Sexual conflict in wing size and shape in *Drosophila melanogaster*

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ABSTRACT

Intralocus sexual conflict occurs when opposing selection pressures operate on loci expressed in both sexes, constraining the evolution of sexual dimorphism and displacing one or both sexes from their optimum. We eliminated intralocus conflict in *Drosophila melanogaster* by limiting transmission of all major chromosomes to males, thereby allowing them to win the intersexual tug-of-war. Here we show that this male-limited (ML) evolution treatment led to the evolution (in both sexes) of masculinized wing morphology, body size, growth rate, wing loading, and allometry. In addition to more male-like size and shape, ML evolution resulted in an increase in developmental stability for males. However females expressing ML chromosomes were less developmentally stable, suggesting that being ontogenetically more male-like was disruptive to development. We suggest that sexual selection over size and shape of the imago may therefore explain the persistence of substantial genetic variation in these characters and the ontogenetic processes underlying them.

Keywords: intralocus sexual conflict**,** ontogenetic sexual conflict, *Drosophila melanogaster*, geometric morphometrics, sexual size dimorphism, experimental evolution

INTRODUCTION

The existence of sexual dimorphism is, in and of itself, evidence that the two sexes have had a history of disruptive selection. Recently it has been suggested that constraints on the evolution of sexual dimorphism as a result of genetic correlations between the sexes may impose a substantial load on the fitness of one or both sexes (Prasad *et al*. 2007; Rice 1984). This 'gender load' may sometimes be detectable as a negative intersexual genetic correlation for fitness, and evidence for such a pattern of covariation across the sexes has accumulated in the last decade in a variety of sexual organisms in both the laboratory and the field (reviewed in Bonduriansky & Chenoweth 2009; and Cox & Calsbeek 2009). Nonetheless, intralocus sexual conflict is, and will probably always be, difficult to measure because of: (1) the composite nature of fitness and the virtual certainty of an admixture of trait-specific intersexual genetic correlations affecting it; (2) the fact that maintenance of sexually antagonistic genetic variation requires specific, locus-dependent (i.e. autosomal or sex-linked) relationships between the selection coefficients on males and females; and (3) a variety of environmental and genetic factors which will tend to make intersexual correlations positive (Bonduriansky & Chenoweth 2009; Cox & Calsbeek 2009).

One way to observe intralocus sexual conflict as an evolutionary force is to manipulate the relative intensity of selection on the two sexes. We followed the approach of Rice (1996) to eliminate female gene expression in *D. melanogaster* by limiting virtually the entire genome (all but the dot chromosome IV; <1% of the genome) to males. Under this male-limited (ML) experimental evolution scheme, the X-chromosome and both the major autosomes behave like a single large Y-chromosome in that they are transferred from father to son and are never expressed in females. This lets us harness the genome-wide power of many loci to augment

the benefits of sex-limitation, and allows loci polymorphic for male-benefit / female-detriment alleles to be positively selected. After a number of generations of ML evolution, the ML-selected chromosomes can then be expressed in both males and females in order to test their effects in a standardized genetic background. ML evolution should generate populations approaching the best masculine phenotypes available from that fraction of the standing variation in the ancestral populations. In accordance with the predictions from intralocus sexual conflict, it has previously been found that release from selection upon female function led to a burst of male-specific adaptation: the fitness of males increased and the fitness of females inheriting ML genotypes decreased (Prasad *et al*. 2007). These evolved fitness differences were accompanied by phenotypic shifts towards the male optimum (inferred from the direction of extant sexual dimorphism) in developmental time and body size (Prasad *et al*. 2007). Gains in male fitness were mediated by increased attractiveness and mating success (Bedhomme *et al*. 2008) and not by postcopulatory sexual selection (S. Bedhomme, unpublished data), therefore directing our attention to aspects of behaviour and the physical phenotype related to courtship and mating.

Because ML evolution resulted in a shift towards the male optimum for previously studied traits, this method should be useful for studying other traits exhibiting substantial sexual dimorphism in *Drosophila*, such as body size. Unlike vertebrates, sexual size dimorphism (SSD) in which females are larger than males is the rule rather than the exception in the Arthropoda, and is proximately explained by differences in growth rate rather than development time (Blanckenhorn *et al*. 2007). The main hypotheses offered to explain this pattern are fecundity selection in females, female anautogeny (where females must feed before oviposition, Blanckenhorn *et al*. 2007), selection for protandry (Maklakov *et al*. 2004), and a higher cost of production of male gonadal tissue (Miller & Pitnick 2003). A fifth

hypothesis has occasionally been advanced, connecting small male size to direct benefits accruing from sexual selection, such as mate-finding (Brandt & Andrade 2007). *Drosophila melanogaster* displays the typical arthropod pattern for SSD, but more strikingly, males are not only smaller than females, but also take longer to mature, making them substantially slower-growing (Blanckenhorn *et al*. 2007). There is evidence that fitness is positively associated with locomotor activity in males, and that this is a sexually antagonistic trait, with more active females experiencing reduced fitness (Long & Rice 2007). One potential explanation for this result is that smaller males excel in chasing, harassment, or courtship displays involving speed or agility, but their daughters inherit only the negative effects of small size on fertility. A second related hypothesis is that while females benefit from rapid growth in terms of fertility selection, males benefit from slower growth because it promotes higher ontogenetic fidelity and resulting morphological quality. This latter 'selection for perfection' model (Chippindale *et al*. 2003), suggests that the risks of rapid growth are not just those associated with increased feeding rate and exposure to predators, but also risks associated with developmental accidents. In this model, the risks associated with rapid growth are outweighed by the benefits for females, but not for males, since male fitness may be substantially negatively impacted by developmental accidents that render them further from the optimal size or shape, and/or more asymmetrical.

Developmental stability is the ability of an organism to buffer its phenotype against genetic or environmental disturbances encountered during development and is usually measured as the inverse of the mean fluctuating asymmetry (FA, Clarke 1998). The selection for perfection model predicts that this sort of developmental buffering should be more important for males than for females. More specifically, in the context of the male-limited (ML) evolution experiment, we expect that ML males will (1) be more symmetrical than Control males and

that (2) evolve to be closer to the male phenotypic optimum inferred from extant sexual dimorphism in size and shape (i.e. have smaller wings which are more masculine in shape). To investigate these hypotheses, we carried out a geometric morphometric analysis of wing morphology. Wing morphology was chosen as an appropriate trait to measure when looking for evidence of intralocus sexual conflict since it is known to be subject to sexual selection in males (Taylor & Kekic 1988) and lends itself well to landmark-based methods (Klingenberg & McIntyre 1998) and fluctuating asymmetry analysis (Breuker *et al*. 2006; Palmer 1994; Palmer & Strobeck 2002).

METHODS

We expressed ML and Control (C) haploid genomes ('hemiclones' consisting of the major autosomes and the X chromosome) from 4 replicate lines in both sexes after 82 generations of experimental ML evolution (Prasad *et al*. 2007). We assayed fitness and investigated intralocus sexual conflict and developmental stability in wing morphology. For more details about ML evolution and the production of flies for fitness and morphological measurements, please see Supplementary Information.

