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

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ABSTRACT

1

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

14

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

17

18 INTRODUCTION

19

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

35

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

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

58

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

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

86

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

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

101

102 METHODS

103

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

110

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

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

120

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

130

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

138

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

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

160

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

168 differences in wing size could simply be attributed to the evolution of differences in body
169 size.
170
171 Developmental stability in wing size was examined using fluctuating asymmetry (FA)
172 analysis (Palmer 1994; Palmer & Strobeck 2002). Because male and female *Drosophila*
173 *melanogaster* differ substantially in size, size-standardized wing size asymmetry values were
174 calculated via $\ln(R)-\ln(L)$ (Palmer & Strobeck 2002). We carried out analysis on both
175 standardized data (i.e. using $\ln(R)-\ln(L)$ values) and raw data (i.e. using raw size and shape
176 values), but since results were qualitatively similar for both datasets, only the standardized
177 analysis is presented in detail here. Before any tests of wing size FA were performed, an
178 ANOVA was carried out to quantify and test the different components of asymmetry: error,
179 FA, and directional asymmetry (DA; see Palmer & Strobeck 2002 for details). FA was large
180 relative to error variance and therefore significant ($F_{964, 1394} = 8034, P < 0.0001$), and although
181 there was significant DA ($F_{1, 1394} = 63.77, P < 0.0001$), this was probably mostly due to the
182 large size of the dataset (Palmer & Strobeck 2002). The side*wing size effect was very small
183 (Cohen's $d = 0.0194$), indicating that DA was much smaller than the average deviation around
184 the mean. It was therefore not deemed necessary to correct for DA (Palmer & Strobeck
185 2002). Signed asymmetry values were normally distributed. Mean absolute asymmetry
186 values for each combination of sex, replicate population, and selection regime were calculated
187 (N=16) and then were analyzed using a factorial ANOVA in JMP, with sex (M or F),
188 selection regime (C or ML), and their interaction (sex*sel) as fixed factors (this is equivalent
189 to Levene's test; Palmer & Strobeck 2002).
190
191 Similarly, mean values for each combination of sex, replicate population, and selection
192 regime were calculated (N=16) for all other univariate traits (wing size, wing loading, body

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

206

207 RESULTS

208

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

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

220

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

232

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

242

243 DISCUSSION

244

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

260

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

268 This is consistent with previous research on *Drosophila melanogaster* which has shown that
269 intersexual genetic correlations for wing and body size traits generally range from 0.6 to 1
270 (Cowley & Atchley 1988; Cowley *et al.* 1986; Karan *et al.* 2000; Karan *et al.* 1999; Reeve &
271 Fairbairn 1996), with a mean around 0.8 (Poissant *et al.* 2009, supplementary information).
272

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

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

300

301 Our results also raise several important questions about the genetic basis of developmental
302 stability, as well as potential causal relationships between FA and fitness. Stressful conditions
303 can increase fluctuating asymmetry (Parsons 1992; Santos *et al.* 2006; Soto *et al.* 2008), so
304 the increase in wing size FA in ML females is consistent with the idea that phenotypic
305 masculinization is stressful for females. An alternative explanation for increased FA in
306 females would be that the ML treatment alters the mutation-selection balance in populations,
307 so that females are free to accumulate mutations at female sex-limited loci. This would make
308 reduced fitness and increased FA a by-product of mutation accumulation at female-specific
309 loci. While we cannot discount this hypothesis outright, only a small proportion of loci are
310 expected to be female limited (Parisi *et al.* 2003), and a previous analysis of the effects of
311 sex-specific selection indicated that most of the decline in the unselected sex could be
312 attributed to a combination of sexually antagonistic loci and mutations that were deleterious
313 in both sexes (Morrow *et al.* 2008). The consistency of results across independent replicate
314 populations also argues against mutation accumulation at female-limited and female-biased
315 loci as the sole explanation for a reduction in female fitness under ML, although it certainly
316 may have played a role. Similarly, although the ML-evolution laboratory protocol does not
317 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

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

348

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

361

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369 Reference List

370

371 Bedhomme, S., N. G. Prasad, P.-P. Jiang and A. K. Chippindale. 2008. Reproductive behavior
372 evolves rapidly when intralocus sexual conflict is removed. *PLoS One*, 3: e2187.

