or less equal number of SNPs for which the effects of natural and sexual selection relative to the control were either antagonistic (7 SNPs) or aligned (6 SNPs) with one another.

Of the 47 SNPs with a significant interaction, only four showed a pattern in which the addition of sexual selection caused a further change in their frequency in the same direction as that under natural selection alone ("interaction: aligned"; Figure 2). For the remaining 43 SNPs, the addition of sexual selection opposed the change in frequency that occurred under natural selection alone ("interaction: antagonistic"; Figure 2). For 21 of these, this was manifested as a reduction of the response compared to that observed under natural selection alone, while for the other 22 SNPs the addition of sexual selection actually reversed the direction of allele frequency change, causing a SNP that increased under natural selection alone to decrease

in frequency in the joint presence of both. Therefore, across all SNPs, the most common class of response, involving 50 of the 80 (62.5%) cases, was one in which sexual selection impeded an increase in the frequency of a SNP that increased under natural selection alone. This includes the seven classically antagonistic SNPs (i.e., opposite effects on sexual versus nonsexual fitness with no interaction), as well as the 43 SNPs in which the effect of sexual selection varied (i.e., a natural × sexual selection interaction) but in the presence of natural selection was antagonistic. Within this group, a comparison of allele frequencies between the natural selection alone and natural plus sexual selection treatments indicated that this hampering effect of sexual selection was statistically significant in more than 70% of the cases (36 of 50 SNPs, $p < 0.05$). The latter group of 43 SNPs is of particular interest because in the majority of cases (35 SNPs) the SNP also increased in frequency under sexual selection alone, despite sexual selection being antagonistic to natural selection when present alongside it. The above patterns remain if higher (5%) or lower (20%) FDR cut-offs are used instead (Table S1).

There are several mechanisms that could underlie such an apparently widespread antagonism of natural and sexual selection. One involves treatment-specific changes in effective population size and their potential impact on genetic drift and selection. In particular, the increased variance in reproductive success that sexual selection generates will decrease the effective population size (N_e), thereby increasing drift and potentially reducing the response to natural selection. Such a process could, in theory, explain the 21 SNPs for which the addition of sexual selection appeared to hamper natural selection-induced allele frequency change. To investigate this, in each of the 12 experimental populations we estimated nucleotide diversity (θ_Π) as a proxy for N_e (see Supplemental Experimental Procedures). Fitting a factorial linear model to these population-level estimates revealed a main effect of natural selection reducing N_e, as expected ($F_{1,8} = 8.34$, $p = 0.020$; Figure S1), but no effect of sexual selection or evidence of an interaction (sexual selection: $F_{1,8} = 0.26$, $p = 0.624$; natural × sexual: $F_{1,8} = 0.873$, $p = 0.377$; Figure S1). The lack of any detectable difference between the natural selection alone and natural plus sexual selection treatments suggests that a sexual selection-induced reduction in N_e is unlikely to have hampered the response to natural selection, consistent with previous results when examining the evolution of phenotypic traits in these populations [4, 38, 39]. Finally, although a sexual selection-induced reduction in N_e could hamper the response to natural selection, increased genetic drift alone should not produce consistent patterns in the direction of allele frequency change across replicate populations within a treatment, such as the 22 SNPs for which the addition of sexual selection reversed the direction of allele frequency change compared to that observed under natural selection alone.

An alternative to differences in N_e involves linkage disequilibrium (LD) among SNPs, which could have caused them to respond in a correlated way to our treatments and by chance resulted in an overestimation of the relative contribution of the antagonistic class of response. In "evolve-and-resequence" studies, LD can extend over much larger distances—several megabases in *Drosophila*—than is typical in natural populations of the study species [41]. While this can make it difficult to distinguish true targets of selection from linked sites, this was not our goal. Although pooled DNA genotyping approaches do not permit direct estimation of LD, if short- to medium-range LD were an issue, we would expect to see a preponderance of SNPs adjacent to each other on genome scaffolds evolving in similar ways. For genome scaffolds where we detected more than one significant SNP (18 scaffolds: range = 2–7 hits, mean = 3 hits per scaffold), we counted the number of times adjacent SNPs were assigned to matching or different evolutionary classes. Across all scaffolds, adjacent SNPs were assigned to different evolutionary classes twice as frequently as to matching classes (24 versus 12 instances). This pattern is in the opposite direction to that predicted under short- to medium-range LD. We note that our longest multiple-hit scaffolds are 6 Mbp long and that distances between significant SNPs on the same scaffolds were as high as 2.4 Mbp. Although it is more difficult to ascertain the extent to which long-range LD could be involved, our use of replicate populations within treatments will help mitigate this because the particular associations that arise are, via sampling, unlikely to be consistent across replicates [42].

If neither changes in N_e nor linkage were responsible, why might sexual selection have been antagonistic to natural selection in its presence yet often aligned with it on its own? Sexual conflict provides an alternative explanation. Sexual conflict occurs when the divergent reproductive interests of males and females generate sex-specific selection on shared traits. If the genetic basis of the trait differs between the sexes, a process of sexually antagonistic coevolution can occur in which traits favored in one sex can be costly to the other [43, 44]. Sexual conflict is prevalent in nature, and there are many examples of traits that increase a male's reproductive success at the expense of female fitness [45]. In our populations, if better adapted males had higher reproductive success (i.e., reproductive success was condition dependent; [3]), then SNPs increasing nonsexual fitness may have increased in frequency under sexual selection alone. However, if males also harmed females, the evolutionary consequences of this harm could only have been manifested when variation in female fitness was allowed (i.e., when natural and sexual selection were both permitted). These combined effects could cause the evolutionary response to sexual selection on its own to align with natural selection but to become antagonistic in the presence of natural selection. A key question is whether this could substantially alter the strength or even direction of total selection on an allele.

Males Prefer and Differentially Harm High-Fitness Females during Courtship and Mating

As with other *Drosophila* [46, 47], the opportunity for sexual selection in our experimental populations was associated with a direct reduction in female fitness [4], indicating that male courtship and/or mating was harmful. If combined with male preferences for high-fecundity mates, this sexual conflict could have hampered adaptation by directing male harm disproportionately toward females of otherwise high intrinsic fitness. This behavioral process could reduce the variance in realized fitness and the efficacy of natural selection [5]. To test this, we raised stock individuals at

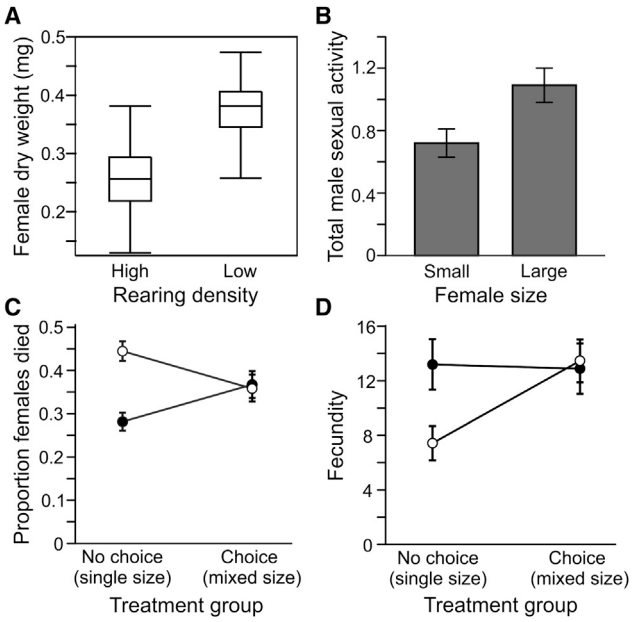

Figure 3. Quantifying Male Mate Preferences and Their Consequence for Female Fitness

(A) Stock individuals were raised at low and high density to generate females that differed in mean body size (paired t test: $t = 15.2$, $df = 95$, $p < 0.0001$).