Female fitness was measured as follows: females were isolated as virgins and housed in groups of 10 along with five competitor females from a replica of the base stock (LH_M) homozygous for the relatively benign recessive scarlet eye marker (called LH_{st}) and were provided with 10 mg of yeast/vial. On day 12 post egg-lay, females were combined with 20 males from LH_{st} for 18 h, after which they were separated from the males and the ML females were allowed to oviposit for 20 h (LH_{st} females were discarded). The progeny eclosing from these vials were counted 12 days later. Female fitness was therefore measured as total

number of adult offspring produced after competition for a limited resource (yeast). Fifteen such vials were set up per population, and final sample size was 119 vials.

To measure male fitness, males were harvested 11 days post-oviposition. Ten males from ML (or C) populations were combined with 10 males from LHst population. Fifteen such vials were set up per population. On day 12 post egg lay, males were combined with 15 virgin clone-generator females and allowed to interact for 18 h after which the females were separated from the males and allowed to oviposit for 18 h. The progeny from the two types of males can be distinguished because of their eye color. Twelve days later, the fraction of progeny sired by the focal males (ML or C) within each vial was scored, and this proportion was used as a fitness measure. Fifteen such vials were set up per population, and final sample size was 115 vials.

Male and female fitness were measured in different currency. In order to be able to include the two fitness measures in a same analysis, we calculated mean values for each sex within each replicate population (ML and C values pooled), and then divided the values for each sample by the appropriate mean in order to get sex-specific relative fitness values. Mean relative fitness values for each combination of sex, replicate population, and selection regime were calculated (N=16) and then were analyzed using a factorial ANOVA in JMP, with sex (M or F), selection regime (C or ML), and their interaction (sex*sel) as fixed factors.

Individuals slated for morphological analysis were frozen and stored individually in eppendorf tubes at -20°C until they could be processed. Wings were mounted by hand on glass microscope slides using double-sided tape. Sample size was 965 individual flies (between 48 and 73 per population/sex/selection regime). After wing removal, flies were

dried for at least 24 hours in a 65°C drying oven before being individually weighed to the nearest 0.0001 g on a Cahn C-31 microbalance. Eleven landmarks were selected for geometric morphometric analysis (Figure 1A). These landmarks are similar to those used in other studies of wing morphology (Breuker *et al*. 2006; Gidaszewski *et al*. 2009). However some landmarks on the proximal part of the wing that have been used in previous studies were not included here as it was sometimes difficult to remove the wing without damaging this area. Wings were photographed and digitized twice (non-successively) to account for error due to distortion by camera/microscope lenses and variation in the placement of landmarks (Klingenberg & McIntyre 1998). Unfortunately it was not possible to entirely control for error caused by the mounting process, but individuals with wings that were damaged or creased in any way were excluded from the analysis. Also, because wings were mounted and digitized in a random order, improvements in mounting/digitizing technique over time cannot be the cause of any systematic differences between groups. Geometric morphometric analysis (digitization of landmarks, procrustes superimposition, relative warp analysis, and visualization of shape differences) was carried out in the tps suite of programs by F. James Rohlf (tpsUtil, tpsDig, tpsRelw, tpsRegr and tpsSplin) which are freely available at http://life.bio.sunysb.edu/morph/.

Centroid size was used as a measure of wing size, and wing shape was analysed using relative warp scores (details below). Note that centroid size, despite being a linear measure, is very highly correlated with wing area ($r = 0.99$, $P < 0.0001$) for this dataset. Wing loading was calculated as dry mass/wing centroid size, and allometric slopes were obtained by regressing wing size on body mass for each combination of sex, replicate population, and selection regime. Because previous results found differences in body mass between ML and Control flies (Prasad *et al*. 2007) we were interested in investigating allometric slopes to see if

differences in wing size could simply be attributed to the evolution of differences in body size.

Developmental stability in wing size was examined using fluctuating asymmetry (FA) analysis (Palmer 1994; Palmer & Strobeck 2002). Because male and female *Drosophila melanogaster* differ substantially in size, size-standardized wing size asymmetry values were calculated via ln(R)-ln(L) (Palmer & Strobeck 2002). We carried out analysis on both standardized data (i.e. using ln(R)-ln(L) values) and raw data (i.e. using raw size and shape values), but since results were qualitatively similar for both datasets, only the standardized analysis is presented in detail here. Before any tests of wing size FA were performed, an ANOVA was carried out to quantify and test the different components of asymmetry: error, FA, and directional asymmetry (DA; see Palmer & Strobeck 2002 for details). FA was large relative to error variance and therefore significant ($F_{964, 1394} = 8034$, $P < 0.0001$), and although there was significant DA ($F_{1, 1394} = 63.77$, $P < 0.0001$), this was probably mostly due to the large size of the dataset (Palmer & Strobeck 2002). The side*wing size effect was very small (Cohen's $d = 0.0194$), indicating that DA was much smaller than the average deviation around the mean. It was therefore not deemed necessary to correct for DA (Palmer & Strobeck 2002). Signed asymmetry values were normally distributed. Mean absolute asymmetry values for each combination of sex, replicate population, and selection regime were calculated (N=16) and then were analyzed using a factorial ANOVA in JMP, with sex (M or F), selection regime (C or ML), and their interaction (sex*sel) as fixed factors (this is equivalent to Levene's test; Palmer & Strobeck 2002).

Similarly, mean values for each combination of sex, replicate population, and selection regime were calculated (N=16) for all other univariate traits (wing size, wing loading, body

mass, allometry, and fitness) and then were analyzed using a factorial ANOVA in JMP, with sex (M or F), selection regime (C or ML), and their interaction (sex*sel) as fixed factors. This design is the same as that used for a previous analysis of data from these populations (Prasad *et al*. 2007). The mean values used in the analysis of univariate traits are reported in Supplementary table S1. For the analysis of wing shape, we carried out a MANCOVA analysis of a similar design, but with centroid size included as a covariate to control for allometry. Because the MANCOVA was performed on mean values there were too few degrees of freedom to calculate standard multivariate statistics for this analysis when carried out on the matrix of all partial warps plus the uniform component. We therefore analysed shape using relative warps (i.e. principal components of shape), and included as many in the model as possible, under the constraints provided by the limited number of degrees of freedom. We were able to include the first 11 relative warps (of 18) as dependent variables in the model, which explained over 95% of the variation in shape in our dataset.

RESULTS

We found evidence of phenotypic masculinization as a result of ML-evolution for all univariate traits. Males had smaller wings than females (Table 1A, Figure 2A), lower body mass (Table S2A, Figure S1A), and lower wing loading (Table S2B, Figure S1B), and parallel changes were seen as a result of ML evolution such that ML individuals of both sexes had smaller wings (Table 1A, Figure 2A), lower body mass (Table S2A, Figure S1A), and lower wing loading (Table S2B, Figure S1B) than Controls. The difference between the sexes in the allometric relationship between wing size and body mass was not significant, but the change in this relationship as a result of ML-evolution was still in the direction of extant sexual dimorphism (Table 1B, Figure 2B), mostly due to an increase in slope in ML females. There

were no significant sex*sel interactions for any of these traits, indicating that the degree of sexual dimorphism was unchanged as a result of ML evolution.