373 Blanckenhorn, W. U., A. F. G. Dixon, D. J. Fairbairn, M. W. Foellmer, P. Gibert, K. van der
374 Linde, R. Meier, S. Nylin, S. Pitnick, C. Schoff, M. Signorelli, T. Teder and C. Wiklund.
375 2007. Proximate causes of Rensch's rule: does sexual size dimorphism in arthropods result
376 from sex differences in development time? *Am. Nat.*, 169: 245-257.

377 Bonduriansky, R. 2009. Condition dependence of developmental stability in the sexually
378 dimorphic fly *Telostylinus angusticollis* (Diptera: Neriidae). *J. Evol. Biol.*, 22: 861-872.

379 Bonduriansky, R. and S. F. Chenoweth. 2009. Intralocus sexual conflict. *Trends Ecol. Evol.*,
380 24: 280-288.

381 Brandt, Y. and M. C. B. Andrade. 2007. What is the matter with the gravity hypothesis?
382 *Funct. Ecol.*, 21: 1182-1183.

383 Breuker, C. J., P. M. Brakefield and M. Gibbs. 2007. The association between wing
384 morphology and dispersal is sex-specific in the glanville fritillary butterfly *Melitaea cinxia*
385 (Lepidoptera: Nymphalidae). *European Journal of Entomology*, 104: 445-452.

386 Breuker, C. J., J. S. Patterson and C. P. Klingenberg. 2006. A single basis for developmental
387 buffering of *Drosophila* wing shape. *PLoS One*, 1: 7.

388 Chippindale, A. K., A. L. Ngo and M. R. Rose. 2003. The devil in the details of life-history
389 evolution: instability and reversal of genetic correlations during selection on *Drosophila*
390 development. *J. Genetics*, 82: 133-145.

391 Clarke, G. M. 1998. The genetic basis of developmental stability. IV. Individual and
392 population asymmetry parameters. *Heredity*, 80: 553-561.

393 Cowley, D. E. and W. R. Atchley. 1988. Quantitative genetics of *Drosophila melanogaster*.
394 II. Heritabilities and genetic correlations between sexes for head and thorax traits. *Genetics*,
395 119: 421-433.

396 Cowley, D. E., W. R. Atchley and J. J. Rutledge. 1986. Quantitative genetics of *Drosophila*
397 *melanogaster*. I. Sexual dimorphism in genetic parameters for wing traits. *Genetics*, 114: 549-
398 566.

399 Cox, R. M. and R. Calsbeek. 2009. Sexually antagonistic selection, sexual dimorphism, and
400 the resolution of intralocus sexual conflict. *Am. Nat.*, 173: 176-187.