(B) When individual stock males were placed together in a vial with one large and one small female (i.e., low- and high-density-reared, respectively), they directed their sexual activity (courtship and mating) disproportionately toward the larger female (paired t test, $df = 99$, $t = 2.57$, $p = 0.012$).

(C) Groups of 16 stock males were held for 66 hr together with 16 large, 16 small, or 8 large and 8 small females. Males were quite harmful during this interaction period, with 436 of the 1,440 females (30.3%) dying, compared to only 41 of the 1,140 males (2.8%). In the absence of variation in female size (i.e., 16 large or 16 small females; "no choice"), the mortality of large females (●) was significantly lower than that of small females (○; $\chi^2 = 27.4$, $df = 1$, $p < 0.001$). However, when females of both sizes were present and hence male choice was possible ("choice"), the mortality of large and small females no longer differed ($\chi^2 = 0.04$, $df = 1$, $p = 0.849$).

(D) When surviving females were subsequently removed and allowed to lay eggs individually for 24 hr, in the absence of an opportunity for male choice the fecundity of large females (●) was significantly higher (78% greater) than that of small females (○; Wilcoxon rank-sum test: $z = -2.25$, $n_{large} = n_{small} = 120$, $p = 0.024$). In contrast, fecundity did not differ between large and small females sampled from the mixed groups in which male choice was possible (Wilcoxon rank-sum test: $z = 0.58$, $n_{large} = 118$, $n_{small} = 114$, $p = 0.561$).

In (B)–(D), treatment means are shown ± 1 SE.

low and high density to generate females that differed in mean body size and, all else being equal, fecundity (Figure 3A). These females were then used in separate assays quantifying male mate choice and its consequences for female fitness.

When individual stock males were held with one large and one small female, they directed their sexual activity disproportionately toward the larger of the two females (Figure 3B), demonstrating a preference for high-fecundity mates that appears common in insects [48]. In a second assay involving mixed-sex groups, large females had lower mortality and higher subsequent fecundity than small females in the absence of an opportunity for male choice, demonstrating their greater intrinsic quality. However, given the opportunity for male choice, these survival and fecundity advantages both vanished, with large and small females having indistinguishable fitness (Figures 3C and 3D). Differential harm by males therefore entirely eliminated a substantial fitness difference between large and small females that would otherwise have existed in the absence of male preferences. Relative to that observed under natural selection alone, the antagonistic effect of the addition of sexual selection may therefore have occurred because the increase in nonsexual fitness provided by the genomic region tagged by the SNP was partially offset or even exceeded by the cost of the increased male attention it garnered for females carrying it.

Conclusions

A longstanding question since Darwin's original description of sexual selection has been its relationship to natural selection. Here, using experimental evolution, genome resequencing, and behavioral assays, we have shown an antagonism between natural and sexual selection that affects a potentially large number of loci and appears to arise from a mating system that features male-induced harm and male mate preferences. Although we did not measure net fitness or its components directly, a consistent change in SNP frequency across replicate populations under natural selection alone strongly implies that the tagged allele is beneficial to nonsexual fitness, and that the observed antagonism of sexual selection with this is therefore maladaptive. Our results thus suggest the existence of a potentially large class of polymorphisms that are hampered or even prevented from contributing to adaptation. Such loci are likely to be invisible to classic approaches to testing the consequences of sexual selection that average the effects across the genome but can be identified with contrasting selection treatments and genotyping. It would be interesting to apply whole-genome resequencing in future experiments to identify and annotate selected variants, giving insight into the molecular pathways subject to antagonistic interactions between natural and sexual selection. However, such experiments will likely need to be significantly larger if causal variants are to be identified [49]. Furthermore, the fates of polymorphisms could be tracked through time and over longer periods, as recent work suggests that while many variants continuously rise in frequency during adaptation, others slow once they reach intermediate frequency [50]. This latter class of loci could be subject to the antagonistic processes identified here. While our experiment addressed adaptation to a novel environment, female-condition-dependent male-induced harm was also recently implicated in hampering the purging of some individual deleterious mutations in an evolution experiment in *D. melanogaster* [23], suggesting that this cost of sexual selection may impact mutation load as well as adaptation. Understanding the broader consequences of these male-female interactions and their relevance to the evolutionary history of various sexual species will require further investigation.

ACCESSION NUMBERS

The NCBI Sequence Read Archive accession number for the sequence data reported in this paper is SRA: SRP048879. The behavior dataset and draft genome scaffolds reported in this paper have been deposited at the Dryad Digital Repository with the http://dx.doi.org/10.5061/dryad.pc762.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three tables, one figure, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2015.05.034.

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Current Biology

Supplemental Information

Genomic Evidence that Sexual Selection Impedes Adaptation to a Novel Environment

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Supplementary Table S1. Classifying the evolutionary response of SNPs to a factorial manipulation of natural selection (NS) and sexual selection (SS) using different false discovery rate (FDR) corrections for multiple comparisons.

		No NS × SS interaction				NS × SS Interaction				Overall
FDR	# SNPS overall model sig	Total number ($p > 0.05$)	No. showing NS alone	No. showing SS alone	No. showing NS and SS	Total # with interaction ($p \leq 0.05$)	Addition of SS aligns with NS	Addition of SS antagonistic to NS: reduced response	Addition of SS antagonistic to NS: reversed response	SS antagonistic
0.05	59	19 (32.2%)	6 (10.2%)	5 (8.5%)	8 (13.6%) -5 antagonistic -3 aligned	40 (67.8%)	2 (3.4%)	19 (32.2%)	19 (32.2%)	5 + 19 + 19 = 43 (72.9%)
0.1	80	33 (41.3%)	11 (13.8%)	9 (11.3%)	13 (16.3%) -7 antagonistic -6 aligned	47 (58.8%)	4 (5.0%)	21 (26.25%)	22 (27.5%)	7 + 21 + 22 = 50 (62.5%)
0.2	120	56 (46.7%)	20 (16.7%)	21 (17.5%)	-15 (12.5%) -9 antagonistic -6 aligned	64 (53.3%)	5 (4.2%)	27 (22.5%)	32 (26.6%)	9 + 27 + 32 = 68 (56.7)

Supplementary Table S2: Summary information for the 80 significant SNPs. Indicated are the physical locations of each on *D. serrata* draft genome scaffolds, population-level allele frequencies, overall model *q*-values, and individual effect (natural selection, sexual selection and their interaction) *p*-values. The classification of the evolutionary response of each SNP is also provided (see Figure 2). [a]Classification of SNPs as responding to natural selection alone (Nat), sexual selection alone (Sex), to both natural and sexual selection in the same way (Aligned) or antagonistically (Antag), or to an interaction between natural and sexual selection (Interact). [b]For SNPs showing an interaction, the addition of sexual to natural selection caused a change in allele frequency that was either aligned with (Aligned), or antagonistic to (Ant), the response under natural selection alone, and when antagonistic, either reduced (red) or reversed (rev) the response compared to that under natural selection alone.