Both the sexes and the selection treatments differed in wing shape (Table 2), and qualitatively similar patterns of phenotypic masculinization appeared to have been achieved via different evolutionary pathways. In males, the size of the proximal part of the wing was reduced and the distal part was increased relative to females (Figure 1B). A similar pattern of reduction of the proximal part of the wing and increase of the distal part was seen in ML individuals relative to Controls (Figure 1C), but this general result was achieved via a different pattern of displacement of wing vein intersections compared to the difference due to sexual dimorphism. Again, there was no indication of any change in the degree of sexual dimorphism in shape for ML individuals. This means that although the visualization in Figure 1C was calculated using pooled data from both sexes, the pattern is the same even if the sexes are plotted separately (consistent with the non-significant sex*selection interaction term in Table 2).

We also found increased fitness in ML males, and decreased fitness of females carrying ML-evolved chromosomes, consistent with earlier results from this system (Prasad *et al*. 2007; Table 1C, Figure 2C). Interestingly, there was a significant sex*selection interaction effect in FA (Table 1D): the rank order of ML and C groups switched between the sexes (Figure 2D) such that ML males had lower FA than C males, while the opposite was true for females. This pattern paralleled the changes seen in fitness (Figure 2C) rather than size (Figure 2A). ML-expressing males were more symmetrical for wing size than Control males were, however females showed decreased developmental stability (higher size FA) when they carried ML chromosomes, despite being smaller than control females (Figure 2A, Table 1).

DISCUSSION

We reproduce the earlier result that male-limited (ML) selection leads to increased total fitness of males, and decreased fitness of females experimentally expressing ML chromosomes. We also found support for our two specific predictions about the evolution of size and wing morphology. First, ML males were indeed more symmetrical than C males, reflecting higher developmental stability. Second, we found that ML evolution proceeded in the direction of extant sexual dimorphism for all univariate traits, and that wing shape evolution evolved in a manner qualitatively similar to the direction of sexual dimorphism. However the change in wing shape as a result of ML evolution was achieved through a different pattern of displacement of wing vein intersections relative to the difference in shape between males and females. These results suggest that the average male in the ancestor or control populations is displaced from the optimal phenotype, presumably by counter-selection in females since evolution in wing morphology occurred once selection on females was removed. Hence, although the effects of selection regime were still generally smaller than sex differences, we saw morphological evidence for a gender load resulting from intralocus sexual conflict.

Results on allometric relationship between wing size and body mass suggest both that a number of inter-related aspects of the developmental program have changed as a result of ML evolution, and that a reduction in body size is not the proximal explanation for the evolution of smaller wings in ML individuals. Our results also provide further experimental evidence that intersexual genetic correlations for wing size/shape and body mass traits must be high, since there was no change in the degree of sexual size dimorphism as a result of ML evolution for these traits (no significant sex*sel interactions, Table 1A-B, Table 2, and Table S2A-B).

This is consistent with previous research on *Drosophila melanogaster* which has shown that intersexual genetic correlations for wing and body size traits generally range from 0.6 to 1 (Cowley & Atchley 1988; Cowley *et al*. 1986; Karan *et al*. 2000; Karan *et al*. 1999; Reeve & Fairbairn 1996), with a mean around 0.8 (Poissant *et al*. 2009, supplementary information).

Previous analysis of wing shape in a number of *Drosophila* species suggests that wing morphology is relatively evolutionarily labile (Gidaszewski *et al*. 2009), and this is consistent with our results since differences in wing size, wing shape, wing loading, and allometry evolved on a short time scale. However the lack of change of the degree of wing shape dimorphism as a result of ML evolution suggests that intersexual genetic correlations for shape are high. Shape changes should therefore evolve much more readily as a result of sexually congruent selection than as a result of sexually antagonistic selection. Wing loading is a trait which exhibits both plastic and genetic variation (Frazier *et al*. 2008; Gilchrist & Huey 2004; Powell *et al*. 2010), so the observed change in wing loading on a short time scale seen here is consistent with previous results but is (to our knowledge) novel in detecting changes in wing loading due to sexual selection rather than ecological adaptation. The wing shape results also suggest that a functionally similar result (i.e. a decrease in the area of the proximal part of the wing and increase in the area of the distal part of the wing) has been achieved via different ontogenetic pathways. This is consistent with previous results for wing size evolution in *Drosophila*, where analogous clines in wing size are found in European and North American populations, but the clines are a result of size increases in different portions of the wing on each continent (Gilchrist *et al*. 2001). Similarly, differences in wing size can be a result of either differences in cell size or in cell number, and contrasting patterns have been found in natural populations (James *et al*. 1995) and as a result of selection experiments (Partridge *et al*. 1994). There do not seem to be strong constraints on the evolution of wing

morphology in *Drosophila* (Gidaszewski *et al*. 2009; Mezey & Houle 2005), so these examples of functionally similar trait values achieved in different ways (both from previous research and from our own results) are probably the result of differences in time scale. Divergence on short time scales (i.e. in the laboratory or in new environments) should proceed in the direction of the most readily available genetic variation (that is, along evolutionary lines of least resistance, Schluter 1996) while divergence on longer (evolutionary) time scales should result in optimization of trait values.

Our results also raise several important questions about the genetic basis of developmental stability, as well as potential causal relationships between FA and fitness. Stressful conditions can increase fluctuating asymmetry (Parsons 1992; Santos *et al*. 2006; Soto *et al*. 2008), so the increase in wing size FA in ML females is consistent with the idea that phenotypic masculinization is stressful for females. An alternative explanation for increased FA in females would be that the ML treatment alters the mutation-selection balance in populations, so that females are free to accumulate mutations at female sex-limited loci. This would make reduced fitness and increased FA a by-product of mutation accumulation at female-specific loci. While we cannot discount this hypothesis outright, only a small proportion of loci are expected to be female limited (Parisi *et al*. 2003), and a previous analysis of the effects of sex-specific selection indicated that most of the decline in the unselected sex could be attributed to a combination of sexually antagonistic loci and mutations that were deleterious in both sexes (Morrow *et al*. 2008). The consistency of results across independent replicate populations also argues against mutation accumulation at female-limited and female-biased loci as the sole explanation for a reduction in female fitness under ML, although it certainly may have played a role. Similarly, although the ML-evolution laboratory protocol does not preclude adaptation to the Y-chromosome and the translocated chromosomes 2 and 3 found in