- 401 Davis, A. K. and A. M. Grosse. 2008. Measuring fluctuating asymmetry in plastron scutes of
402 yellow-bellied sliders: the importance of gender, size and body location. *Am. Midl. Nat.*, 159:
403 340-348.
- 404 Fraser, H. B. and E. E. Schadt. 2010. The quantitative genetics of phenotypic robustness.
405 *PLoS One*, 5: e8635.
- 406 Frazier, M. R., J. F. Harrison, S. D. Kirkton and S. P. Roberts. 2008. Cold-rearing improves
407 cold-flight performance in *Drosophila* via changes in wing morphology. *J. Exp. Biol.*, 211:
408 2116-2122.
- 409 Gidaszewski, N. A., M. Baylac and C. P. Klingenberg. 2009. Evolution of sexual
410 diimorphism of wing shape in the *Drosophila melanogaster* subgroup. *BMC Evolutionary*
411 *Biology*, 9: 110.
- 412 Gilchrist, G. W. and R. B. Huey. 2004. Plastic and genetic variation in wing loading as a
413 function of temperature within and among parallel clines in *Drosophila subobscura*.
414 *Integrative and Comparative Biology*, 44: 461-470.
- 415 Gilchrist, G. W., R. B. Huey and L. Serra. 2001. Rapid evolution of wing size clines in
416 *Drosophila subobscura*. *Genetica*, 112-113: 273-286.
- 417 Hansen, T. F., A. J. R. Carter and C. Pélabon. 2006. On adaptive accuracy and precision in
418 natural populations. *Am. Nat.*, 168: 168-181.
- 419 Houle, D. 1998. High enthusiasm and low *r*-squared. *Evolution*, 52: 1872-1876.
- 420 James, A. C., R. B. R. Azevedo and L. Partridge. 1995. Cellular basis and developmental
421 timing in a size cline of *Drosophila melanogaster*. *Genetics*, 140: 659-666.
- 422 Karan, D., J.-P. Morin, P. Gibert, B. Moreteau, S. M. Scheiner and J. R. David. 2000. The
423 genetics of phenotypic plasticity. IX. Genetic architecture, temperature, and sex differences in
424 *Drosophila melanogaster*. *Evolution*, 54: 1035-1040.
- 425 Karan, D., J.-P. Morin, E. Gravot, B. Moreteau and J. R. David. 1999. Body size reaction
426 norms in *Drosophila melanogaster*: temporal stability and genetic architecture in a natural
427 population. *Genetics, Selection, Evolution*, 31: 491-508.
- 428 Klingenberg, C. P. and G. S. McIntyre. 1998. Geometric morphometrics of developmental
429 instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. *Evolution*,
430 52: 1363-1375.
- 431 Long, T. A. F. and W. R. Rice. 2007. Adult locomotory activity mediates intralocus sexual
432 conflict in a laboratory-adapted population of *Drosophila melanogaster*. *Proc. R. Soc. Lond.*
433 *B Biol. Sci.*, 274: 3105-3112.
- 434 Maklakov, A. A., T. Bilde and Y. Lubin. 2004. Sexual selection for increased male body size
435 and protandry in a spider. *Anim. Behav.*, 68: 1041-1048.
- 436 Markow, T. A. and J. P. Ricker. 1992. Male size, developmental stability, and mating success
437 in natural populations of three *Drosophila* species. *Heredity*, 69: 122-127.

- 438 Mezey, J. G. and D. Houle. 2005. The dimensionality of genetic variation for wing shape in
439 *Drosophila melanogaster*. *Evolution*, 59: 1027-1038.
- 440 Miller, G. T. and S. Pitnick. 2003. Sperm-female coevolution in *Drosophila*. *Science*, 298:
441 1230-1233.
- 442 Møller, A. P. and R. Thornhill. 1998. Bilateral symmetry and sexual selection: a meta-
443 analysis. *Am. Nat.*, 151: 174-192.
- 444 Morrow, E. H., A. D. Stewart and W. R. Rice. 2008. Assessing the extent of genome-wide
445 intralocus sexual conflict via experimentally enforced gender-limited selection. *J. Evol. Biol.*,
446 21: 1046-1054.
- 447 Palmer, A. R. 1994. Fluctuating asymmetry analyses: a primer in Markow, T. A. (ed)
448 *Developmental instability: its origins and evolutionary implications*. Kluwer, Dordrecht,
449 Netherlands.
- 450 Palmer, A. R. and C. Strobeck. 2002. Fluctuating asymmetry analyses revisited in Polak, M.
451 (ed) *Developmental instability: causes and consequences*. Oxford University Press, Oxford,
452 UK.
- 453 Parisi, M., R. Nuttall, D. Naiman, G. Bouffard, J. Malley, J. Andrews, S. Eastman and B.
454 Oliver. 2003. Paucity of genes on the *Drosophila* X chromosome showing male-biased
455 expression. *Science*, 299: 697-700.
- 456 Parsons, P. A. 1992. Fluctuating asymmetry: a biological monitor of environmental and
457 genomic stress. *Heredity*, 68: 361-368.
- 458 Partridge, L., B. Barrie, K. Fowler and V. French. 1994. Evolution and development of body
459 size and cell size in *Drosophila melanogaster* in response to temperature. *Evolution*, 48:
460 1269-1276.
- 461 Poissant, J., A. J. Wilson and D. W. Coltman. 2009. Sex-specific genetic variance and the
462 evolution of sexual dimorphism: a systematic review of cross-sex genetic correlations.
463 *Evolution*, 64: 97-107.
- 464 Powell, A. M., M. Davis and J. R. Powell. 2010. Phenotypic plasticity across 50 MY of
465 evolution: *Drosophila* wing size and temperature. *Journal of Insect Physiology*, 56: 380-382.
- 466 Prasad, N. G., S. Bedhomme, T. Day and A. K. Chippindale. 2007. An evolutionary cost of
467 separate genders revealed by male-limited expression. *Am. Nat.*, 169: 29-37.
- 468 Reeve, H. K. and D. J. Fairbairn. 1996. Sexual size dimorphism as a correlated response to
469 selection on body size: an empirical test of the quantitative genetic model. *Evolution*, 50:
470 1927-1938.
- 471 Rice, W. R. 1984. Sex chromosomes and the evolution of sexual dimorphism. *Evolution*, 38:
472 735-742.
- 473 Rice, W. R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of
474 female evolution. *Nature*, 381: 232-234.