Table S2. Summary Information for the 80 Significant SNPs

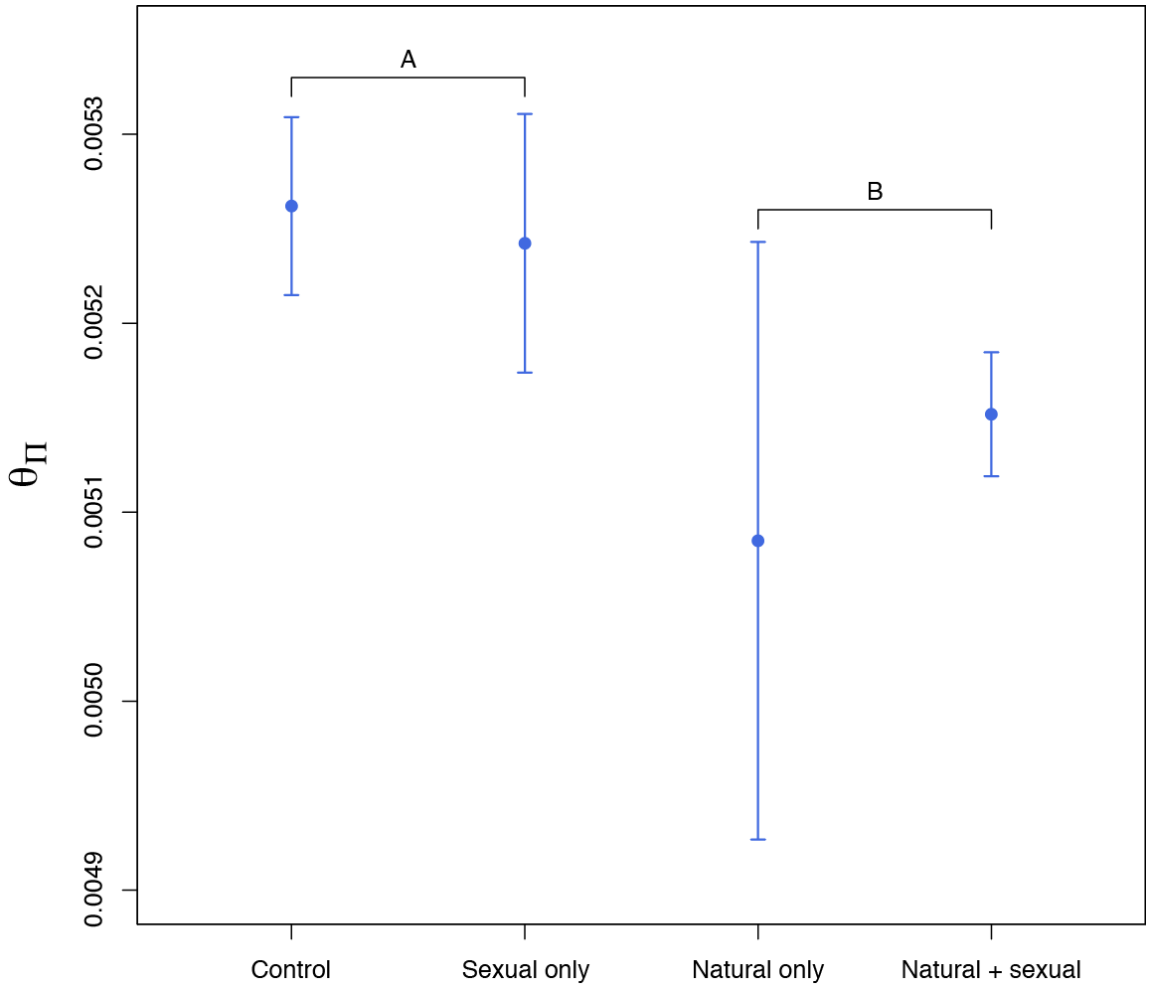
SNP ID	Scaffold ID	Scaffold size (bp)	Position (bp)	Control	Natural	Sexual	Natural × sexual	Model *q*-value	$P_{natural}$	P_{sexual}	$P_{natural \times sexual}$	Class[a]	adding sexual sel[b]
24	1.1	6200209	2808490	0.12, 0.06, 0.11	0.23, 0.09, 0.10	0.04, 0.05, 0.03	0.13, 0.14, 0.30	4.34×10^{-2}	1.14×10^{-3}	4.27×10^{-1}	5.85×10^{-2}	Nat	
25	1.1	6200209	2986851	0.86, 0.62, 0.79	0.96, 0.85, 0.92	0.68, 0.63, 0.72	0.95, 0.93, 0.96	7.02×10^{-5}	4.23×10^{-8}	9.20×10^{-1}	1.53×10^{-1}	Nat	
29	1.1	6200209	3213685	0.11, 0.11, 0.07	0.00, 0.00, 0.04	0.11, 0.03, 0.14	0.57, 0.19, 0.06	4.14×10^{-2}	1.82×10^{-1}	1.41×10^{-2}	7.01×10^{-3}	Interact	AntAnt (rev)
33	1.1	6200209	3368691	0.88, 0.68, 0.79	0.96, 0.84, 0.86	0.75, 0.67, 0.78	0.95, 0.90, 0.94	4.14×10^{-2}	2.13×10^{-4}	8.41×10^{-1}	2.49×10^{-1}	Nat	
56	1.1	6200209	4942471	0.72, 0.66, 0.72	0.86, 0.83, 0.70	0.82, 0.67, 0.41	0.36, 0.40, 0.28	9.27×10^{-4}	2.84×10^{-1}	7.62×10^{-5}	3.29×10^{-3}	Interact	Ant (rev)
80	10.1	6200214	808652	0.94, 0.87, 0.74	0.77, 0.58, 0.73	1.00, 0.99, 0.97	1.00, 1.00, 0.99	1.18×10^{-4}	5.72×10^{-1}	1.95×10^{-7}	7.36×10^{-2}	Sex	
115	107.1	6200215	245338	0.24, 0.22, 0.18	0.25, 0.24, 0.28	0.15, 0.20, 0.16	0.22, 0.22, 0.19	1.26×10^{-2}	2.53×10^{-3}	2.07×10^{-3}	1	Antag	
153	111.1	6200216	61940	0.51, 0.46, 0.38	0.76, 0.72, 0.73	0.58, 0.58, 0.38	0.51, 0.66, 0.56	8.20×10^{-4}	3.76×10^{-5}	1.81×10^{-1}	7.97×10^{-3}	Interact	Ant (red)
159	116.1	256239	187595	0.55, 0.39, 0.64	0.84, 0.91, 0.90	0.62, 0.54, 0.63	0.44, 0.71, 0.49	1.98×10^{-5}	9.36×10^{-4}	3.15×10^{-3}	5.61×10^{-5}	Interact	Ant (red)
206	13.1	2357508	1152773	0.32, 0.21, 0.32	0.02, 0.06, 0.00	0.26, 0.19, 0.36	0.17, 0.44, 0.17	3.33×10^{-5}	2.94×10^{-4}	1.42×10^{-3}	8.31×10^{-4}	Interact	Ant (red)
210	13.1	2357508	1528594	0.77, 0.58, 0.59	0.88, 0.93, 0.94	0.82, 0.93, 0.67	0.77, 0.73, 0.67	1.24×10^{-2}	4.89×10^{-2}	3.77×10^{-1}	1.72×10^{-4}	Interact	Ant (red)
219	13.1	2357508	2344977	0.30, 0.19, 0.30	0.30, 0.40, 0.49	0.13, 0.13, 0.20	0.32, 0.23, 0.48	4.21×10^{-2}	9.99×10^{-4}	6.93×10^{-2}	3.80×10^{-1}	Nat	
259	149.1	131664	33603	0.67, 0.61, 0.59	0.50, 0.77, 0.63	0.87, 0.89, 0.89	0.38, 0.54, 0.58	1.33×10^{-6}	2.83×10^{-5}	4.04×10^{-2}	7.09×10^{-6}	Interact	Ant (rev)
264	15.1	2398211	829563	0.90, 0.57, 0.83	0.89, 0.83, 0.99	0.85, 0.83, 0.73	0.99, 0.99, 0.95	6.99×10^{-2}	1.13×10^{-3}	2.02×10^{-1}	2.52×10^{-1}	Nat	
276	16.1	3217378	1478	0.18, 0.16, 0.16	0.18, 0.19, 0.14	0.17, 0.19, 0.20	0.13, 0.13, 0.13	3.28×10^{-2}	1.41×10^{-2}	3.37×10^{-1}	3.08×10^{-3}	Interact	Ant (rev)
290	16.1	3217378	943310	0.27, 0.23, 0.16	0.40, 0.59, 0.41	0.42, 0.36, 0.41	0.14, 0.37, 0.30	7.24×10^{-2}	3.03×10^{-1}	8.88×10^{-1}	4.48×10^{-4}	Interact	Ant (red)
314	16.1	3217378	3197250	0.82, 0.80, 0.78	0.88, 1.00, 0.97	0.75, 0.83, 0.84	0.69, 0.80, 0.79	3.05×10^{-2}	5.16×10^{-2}	1.66×10^{-2}	1.12×10^{-2}	Interact	Ant (rev)