318 the clone generator females (see Supplementary Information for more details), such
319 adaptation would not explain the sex-specific nature of the fitness and FA results. The
320 selection for perfection model suggests that males should be selected for increased
321 developmental stability relative to females, but other studies have found higher FA in males
322 in a number of different taxa (Bonduriansky 2009; Breuker *et al*. 2007; Davis & Grosse 2008;
323 Söderman *et al*. 2006; Vishalakshi & Singh 2006), and mean male wing size FA was indeed
324 slightly higher than mean female wing size FA in our Control populations. This makes the
325 increase in developmental stability we observed in ML males particularly striking, since it
326 suggests that intralocus sexual conflict is an important factor in determining levels of
327 developmental stability between the sexes.
328
329 The role of FA in mate choice has been widely discussed, and, in particular, the application of
330 this population parameter to the study of individual variation has been called into question
331 (e.g. Houle 1998, but see also Hansen et al. 2006). We unfortunately cannot deduce from the
332 data at hand whether wing size FA contributed directly to increases in ML male fitness via
333 female choice of more symmetrical males, or increased success in intrasexual competition
334 (Møller & Thornhill 1998). Alternatively, FA may simply serve as an indicator trait of high
335 genetic quality/attractiveness, for example if FA is not under direct selection but is negatively
336 correlated with other sexually selected traits (Bonduriansky 2009; Markow & Ricker 1992).
337 ML males evolved increased fitness through higher mating frequency, and behavioural
338 observations have shown that they obtain matings with females with lower courtship effort
339 per copulation (Bedhomme *et al*. 2008). This does not appear to be related to differences
340 between ML and C populations in CHCs (cuticular hydrocarbons; S. Bedhomme, A.K.
341 Chippindale, N.G. Prasad, M. Delcourt, J.K. Abbott, M.A. Mallet and H.D. Rundle,
342 unpublished data), so we can conclude that some other aspect of attractiveness or general

vigour related to precopulatory sexual selection has improved. Interestingly, recent research has shown that in mice, loci coding for environmental robustness (insensitivity of the trait to environmental variation) are almost universally sex-specific (Fraser & Schadt 2010). Whether this is also true in *Drosophila* is currently unknown, but sex-specificity of environmental robustness loci is certainly consistent with our results.

Intralocus sexual conflict will manifest itself when positive intersexual genetic correlations prohibit a response to disruptive selection on the sexes for different phenotypic optima. Consistent with this, ML selection not only led to smaller males, but to increased development time, reflecting a decrease in growth rate through both of its components. At the same time, the wing generally evolved increased phenotypic masculinization (both in terms of size and shape), and the developmental stability of ML males increased. Both of these general results were consistent with our expectations from the selection for perfection model discussed above. Because we saw coordinated changes in female morphology when expressing ML chromosomes, but reduced fitness and lower levels of developmental stability, this provides experimental evidence of strong intersexual genetic correlations for the characters themselves but to differing mechanisms of homeostasis in growth and ontogeny within the two sexes.

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Table 1: Statistical significance of analysis of A. Wing size, B. The slope of the allometric relationship between body mass and wing size, C. Relative fitness, and D. Wing size asymmetry. All measures were analysed using factorial ANOVAs on population mean values in JMP, with sex (M or F), selection regime (C or ML), and their interaction (sex*sel) as fixed factors. Degrees of freedom, sums of squares, F-ratios and *P*-values are reported for all effects.

Effect	DF	SS	F-ratio	*P*-value
A. Wing size				
Sex	1	0.3127	528.1	<0.0001
Selection	1	0.0029	4.818	0.0486
Sex*sel	1	$3.36*10^{-6}$	0.0057	0.9412
Error	12	0.0071		
B. Allometry				
Sex	1	0.1444	1.844	0.1995
Selection	1	0.3833	4.894	0.0471
Sex*sel	1	0.0923	1.178	0.2990
Error	12	0.9399		
C. Relative fitness				
Sex	1	0.0010	0.3540	0.5629
Selection	1	$1.98*10^{-5}$	0.0068	0.9358
Sex*sel	1	0.0284	9.691	0.0090
Error	12	0.0352		
D. Wing size asymmetry				
Sex	1	$2.53*10^{-7}$	1.564	0.2350

Selection	1	$4.26*10^{-8}$	0.2640	0.6167
Sex*sel	1	$9.03*10^{-7}$	5.594	0.0357
Error	12	$1.94*10^{-6}$		

Table 2: Results of MANCOVA analysis of wing shape. Wing shape was analysed using the first 11 relative warps (i.e. principal components of shape) as the dependent variables, with sex (M or F), selection regime (C or ML), and their interaction (sex*sel) as fixed factors. Wing size (centroid size) was also included as a covariate to control for shape differences due to allometric effects. Numerator and denominator degrees of freedom, test statistics (Wilks' λ or F-ratio), and *P*-values are reported for all effects; Wilks' λ is reported for effects with DF > 1, and F-ratio is reported for effects with DF = 1. There were significant effects of both sex and selection regime on wing shape, as well a significant allometric effect of wing size on wing shape.

Effect	Num DF	Den DF	Wilks' λ	F-ratio	*P*-value
Whole model	44	5.78	$9.04*10^{9}$		0.0012
Intercept	11	1		754.8	0.0284
Sex	11	1		1928	0.0178
Selection	11	1		3157	0.0139
Sex*sel	11	1		29.85	0.1419
Wing size	11	1		760.6	0.0283

Figure 1: Landmark locations (A) and wing shape differences (B-C). A. Locations of the 11 landmarks used in this study. B. Visualization of the difference in wing shape between the sexes. Arrows indicate the direction of change from female configuration to male in Control individuals. For the sake of clarity, the difference in shape between the sexes has been exaggerated by a factor of three. C. Visualization of the change in wing shape as a result of male-limited (ML) evolution (males and females pooled). Arrows indicate the direction of change from Control configuration to ML for both sexes. The difference in shape between selection regimes is smaller than between the sexes, so the difference in shape between ML and C groups has been exaggerated by a factor of 10 for the sake of clarity. The change in shape resulting from ML evolution is qualitatively similar to the extant sexual dimorphism for shape, in that both involve an increase in the size of the distal part of the wing, and a decrease in the size of the proximal part of the wing.

Figure 2: Sex by selection interaction in A. Wing size, B. Allometry, C. Relative fitness, and D. Developmental stability (measured as the inverse of the population mean fluctuating asymmetry of wing size). A. Males have smaller wings than females, and ML individuals have smaller wings than Control individuals. This is consistent with previous results for body size. B. The slope of the regression of wing size on body mass was higher for ML flies than for C flies. This suggests an evolutionary change not only in isolated traits, but in a number of interrelated aspects of the developmental program. C. Male fitness was measured as the proportion of the progeny sired by experimental males when in competition with standard competitor males for the access to females. Female fitness was measured as the total progeny produced after experimental females had been in competition with standard competitor females for access to food resources. To make male and female data comparable, fitness is expressed relative to the mean fitness for each sex within each replicate population. The ML

evolution procedure led to an increase in male fitness and a decrease in female fitness, confirming the presence in the ancestral population of sexually antagonistic variation and a gender load. D. ML males have higher developmental stability than C males, while the pattern is reversed for females (i.e. ML females have higher FA than C females; data shown is standardized for size differences, but the pattern is similar for raw data). This suggests that experimental ML evolution has resulted in an increase in developmental stability in males at the cost of a decrease in developmental stability in females. Error bars denote SEs.