- 475 Santos, M., D. Brites and H. Laayouni. 2006. Thermal evolution of pre-adult life history
476 traits, geometric size and shape, and developmental stability in *Drosophila subobscura*. *J.*
477 *Evol. Biol.*, 19: 2006-2021.
- 478 Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. *Evolution*, 50:
479 1766-1774.
- 480 Söderman, F., S. van Dongen, S. Pakkasmaa and J. Merilä. 2006. Environmental stress
481 increases skeletal fluctuating asymmetry in the moor frog *Rana arvalis*. *Oecologia*, 151: 593-
482 604.
- 483 Soto, I. M., V. P. Carreira, E. M. Soto and E. Hasson. 2008. Wing morphology and
484 fluctuating asymmetry depend on the host plant in cactophilic *Drosophila*. *J. Evol. Biol.*, 21:
485 298-609.
- 486 Taylor, C. E. and V. Kekic. 1988. Sexual Selection in a Natural Population of *Drosophila*
487 *melanogaster*. *Evolution*, 42: 197-199.
- 488 Vishalakshi, C. and B. N. Singh. 2006. Fluctuating asymmetry in certain morphological traits
489 in laboratory populations of *Drosophila ananassae*. *Genome*, 49: 777-785.
490
491

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	<i>P</i> -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	<i>P</i> -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.