413	2.1	6993494	1036934	0.11, 0.17, 0.13	0.02, 0.17, 0.18	0.24, 0.26, 0.36	0.55, 0.28, 0.33	8.99×10^{-2}	9.20×10^{-1}	6.77×10^{-4}	3.06×10^{-1}	Sex	
414	2.1	6993494	1085331	0.58, 0.53, 0.52	0.33, 0.51, 0.48	0.59, 0.66, 0.60	0.83, 0.71, 0.64	4.57×10^{-3}	7.64×10^{-1}	8.29×10^{-5}	1.51×10^{-2}	Interact	Ant (rev)
487	20.1	1829100	725791	0.16, 0.27, 0.07	0.05, 0.02, 0.00	0.20, 0.30, 0.17	0.20, 0.09, 0.04	2.44×10^{-2}	1.50×10^{-3}	2.58×10^{-2}	2.12×10^{-1}	Antag	
490	20.1	1829100	799174	0.80, 0.93, 0.83	0.84, 0.79, 0.77	1.00, 0.93, 1.00	0.98, 0.93, 0.82	7.61×10^{-2}	6.60×10^{-2}	4.63×10^{-3}	1.83×10^{-1}	Sex	
502	21.1	1616703	258780	0.40, 0.51, 0.45	0.49, 0.63, 0.43	0.31, 0.34, 0.25	0.61, 0.61, 0.47	4.39×10^{-3}	1.43×10^{-4}	2.02×10^{-1}	1.73×10^{-2}	Interact	Aligned
515	21.1	1616703	1177925	0.71, 0.80, 0.74	0.82, 0.49, 0.79	0.65, 0.79, 0.80	0.97, 1.00, 0.91	2.51×10^{-3}	1.28×10^{-2}	4.17×10^{-3}	3.32×10^{-3}	Interact	Ant (rev)
517	21.1	1616703	1346784	0.69, 0.58, 0.83	0.44, 0.50, 0.66	0.79, 0.75, 0.85	0.51, 0.58, 0.57	2.53×10^{-2}	1.41×10^{-4}	2.49×10^{-1}	4.17×10^{-1}	Nat	
595	27.1	3936571	619472	0.27, 0.26, 0.12	0.14, 0.16, 0.17	0.26, 0.34, 0.23	0.13, 0.13, 0.04	7.54×10^{-2}	1.97×10^{-3}	6.63×10^{-1}	6.21×10^{-2}	Nat	
600	27.1	3936571	1604917	0.79, 0.63, 0.72	0.71, 0.66, 0.73	0.47, 0.69, 0.42	0.45, 0.22, 0.57	8.94×10^{-2}	3.54×10^{-1}	7.71×10^{-4}	4.84×10^{-1}	Sex	
603	27.1	3936571	1795584	0.16, 0.10, 0.24	0.19, 0.26, 0.07	0.09, 0.06, 0.10	0.00, 0.02, 0.00	1.86×10^{-4}	6.17×10^{-3}	1.43×10^{-4}	6.07×10^{-3}	Interact	Ant (rev)
613	27.1	3936571	3056300	0.71, 0.61, 0.51	0.52, 0.51, 0.30	0.66, 0.70, 0.65	0.52, 0.38, 0.41	4.14×10^{-2}	1.49×10^{-4}	5.97×10^{-1}	5.32×10^{-1}	Nat	
651	3.1	5153792	105444	0.12, 0.10, 0.26	0.08, 0.04, 0.04	0.20, 0.27, 0.33	0.05, 0.21, 0.13	2.33×10^{-2}	1.15×10^{-3}	1.36×10^{-2}	7.08×10^{-1}	Antag	
694	3.1	5153792	4727901	0.48, 0.37, 0.56	0.58, 0.42, 0.33	0.35, 0.49, 0.48	0.14, 0.32, 0.19	5.19×10^{-2}	2.13×10^{-2}	1.93×10^{-2}	5.99×10^{-2}	Aligned	
754	34.1	1133726	95753	0.66, 0.73, 0.72	0.88, 0.82, 0.92	0.71, 0.89, 0.85	0.58, 0.67, 0.78	6.38×10^{-2}	5.66×10^{-1}	3.15×10^{-1}	4.22×10^{-4}	Interact	Ant (rev)
757	34.1	1133726	177839	0.66, 0.71, 0.73	0.90, 0.79, 0.83	0.79, 0.82, 0.87	0.55, 0.60, 0.62	8.61×10^{-8}	2.44×10^{-1}	4.26×10^{-2}	6.68×10^{-11}	Interact	Ant (rev)
794	36.1	2068437	880864	0.85, 0.85, 0.81	0.68, 0.63, 0.59	0.81, 0.68, 0.69	0.60, 0.75, 0.77	2.33×10^{-2}	2.29×10^{-3}	4.71×10^{-1}	8.38×10^{-3}	Interact	Ant (red)
820	38.1	1742166	1091964	0.34, 0.27, 0.23	0.37, 0.82, 0.73	0.60, 0.45, 0.48	0.15, 0.13, 0.45	8.84×10^{-2}	7.40×10^{-1}	2.88×10^{-1}	7.00×10^{-4}	Interact	Ant (rev)
833	39.1	1068078	760595	0.65, 0.72, 0.62	0.75, 0.81, 0.79	0.68, 0.61, 0.68	0.63, 0.58, 0.60	3.99×10^{-5}	9.66×10^{-2}	4.59×10^{-5}	1.48×10^{-4}	Interact	Ant (rev)
834	39.1	1068078	766720	0.71, 0.72, 0.75	0.82, 0.79, 0.61	0.97, 0.82, 0.96	0.62, 0.69, 0.70	8.13×10^{-3}	6.93×10^{-3}	6.25×10^{-2}	2.23×10^{-3}	Interact	Ant (rev)
877	4.1	7414480	4398479	0.14, 0.17, 0.18	0.05, 0.00, 0.07	0.21, 0.14, 0.10	0.27, 0.25, 0.11	8.47×10^{-3}	5.06×10^{-2}	7.50×10^{-3}	2.74×10^{-3}	Interact	Ant (rev)