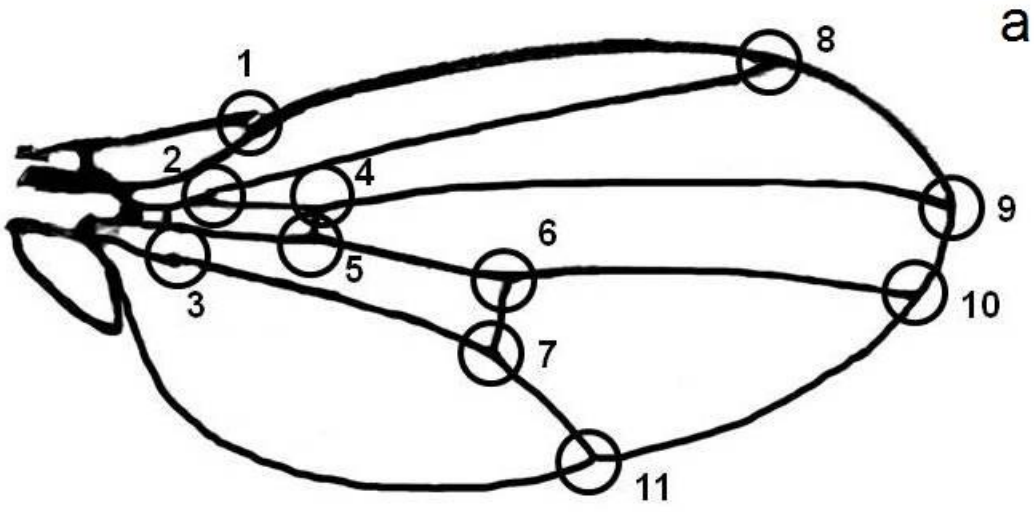

Figure 1A

Figure 1B

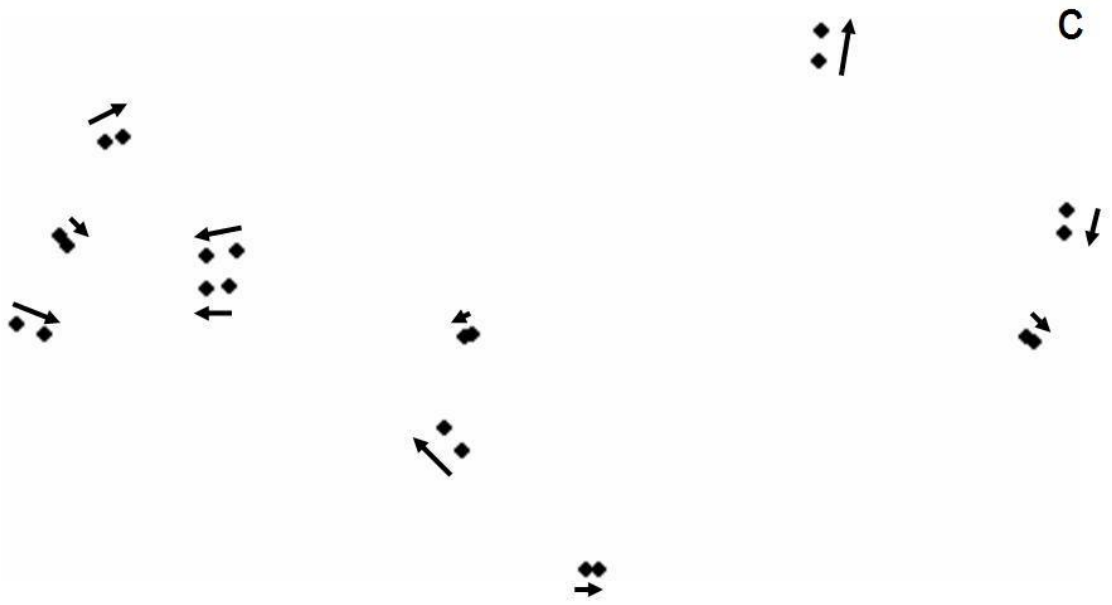

Figure 1C

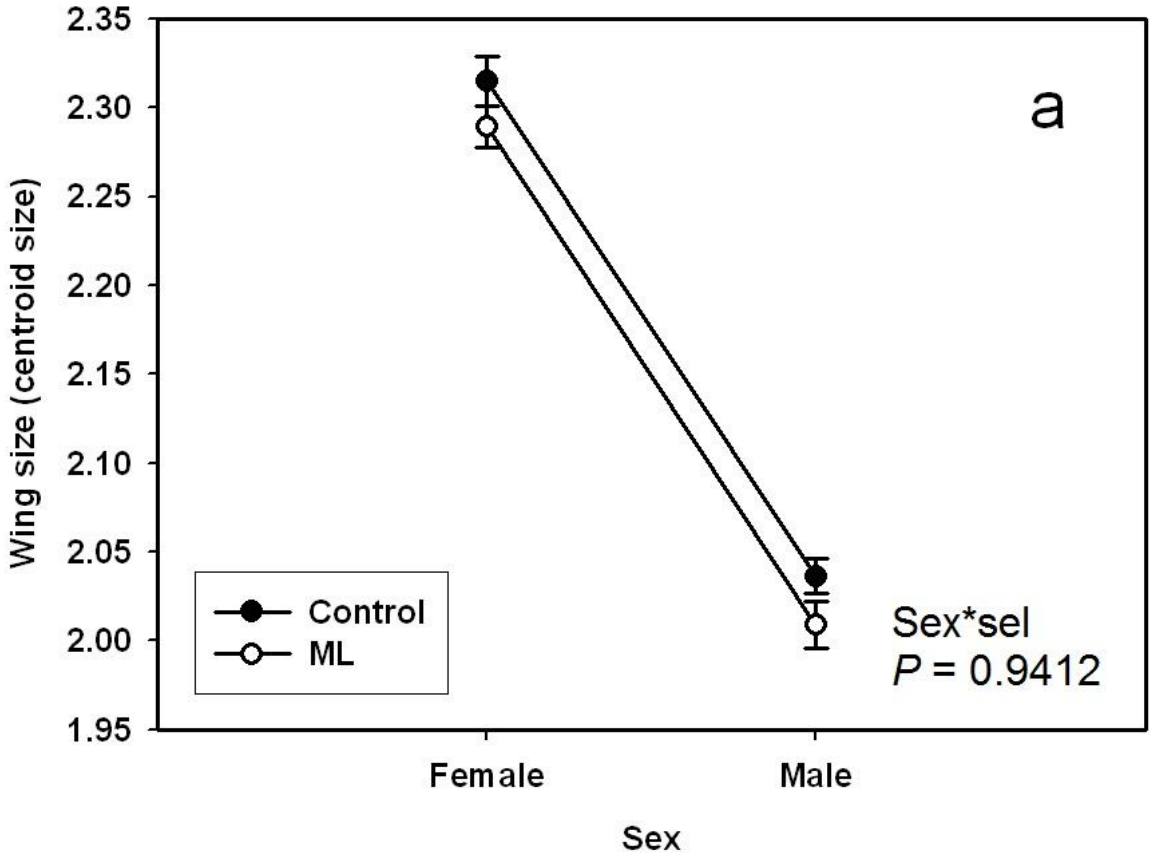
2.35
2.30
2.25
2.20
2.15
2.10
2.05
2.00
1.95
Wing size (centroid size)
a
Control
ML
Sex*sel
P = 0.9412
Female
Male
Sex

Figure 2A

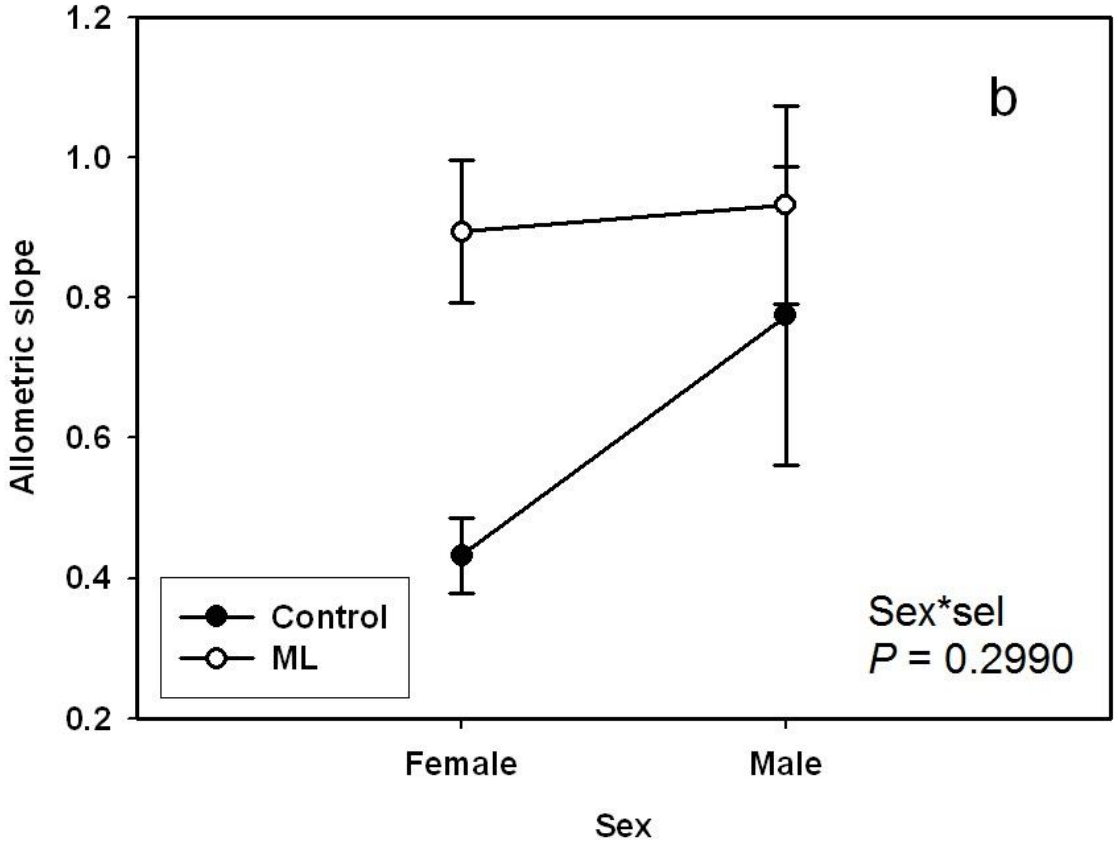
b
Allometric slope
1.2
1.0
0.8
0.6
0.4
0.2
Control
ML
Sex*sel
P = 0.2990
Female
Male
Sex

Figure 2B

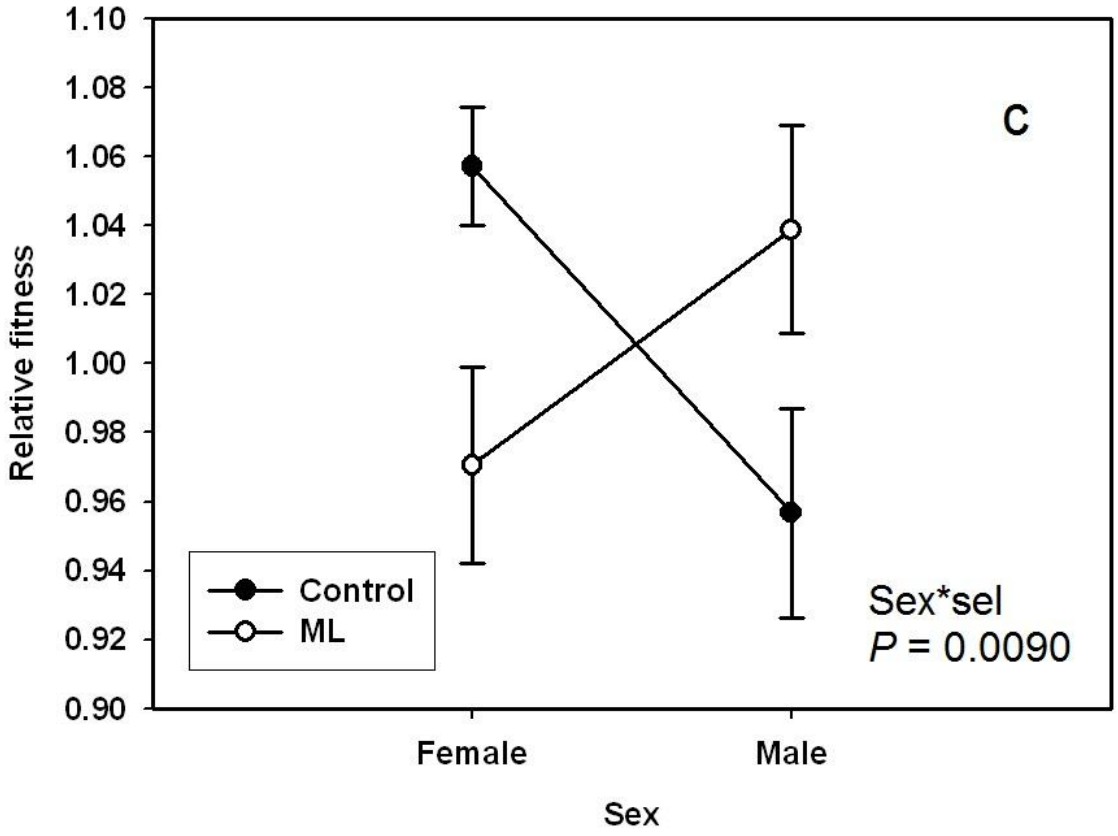
1.10
1.08
1.06
1.04
1.02
1.00
0.98
0.96
0.94
0.92
0.90
Relative fitness
C
Control
ML
Sex*sel
P = 0.0090
Female
Male
Sex