891	4.1	7414480	6282208	0.48, 0.53, 0.59	0.23, 0.14, 0.21	0.44, 0.20, 0.24	0.12, 0.06, 0.31	1.90×10^{-2}	4.41×10^{-4}	4.31×10^{-2}	3.03×10^{-1}	Aligned	
897	4.1	7414480	6646499	0.31, 0.07, 0.17	0.01, 0.00, 0.04	0.20, 0.09, 0.20	0.05, 0.24, 0.27	4.75×10^{-2}	2.52×10^{-2}	3.12×10^{-2}	2.65×10^{-2}	Interact	Ant (red)
922	40.1	1619687	1008893	0.64, 0.82, 0.69	0.81, 0.96, 0.96	1.00, 1.00, 0.95	0.86, 0.86, 0.95	1.57×10^{-2}	3.17×10^{-1}	7.21×10^{-3}	1.69×10^{-3}	Interact	Ant (red)
927	40.1	1619687	1508441	0.81, 0.64, 0.57	0.90, 1.00, 1.00	0.60, 0.83, 0.79	0.66, 0.67, 0.81	2.05×10^{-3}	5.00×10^{-3}	1.44×10^{-2}	1.85×10^{-3}	Interact	Ant (red)
934	41.1	1238660	1108186	0.29, 0.20, 0.19	0.23, 0.24, 0.32	0.02, 0.05, 0.17	0.22, 0.24, 0.12	4.21×10^{-2}	3.29×10^{-2}	4.65×10^{-3}	1.26×10^{-1}	Antag	
936	42.1	1055251	43242	0.31, 0.60, 0.42	0.16, 0.15, 0.22	0.56, 0.27, 0.48	0.25, 0.25, 0.06	8.94×10^{-2}	3.82×10^{-4}	8.62×10^{-1}	1	Nat	
955	43.1	1015039	681494	0.18, 0.27, 0.36	0.14, 0.14, 0.04	0.36, 0.37, 0.43	0.13, 0.45, 0.42	9.31×10^{-2}	3.77×10^{-2}	8.62×10^{-3}	2.62×10^{-1}	Antag	
966	44.1	1006440	483251	0.37, 0.45, 0.15	0.23, 0.34, 0.36	0.08, 0.08, 0.05	0.19, 0.34, 0.11	9.84×10^{-3}	6.68×10^{-2}	3.27×10^{-4}	7.36×10^{-2}	Sex	
985	46.1	902586	732137	0.72, 0.79, 0.69	0.78, 0.68, 0.92	0.65, 0.80, 0.87	0.97, 0.95, 0.93	1.02×10^{-2}	2.23×10^{-3}	9.43×10^{-3}	7.23×10^{-2}	Aligned	
987	46.1	902586	880986	0.64, 0.60, 0.43	0.48, 0.54, 0.75	0.70, 0.73, 0.42	0.81, 0.88, 0.88	2.56×10^{-2}	1.81×10^{-2}	6.90×10^{-3}	6.06×10^{-2}	Aligned	
994	47.1	862639	338258	0.61, 0.53, 0.57	0.21, 0.22, 0.16	0.43, 0.37, 0.34	0.23, 0.40, 0.31	4.00×10^{-11}	2.57×10^{-11}	5.78×10^{-1}	4.27×10^{-6}	Interact	Ant (red)
1004	48.1	871544	126697	0.11, 0.28, 0.37	0.50, 0.18, 0.50	0.12, 0.19, 0.36	0.09, 0.00, 0.00	2.09×10^{-2}	7.23×10^{-2}	6.34×10^{-3}	1.10×10^{-2}	Interact	Ant (rev)
1017	48.1	871544	839087	0.00, 0.10, 0.00	0.41, 0.19, 0.45	0.10, 0.11, 0.34	0.19, 0.21, 0.26	2.09×10^{-2}	3.63×10^{-3}	9.66×10^{-2}	1.50×10^{-2}	Interact	Ant (red)
1034	5.1	3510882	799359	0.55, 0.38, 0.31	0.58, 0.39, 0.35	0.63, 0.88, 0.95	0.87, 0.67, 0.80	9.84×10^{-3}	7.08×10^{-1}	1.39×10^{-5}	4.93×10^{-1}	Sex	
1038	5.1	3510882	978921	0.68, 0.82, 0.47	0.57, 0.56, 0.77	0.97, 0.98, 0.90	0.69, 0.98, 0.98	7.79×10^{-2}	6.99×10^{-1}	3.32×10^{-4}	8.62×10^{-1}	Sex	
1043	5.1	3510882	1093131	0.66, 0.91, 0.73	0.53, 0.58, 0.82	0.93, 0.95, 0.91	0.76, 0.89, 0.89	5.19×10^{-2}	4.55×10^{-2}	1.37×10^{-3}	8.06×10^{-1}	Antag	
1045	5.1	3510882	1229876	0.23, 0.30, 0.46	0.21, 0.11, 0.51	0.07, 0.03, 0.09	0.10, 0.13, 0.10	3.05×10^{-2}	6.99×10^{-1}	2.04×10^{-4}	1.56×10^{-1}	Sex	
1046	5.1	3510882	1511137	0.22, 0.36, 0.17	0.03, 0.00, 0.00	0.08, 0.12, 0.06	0.28, 0.10, 0.38	3.27×10^{-4}	1.33×10^{-2}	1.81×10^{-2}	5.46×10^{-5}	Interact	Ant (rev)
1094	55.1	759158	351729	0.17, 0.42, 0.25	0.73, 0.61, 0.46	0.25, 0.31, 0.30	0.39, 0.24, 0.29	1.77×10^{-2}	6.71×10^{-3}	3.62×10^{-2}	1.41×10^{-2}	Interact	Ant (red)