Figure 2C

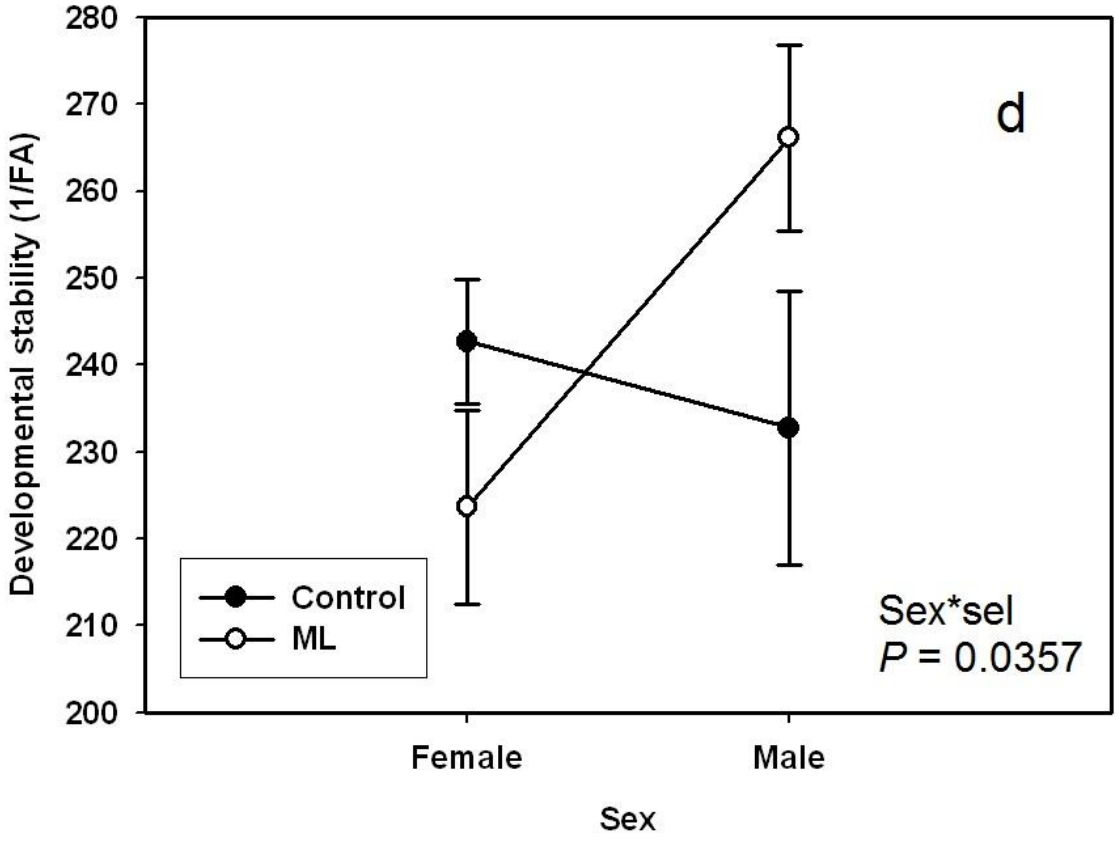
280
270
260
250
240
230
220
210
200
Developmental stability (1/FA)
d
Control
ML
Sex*sel
P = 0.0357
Female
Male
Sex

Figure 2D

Sexual conflict in wing size and shape in *Drosophila melanogaster*: Supplementary information

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SUPPLEMENTARY METHODS

Male-limited evolution protocol

The derivation of the male-limited (ML) lines and their matching controls (C) is described in detail elsewhere (Prasad *et al.*, 2007). Briefly, the ancestral population is the LH_M population, a laboratory-adapted outbred population (Chippindale & Rice, 2001). Four large subpopulations were derived from the ancestral population and maintained in isolation for 10 generations. From each of these populations, one pair of selected (ML_{1-4}) and control (C_{1-4}) populations was initiated. Selected and control populations bearing the same numerical subscript were therefore more closely related to one another through their common ancestry and subsequent handling than to other selected or control populations. To initiate an ML population, 1040 haplotypes, consisting of chromosomes I (X), II, and III, but not the tiny chromosome IV (i.e. more than 99% of the genome in total, hereafter referred to as haplotypes) were sampled using "clone generator females" carrying a compound X(C(1)DX, *y, f*), a Y chromosome from the LH_M base population, and a homozygous-viable translocation of the two major autosomes (T(2:3)*rdgc st in ri* p^p *bw*). These chromosomal constructs and the absence of molecular recombination in male *D. melanogaster* mediate the transmission of the haplotypes from father to son. The males carrying a translocation and a wildtype haplotype originally sampled from LH_M were crossed each generation to "clone generator females". In this way, these haplotypes were transmitted from father to son only, the grand-maternal haplotypes being discarded every generation. Efforts were made to standardize the effective population size between selected (ML) and control (C) populations by maintaining the same number of haploid genomes in each. Finally, the same maintenance protocol was used for C and ML populations, except that the C populations had normal transmission of

genetic material from one generation to the next, via both males and females. This experimental protocol completely prevented recombination in the ML populations, which could slow down their rate of adaptation due to genetic hitchhiking, mutation accumulation, and background selection. To prevent this, in each generation 4% of the genomes were passed through a series of crosses in which the ML haplotypes were expressed in females, allowing them to recombine (Prasad *et al.*, 2007). Because this 'recombination loop' constantly received new ML-selected chromosomes, females in it were carrying ML chromosomes from the previous generations of selection. These recombined ML haplotypes were then reintroduced into the general ML population.

All flies were reared at 25°C in 50% relative humidity in a 12:12h light/dark cycle under moderate densities of approximately 150 larvae per vial.

Generation of males and females expressing ML and C genotypes.

At generation 82 of experimental evolution, flies were collected to start a series of three crosses necessary to generate the individuals for fitness measurements and wing morphology analysis. Males from the ML selection treatment were first crossed to the clone generator females described in the main text. The F1 males produced from this cross were then mated to females that were homozygous for a balancer X chromosome (FM7) and translocation (T (2 : 3)*rdgc st in ri pp bw*). F2 females that were heterozygous for the balancer X but homozygous for the translocation were then back-crossed to the F1 males. The offspring of this third cross were therefore males and females carrying one ML or C haplotype and the translocation of chromosomes 2 and 3 used to evolve the ML populations.

SUPPLEMENTARY RESULTS

Both the sexes and the experimental groups differed in dry body mass (Table S1A). Males were significantly smaller than females, and ML individuals were smaller than C individuals (Figure S2A). This is similar to previous results for dry body mass (Prasad *et al.*, 2007). The pattern was the same for wing loading. Females had higher wing loading than males and C had higher wing loading than ML (Table S2B, Figure S1B).

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Table S1: Means for each combination of population, sex, and selection regime for all univariate traits. Loading is short for wing loading.

Population	Sex	Selection	Body mass	Wing size	Loading	Allometry	Fitness	FA
1	Female	Control	0.3864	2.280	0.1694	0.3937	1.061	0.0042
1	Male	Control	0.2480	2.031	0.1221	1.256	0.9461	0.0050
2	Female	Control	0.4388	2.317	0.1892	0.4202	1.103	0.0038
2	Male	Control	0.2453	2.039	0.1203	0.3993	0.8998	0.0042
3	Female	Control	0.4261	2.347	0.1816	0.3331	1.040	0.0044
3	Male	Control	0.2469	2.061	0.1198	1.007	1.042	0.0047
4	Female	Control	0.4103	2.316	0.1770	0.5837	1.024	0.0042
4	Male	Control	0.2358	2.014	0.1172	0.4355	0.9386	0.0037
1	Female	ML	0.3930	2.289	0.1715	0.7412	1.038	0.0046
1	Male	ML	0.2312	1.996	0.1158	1.323	1.036	0.0039
2	Female	ML	0.3629	2.319	0.1564	1.133	0.8989	0.0046
2	Male	ML	0.2304	2.048	0.1124	0.8471	1.100	0.0036
3	Female	ML	0.3813	2.263	0.1686	0.9944	0.9679	0.0039

3	Male	ML	0.2265	1.991	0.1138	0.9049	0.9575	0.0034
4	Female	ML	0.3675	2.287	0.1606	0.7077	0.9776	0.0049
4	Male	ML	0.2266	1.999	0.1131	0.6538	1.061	0.0041

Table S2: Statistical significance of analysis of A. Body mass, and B. Wing loading. Mean values for each combination of sex, replicate population, and selection regime were first calculated and then were analyzed using a factorial ANOVA in JMP, with sex (M or F), selection regime (C or ML), and their interaction (sex*sel) as fixed factors. Degrees of freedom, SS, F-ratios and *P*-values are reported for all effects.