1108	57.1	717862	343872	0.07, 0.10, 0.49	0.07, 0.00, 0.00	0.17, 0.17, 0.10	0.24, 0.19, 0.10	7.51×10^{-2}	4.26×10^{-2}	4.37×10^{-2}	2.38×10^{-2}	Interact	Ant (red)
1115	58.1	706328	404143	0.35, 0.33, 0.42	0.21, 0.34, 0.20	0.37, 0.41, 0.17	0.15, 0.18, 0.07	7.24×10^{-2}	2.79×10^{-3}	5.85×10^{-2}	3.45×10^{-1}	Nat	
1118	58.1	706328	617432	0.97, 0.91, 0.90	0.63, 0.78, 0.74	0.69, 0.80, 0.69	0.81, 0.72, 0.74	9.92×10^{-5}	2.09×10^{-3}	4.53×10^{-3}	2.23×10^{-4}	Interact	Ant (red)
1129	6.1	3379143	516608	0.85, 0.87, 0.96	0.98, 0.93, 0.97	0.82, 0.83, 0.79	0.77, 0.60, 0.66	4.63×10^{-5}	5.12×10^{-1}	4.66×10^{-7}	6.67×10^{-3}	Interact	Ant (rev)
1132	6.1	3379143	637458	0.05, 0.21, 0.03	0.18, 0.22, 0.18	0.37, 0.12, 0.30	0.41, 0.35, 0.30	7.79×10^{-2}	4.04×10^{-2}	3.77×10^{-3}	4.93×10^{-1}	Aligned	
1135	6.1	3379143	782046	0.94, 0.87, 0.97	0.81, 0.68, 0.85	0.63, 0.83, 0.64	0.75, 0.84, 0.74	1.90×10^{-2}	1.28×10^{-1}	4.63×10^{-3}	7.33×10^{-3}	Interact	Ant (red)
1143	6.1	3379143	1422232	0.77, 0.89, 0.94	0.65, 0.55, 0.54	0.54, 0.70, 0.77	0.94, 0.88, 0.77	1.99×10^{-2}	5.22×10^{-1}	5.97×10^{-1}	4.47×10^{-5}	Interact	Ant (red)
1199	63.1	648220	484038	0.68, 0.63, 0.74	0.79, 0.49, 0.76	0.84, 0.97, 0.82	0.49, 0.60, 0.63	7.61×10^{-2}	1.69×10^{-2}	2.37×10^{-1}	1.37×10^{-2}	Interact	Aligned
1207	65.1	625880	8705	0.15, 0.15, 0.09	0.06, 0.00, 0.03	0.24, 0.14, 0.13	0.22, 0.12, 0.14	4.57×10^{-3}	1.28×10^{-2}	1.80×10^{-3}	1.85×10^{-2}	Interact	Ant (rev)
1272	74.1	801886	203100	0.24, 0.45, 0.48	0.20, 0.08, 0.12	0.54, 0.56, 0.45	0.27, 0.20, 0.40	3.85×10^{-4}	1.12×10^{-5}	5.80×10^{-3}	4.31×10^{-1}	Antag	
1280	75.1	517822	255743	0.26, 0.11, 0.17	0.13, 0.12, 0.16	0.09, 0.05, 0.04	0.22, 0.25, 0.19	1.86×10^{-4}	4.24×10^{-3}	1.12×10^{-1}	1.60×10^{-5}	Interact	Ant (rev)
1300	8.1	5657471	41644	0.26, 0.42, 0.32	0.14, 0.26, 0.21	0.30, 0.31, 0.37	0.40, 0.38, 0.66	5.64×10^{-2}	9.20×10^{-1}	9.27×10^{-3}	6.82×10^{-3}	Interact	Ant (rev)
1314	8.1	5657471	1223546	0.68, 0.60, 0.80	0.70, 0.80, 0.76	0.63, 0.50, 0.36	0.80, 0.72, 0.87	2.33×10^{-2}	1.05×10^{-3}	2.73×10^{-1}	2.83×10^{-2}	Interact	Aligned
1334	8.1	5657471	2458869	0.93, 0.81, 0.88	1.00, 0.98, 0.98	0.95, 0.78, 0.92	0.95, 0.82, 0.87	1.26×10^{-2}	1.42×10^{-2}	2.07×10^{-2}	7.05×10^{-3}	Interact	Ant (red)
1346	8.1	5657471	4386942	0.84, 0.84, 0.95	1.00, 1.00, 1.00	0.97, 0.89, 0.89	0.62, 0.85, 0.90	3.94×10^{-8}	1.35×10^{-3}	2.75×10^{-5}	1.94×10^{-6}	Interact	Ant (rev)
1359	8.1	5657471	5334512	0.20, 0.29, 0.35	0.23, 0.27, 0.18	0.30, 0.42, 0.43	0.38, 0.40, 0.56	3.76×10^{-2}	1	2.08×10^{-4}	2.03×10^{-1}	Sex	
1360	8.1	5657471	5376780	0.77, 0.50, 0.79	0.88, 0.89, 1.00	1.00, 0.97, 0.96	0.98, 0.93, 0.87	9.31×10^{-2}	4.71×10^{-1}	1.30×10^{-2}	1.60×10^{-2}	Interact	Aligned
1361	8.1	5657471	5389321	0.50, 0.6, 0.35	0.21, 0.09, 0.19	0.45, 0.56, 0.32	0.52, 0.37, 0.52	1.47×10^{-3}	5.77×10^{-3}	1.03×10^{-2}	1.44×10^{-3}	Interact	Ant (red)
1363	80.1	488845	82858	0.32, 0.15, 0.17	0.03, 0.03, 0.13	0.18, 0.13, 0.29	0.02, 0.12, 0.01	8.84×10^{-2}	4.70×10^{-4}	5.54×10^{-1}	6.89×10^{-1}	Nat	
1367	82.1	501863	351001	0.32, 0.30, 0.32	0.04, 0.18, 0.07	0.35, 0.19, 0.16	0.36, 0.25, 0.26	1.24×10^{-2}	3.25×10^{-2}	7.36×10^{-2}	8.77×10^{-4}	Interact	Ant (red)
1379	84.1	475645	224984	0.04, 0.14, 0.08	0.27, 0.22, 0.19	0.16, 0.27, 0.37	0.17, 0.08, 0.14	4.21×10^{-2}	6.17×10^{-1}	2.54×10^{-1}	2.57×10^{-4}	Interact	Ant (red)
1384	85.1	471672	431047	0.65, 0.56, 0.52	0.80, 0.61, 0.50	0.79, 0.57, 0.47	0.91, 0.79, 0.91	6.27×10^{-2}	1.26×10^{-2}	2.70×10^{-2}	9.85×10^{-2}	Aligned	
1395	88.1	444646	5479	0.56, 0.42, 0.61	0.59, 0.61, 0.79	0.50, 0.30, 0.47	0.24, 0.19, 0.38	5.64×10^{-3}	8.41×10^{-1}	8.29×10^{-5}	1.98×10^{-2}	Interact	Ant (rev)
1434	9.1	2677374	2243728	0.13, 0.19, 0.14	0.47, 0.37, 0.41	0.37, 0.62, 0.50	0.49, 0.21, 0.12	3.10×10^{-2}	6.80×10^{-1}	1.64×10^{-1}	2.08×10^{-4}	Interact	Ant (red)