	Effect	DF	SS	F-ratio	*P*-value
A. Body mass					
	Sex	1	0.1017	555.5	<0.0001
	Selection	1	0.0030	16.27	0.0017
	Sex*sel	1	0.0006	3.119	0.1028
	Error	12	0.0022		
B. Wing loading					
	Sex	1	0.0121	390.3	<0.0001
	Selection	1	0.0004	14.46	0.0025
	Sex*sel	1	$8.1*10^{-5}$	2.617	0.1317
	Error	12	0.0004		

Figure S1: Differences between the sexes and experimental groups in A. Dry body mass, and B. Wing loading. Males were smaller than females, and ML individuals were smaller than C individuals. Similarly, females had higher wing loading than males and C had higher wing loading than ML. Error bars denote SEs.

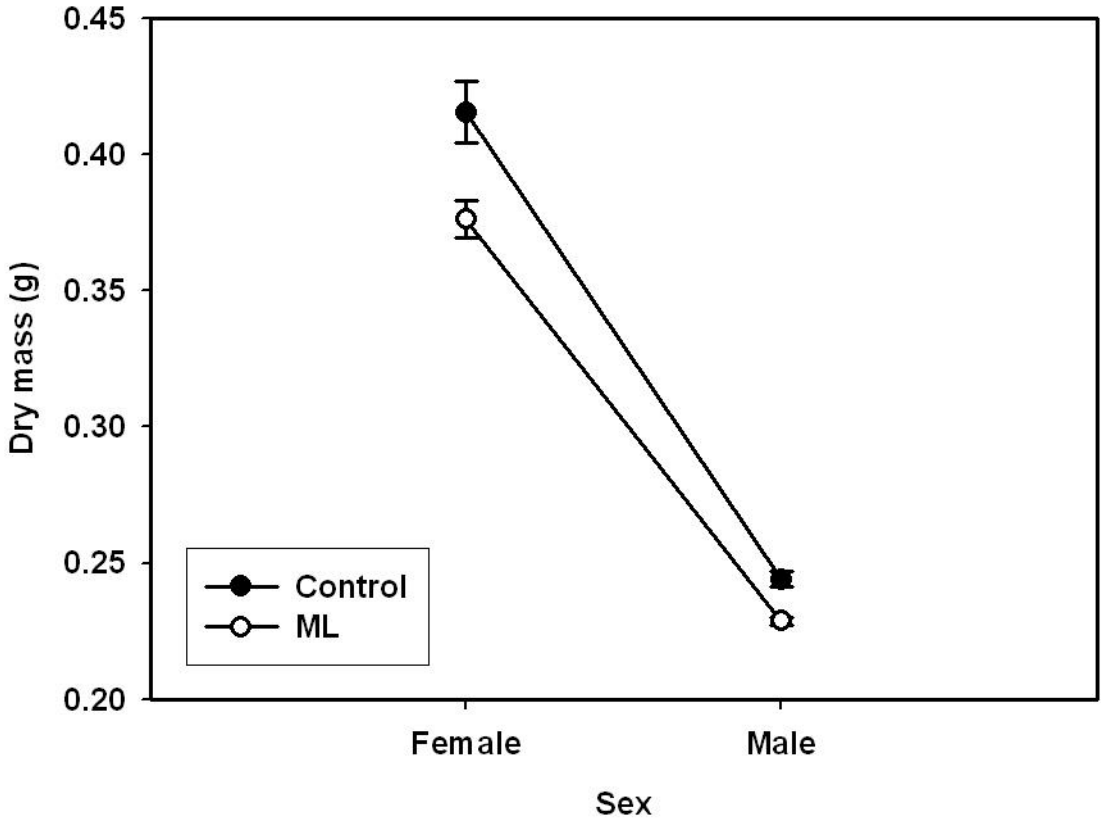

Figure S1A

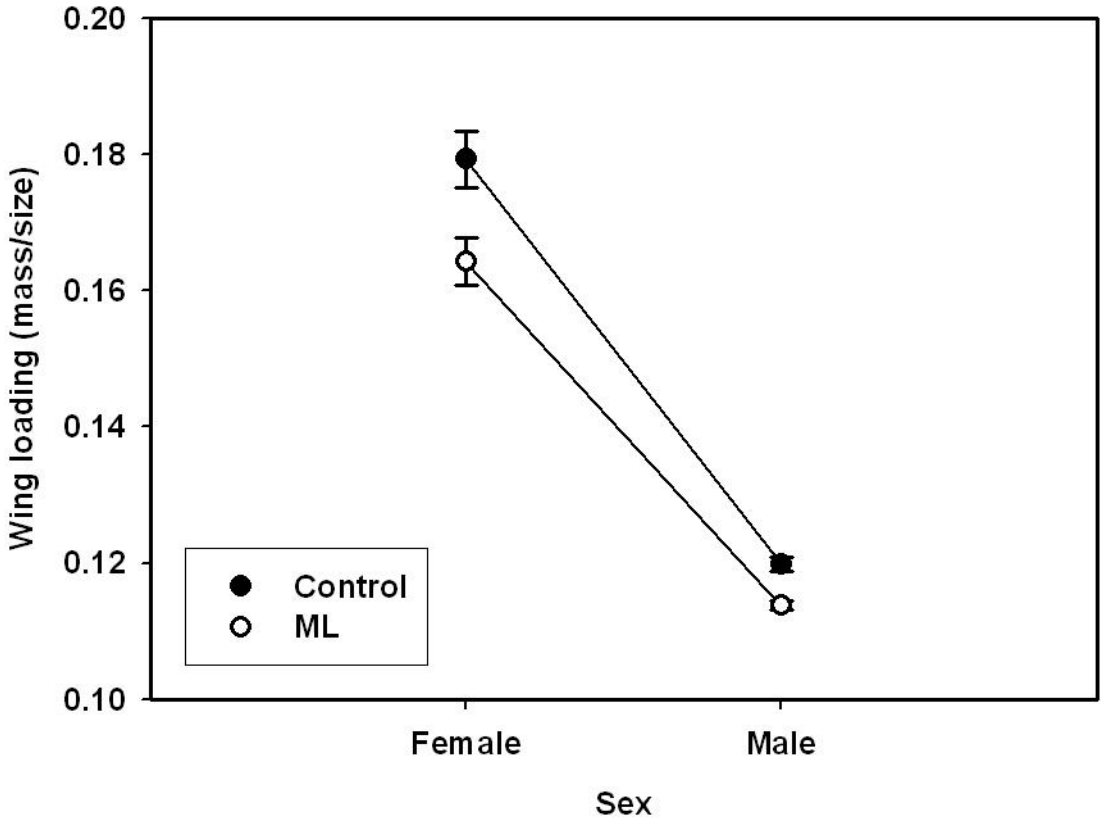
0.20
0.18
0.16
0.14
0.12
0.10
Wing loading (mass/size)
Control
ML
Female
Male
Sex

Figure S1B