Supplementary Table S3. Number of reads attained from each sequencing library.

Treatment	Population	No. reads
Control	1	11,249,930
Control	2	11,358,891
Control	3	12,115,669
Sexual only	1	7,165,497
Sexual only	2	8,899,122
Sexual only	3	8,707,475
Natural only	1	10,369,180
Natural only	2	10,288,620
Natural only	3	6,985,610
Natural and sexual	1	14,891,855
Natural and sexual	2	14,765,344
Natural and sexual	3	14,812,166
	Total reads	**131,609,359**

Supplementary Figure S1. Treatment-level means (± 95% C.I.) for genome-wide estimates of θ_{Π}. Brackets group treatments that could not be distinguished from each other statistically (p <0.05).



Supplemental Experimental Procedures

Experimental Evolution

The derivation and maintenance of the experimental populations is described in detail in [S1]. In brief, 12 replicate populations were derived from a lab-adapted, outbred stock population and housed on a novel corn-based larval food [S2]. Following [S3], these populations were randomly allocated to one of four treatment combinations that independently manipulated the opportunities for natural and sexual selection in a factorial design, yielding: three populations that experienced both natural and sexual selection; three populations that experienced natural selection and reduced sexual selection; three populations that experienced sexual selection and reduced natural selection; and three populations that experienced reduced natural and reduced sexual selection. Population were propagated each (non-overlapping) generation by allowing each of 55 females to individually oviposit in one of 55 vials. Offspring were collected from a maximum of 50 (randomly chosen) of these vials, with the collection procedure determining the opportunity for natural selection and their subsequent mating treatment altering the opportunity for sexual selection (Figure 1).

Natural selection was permitted each generation by pooling all offspring from the 50 vials prior and then randomly selecting 55 of each sex to become the following generation. This ensured that females tended to contribute to the next generation in proportion to the number of offspring that they produced: offspring from high productivity females were more likely to have their offspring sampled than low productivity females. Natural selection was reduced by equalising each female's contribution to the next generation to two offspring – one male and one female. Once the next generation was selected they were passed to a mating phase that manipulated the opportunity for sexual selection. Sexual selection was permitted by placing all individuals in a single bottle, allowing both mate choice and male-male competition to occur (pre- and post-copulatory). Sexual selection was reduced by randomly assigning a single male to a single female in a vial for mating. Essentially all females eventually mate with their random partner under these conditions, eliminating any variance among the males in their reproductive success. After the mating period, females were then transferred to individual vials to lay their eggs. Flies

from generation 13 were stored at -80˚C for later genetic analysis (approximately 30 individuals/line).

DNA Extraction, Pooling and RAD Sequencing

From a sample of generation 12 flies that had been frozen at -80˚C, 24 females were randomly chosen from each population. DNA was extracted from groups of eight individuals using QIAGEN's DNAeasy Blood and Tissue Kit, Animal Tissue (Spin-Column) Protocol (with a 2.5 h incubation and including RNase A digestion and two elutions to maximise the purity and volume of the end product). Male *D. serrata* are hemizygous so by using only females we equalised the number of X chromosomes and autosomes sampled from each population. Equimolar amounts of DNA were pooled from each of three DNA extractions per line to provide the starting material to produce 12 barcoded RAD sequencing libraries. The restriction enzyme SgrAI was used for digestions, and RAD genotyping was performed by Floragenex Inc. (Oregon, USA) with sequencing performed on a single lane of an Illumina Hi-Seq 2000 platform (101 bp single-end reads). We attained a total of 131,609,359 reads for the entire set of 12 libraries (Table S3). Raw sequence data are available from the NCBI Short Read Archive (SRP048879).

RAD Data Analysis

Fastq files were split by barcode first using the RADpools script in the RADtools package v. 1.2.2 [S4], resulting in 12 fastq files. This script also removed erroneous reads that did not contain a cut site (~ 0.9% of all tags). We then aligned the reads to the *D. serrata* reference genome scaffolds (S. F. Chenoweth unpublished; 5799 unordered scaffolds spanning 177 Mbp the genome N50 = 1.62 Mbp. Scaffolds are freely available from the authors (http://www.chenowethlab.org/Site/Resources.html) using bwa v. 0.5.9rc1 [S5]. The 12 sam files were then converted into a single mpileup file for further analysis using samtools v. 0.1.18 [S6]. To score SNPs in the 12 pools we used the tools in the Popoolation2 package to parse the mpileup file [S7]. SNPs were filtered for base quality (mpileup2sync.jar; min base quality = 20) and coverage (snp-frequency-diff.pl; Min. count = 2; Min coverage = 6; Max. coverage per pool

= 3000). The data were then further parsed using custom Perl scripts to remove non-biallelic SNPs, and to apply additional minimum coverage criteria in all replicates (minimum of 48 reads per population; maximum of 3000). Due the complementary nature of the restriction enzyme's binding site (CCGG), tags are sequenced both up and downstream of a cut-site and there was often more than one SNP available for analysis in the sequenced region surrounding a cut site. In these cases we randomly selected a single SNP for analysis. A total of 1460 biallelic SNPs were available for analysis after quality filtering. These SNPs were distributed across 142 *D. serrata* draft *de novo* genome scaffolds (range: 1 - 67 SNPs per scaffold). When we observed multiple SNPs per scaffold the average distance between SNPs was 90,300 bp and, as expected, there was a positive relationship between SNP count and scaffold length (Pearson's $r = 0.95$, $t = 36.26$, d.f. = 140, $p < 0.0001$). We note that allele frequency estimates from pool-based sequencing tend to closely match that obtained from individual-based genotyping when coverage values are above 20× [S8]. As we did not analyze SNPs with coverage less than 48× per population, we expect our pool-based estimates are reasonable.

We analyzed SNP data for selection-induced allele frequency change in two ways: all 1460 SNPs at once and via a SNP-by-SNP approach. First, to provide an overall genome-wide analysis of the evolutionary response, we used a multivariate analysis of dissimilarity implemented in the *adonis* function of the R/vegan package [S9]. This enabled us to fit a linear model that included the main effect factors of natural and sexual selection, plus their interaction, while using all information in the data set. This analysis was performed on a distance matrix between the SNP frequency vector of each of the 12 populations, calculated using Reynolds' genetic distances [S10].

Second, we performed single-SNP analyses using a generalized linear mixed model that accurately reflected the design of the experiment. For the single-marker analysis, we fit the following model separately to each SNP:

$$read_i = \mu + Nat + Sex + Nat \times Sex + pop_j(Nat \times Sex) + \varepsilon, \qquad [1]$$

where $read_i$ is the allele of the ith sequence read within the jth population and is coded as 0 or 1 for the minor or major base respectively. We assumed that $read_i \sim$ Bernoulli(1, p) where p is the experiment wide allele frequency. *Nat* is the main effect of natural selection, *Sex* is the main effect of sexual selection, *pop* is the effect of the replicate population nested with a combination of natural and sexual selection treatments and accounts for random differences in allele frequency between replicates within treatments (e.g. due to drift), and ε is unexplained error. All factors were fixed except for the *pop* and residual, ε, terms, which were considered random. Models were fit using the GLIMMIX procedure in SAS version 9.3 (SAS Institute. Cary, NC). To correct for multiple testing in these analyses we took a false-discovery rate approach. Full model p-values were converted to q-values using the R/qvalue package [S11, 12]. For all SNPs with full model q-values less than 0.1, we further analyzed the nominal significance of the interaction and main effect terms ($p \leq 0.05$).

As opposed to gene discovery, our goal was to categorize SNPs according to how natural and sexual selection affected allele frequency trajectories, thereby summarizing effects across the genome. We first distinguished SNPs based on the presence vs. absence of a significant interaction between natural and sexual selection (i.e. *Nat* × *Sex* in Eqn. 1). Then, for those with non-significant interactions, we assessed the main effects of natural and sexual selection. SNPs significantly affected by only a single process were categorised as *natural only* or *sexual only*, and when both processes were significant, we compared the direction of allele frequency change and classified them as either *aligned* or *antagonistic*.

To further categorise the SNPs with significant interaction terms, we again classified these by comparing the direction of allele frequency change. While interpretation in the presence of an interaction can be more complex, given our interest in the effects of sexual selection on adaptation, we focused on how the response to natural selection (i.e. the difference between the control and natural selection only treatments) changed with the addition of sexual selection, or in other words, on the effects of the addition of sexual to natural selection. These SNPs with

significant interactions were subdivided into one of two groups: 1) *interaction: aligned*, when relative to the effect of natural selection alone, the additional presence of sexual selection caused the SNP to change frequency in the same direction and with an increased magnitude; or 2) *interaction: antagonistic*, when the addition of sexual selection reduced, eliminated, or even reversed the response compared with that observed under natural selection alone.

To compare effective population sizes (N_e) between treatments, we calculated pool-based estimates of nucleotide diversity, θ_Π, ($\theta_\Pi = 4N_e\mu$ for diploids) as a proxy for N_e based on the fact that mutation rates (μ) were unlikely to differ between populations in such a short-term experiment. Because sequencing from pooled DNA samples involves both sampling individuals from a population and then sampling sequenced reads from individuals within each DNA pool, there is increased uncertainty. To correct for this, θ_Π is divided by a normalization factor using NPStat [S13]. Population level mpileup files were generated using samtools with BAQ computation disabled and a mapping depth threshold of 30,000. The mpileup files were used as input for NPStat which estimated a pooled sample corrected θ_Π value in non-overlapping 10,000 bp windows across the genome, a minimum coverage of 48 reads, a maximum coverage of 3000, minimum base quality of 20, and the omission of alleles that occurred less than four times. We then compared population mean θ_Π values among treatments using a two-way factorial linear model with main effects of natural selection, sexual selection and their interaction.

Behavioural Assays

Large/small virgin female were collected at emergence using light CO_2 anaesthesia from vials in which either 5/40 stock females had oviposited for 24 h. Males were collected from a third set of intermediate density vials in which groups of 15-20 mixed sex flies had oviposited for 24 h. Flies were held separately by sex and treatment in groups of five flies/vials. After 48 h, females were anaesthetized with light CO_2 and a small portion of the outer corner of either their right or left wing was clipped. Females were then combined into 100 replicate pairs, each consisting of one

high and one low-density reared female. In 50 of these pairs, the high (low) density female had their right (left) wing clipped and in the other 50 this was reversed. Flies were held for a further 72 h to recover before the assay.

The assay commenced when a single male was introduced via aspiration (without CO_2) into each of the 100 vials. Vials were checked every 30 min for a total of 15 observations. During each observation, we recorded whether or not the male was directing any sexual activity towards either female. Following [S14], sexual activity was defined as situations in which a male was: 1) oriented towards a female and within approximately 5 mm of her; 2) courting a female (i.e. pursuing, attempting to mount, and/or performing a wing display); or 3) mating with a female. Following the trials, all females were individually dried at 65°C for 24 h and then weighed to ±1 μg on a MX5 Microbalance (Mettler Toledo, Columbus, USA). The difference in sexual activity directed toward large vs. small females was tested using a two-sided paired *t*-test, treating vials as replicates.

To test the fitness consequences to females of differential harassment arising from male mate preferences, large/small virgin female offspring were again collected using light CO_2 anaesthesia from vials in which 5/60 stock females respectively had oviposited for 24 h. Virgin males were also collected from the low density vials. Flies were stored separately by sex and female treatment in groups of seven flies/vial. 72 h later, we created three different mating treatments involving 16 males together in a vial with either: 1) 16 large (i.e. low density-reared) females; 2) 16 small (i.e. high density-reared) females; or 3) mixed groups of eight large and eight small females. Males in treatment #3 have choice and can express a preference for larger, more fecund females, whereas the opportunity to do this is greatly reduced within treatments #1 and #2 because all females were raised at the same density and thus are of similar size. Thirty replicates were created for each treatment combination. Females in the mixed treatment had a small portion of the outer corner of either their right (small females) or left (large females) wing clipped for identification. After approximately 66 h in their respective mating treatments, the number, sex, and size (large vs. small females) of dead flies was recorded for each replicate vial and a subsample of the surviving females was then removed and the females were placed individually

in new vials for 24 h of egg laying. An average of 4.0 ± 0.1 (SE) large females were sampled from each vial in treatments #1 and #3, and 4.0 ± 0.1 (SE) small females from each vial in treatments #2 and #3. The number of adult offspring that emerged from each of these vials was counted 11 d later. A χ^2 test of independence was used to compare the survival of females between the no-choice and choice mating treatments separately for large and for small females. Productivity data were non-normally distributed because some females produced few or no offspring. A non-parametric Wilcoxon rank sum test was therefore used to compare the performance of females between the choice and no-choice treatments separately for large and small females.

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