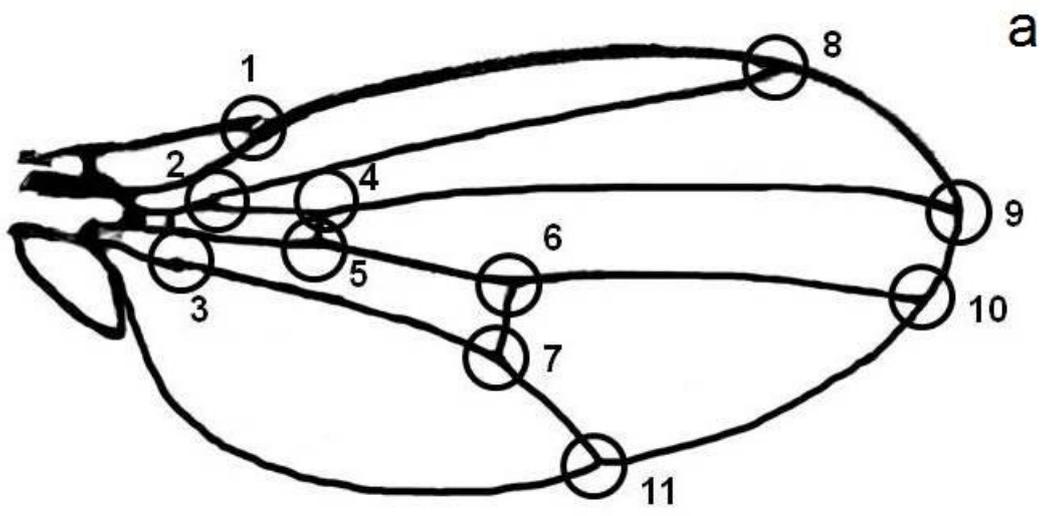


Figure 1A

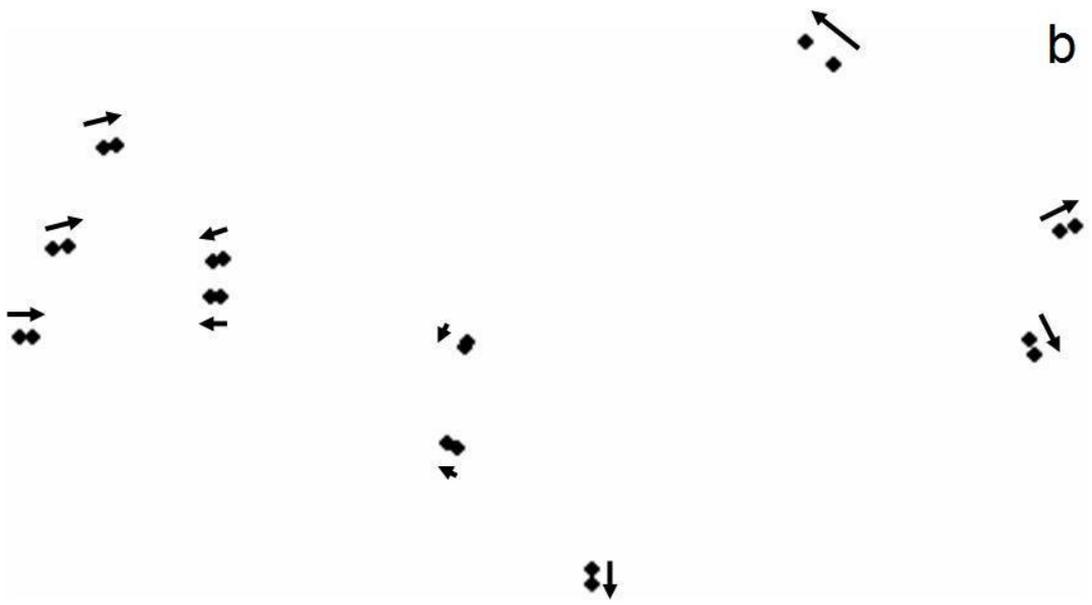


Figure 1B

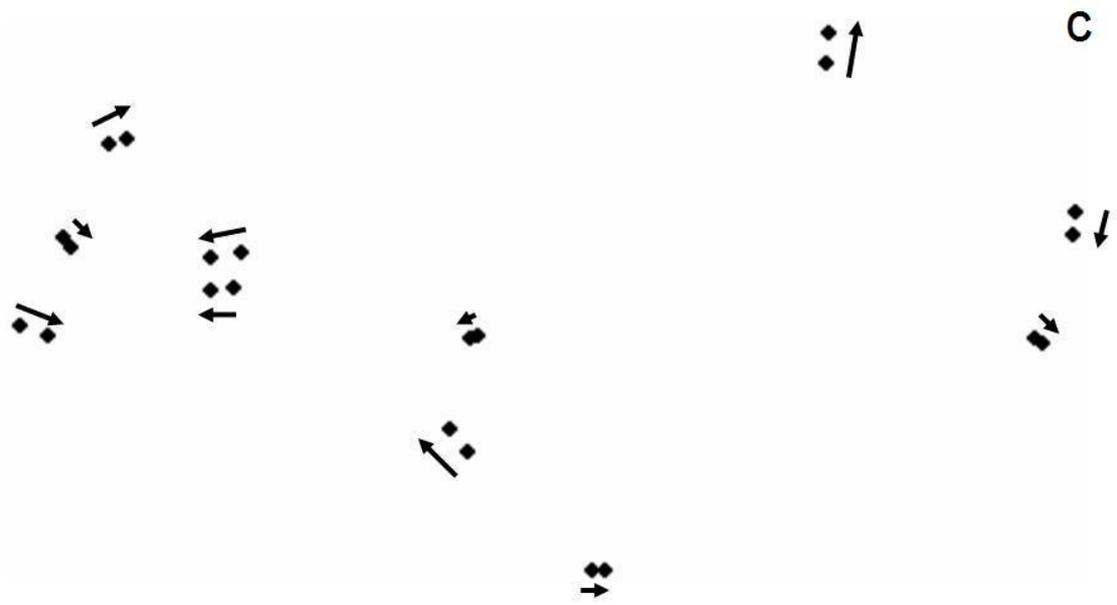


Figure 1C

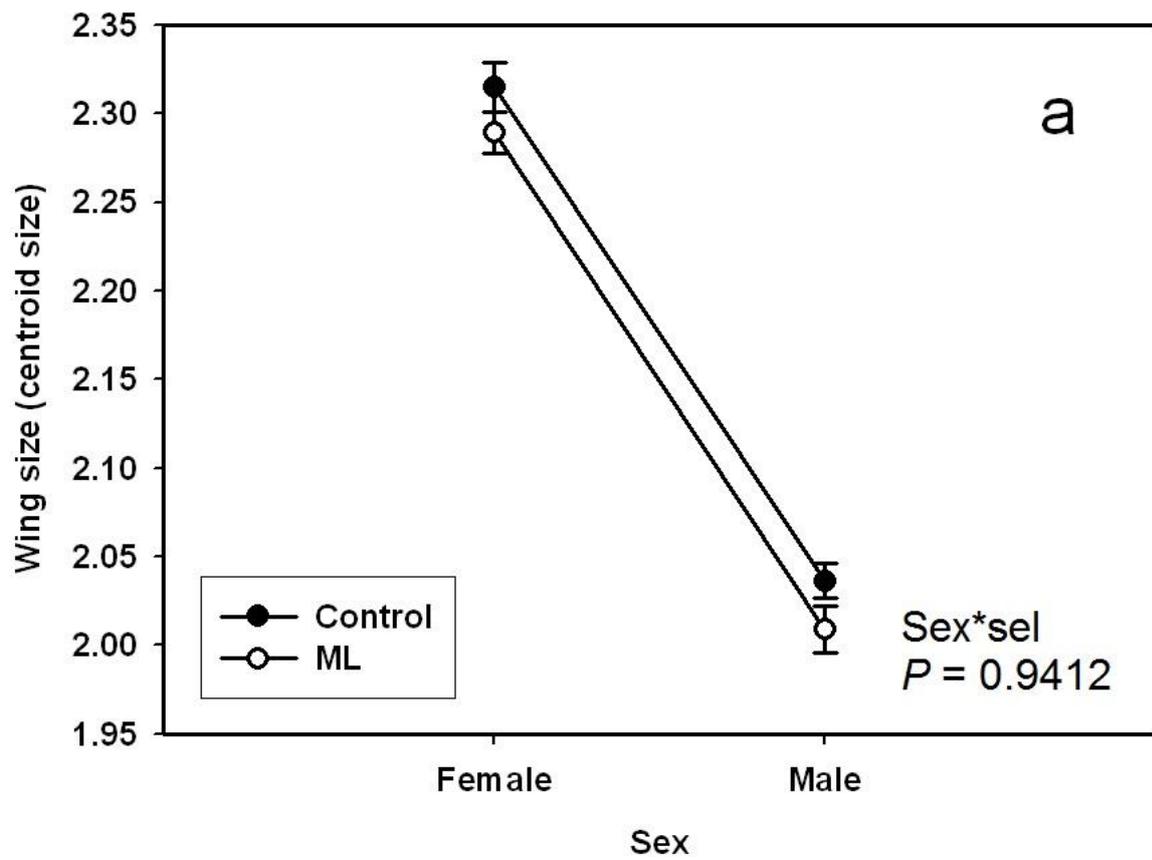


Figure 2A

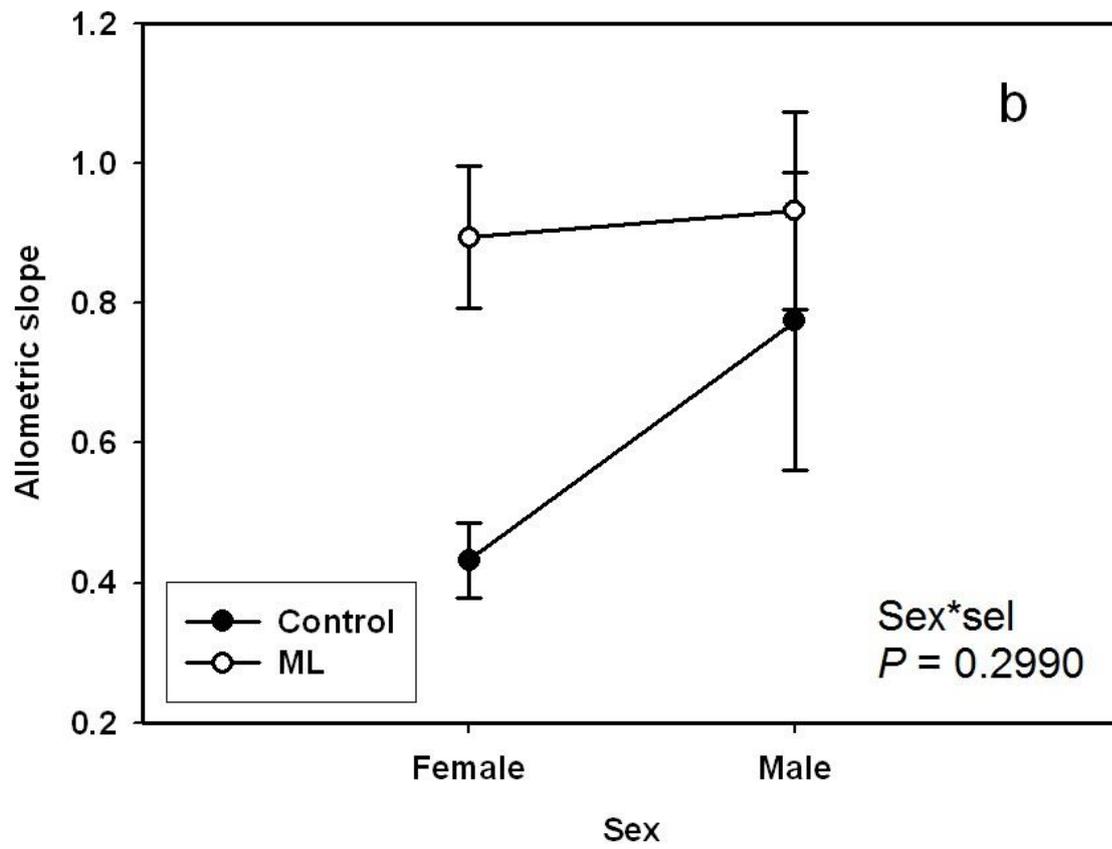


Figure 2B

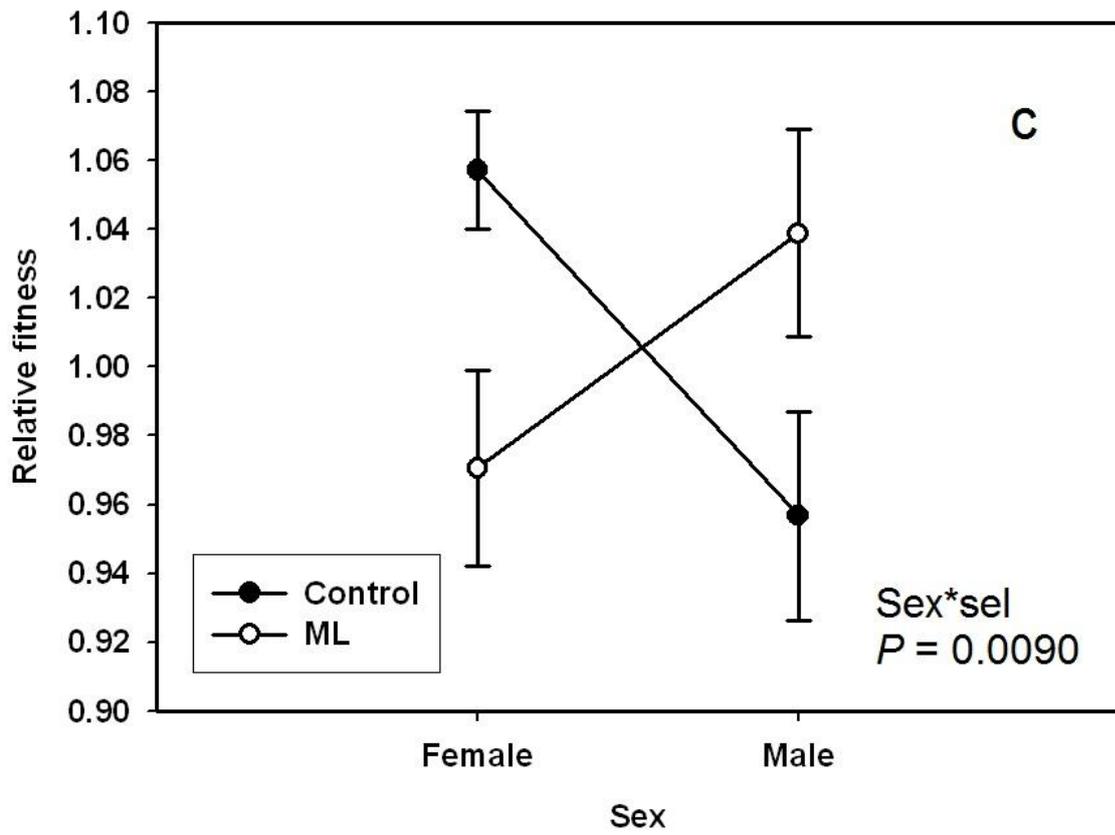


Figure 2C

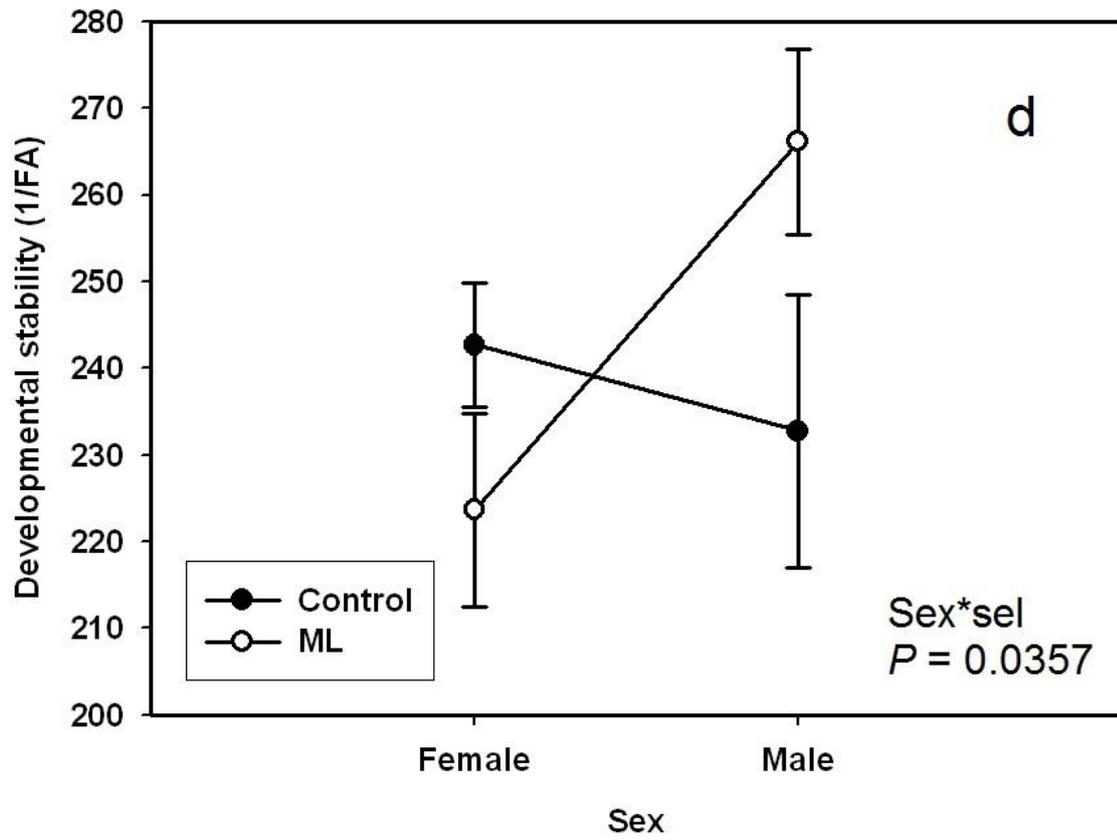


Figure 2D

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

Supplementary information

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493 SUPPLEMENTARY METHODS

494

495 Male-limited evolution protocol

496

497 The derivation of the male-limited (ML) lines and their matching controls (C) is described in
498 detail elsewhere (Prasad *et al.*, 2007). Briefly, the ancestral population is the LH_M population,
499 a laboratory-adapted outbred population (Chippindale & Rice, 2001). Four large
500 subpopulations were derived from the ancestral population and maintained in isolation for 10
501 generations. From each of these populations, one pair of selected (ML₁₋₄) and control (C₁₋₄)
502 populations was initiated. Selected and control populations bearing the same numerical
503 subscript were therefore more closely related to one another through their common ancestry
504 and subsequent handling than to other selected or control populations. To initiate an ML
505 population, 1040 haplotypes, consisting of chromosomes I (X), II, and III, but not the tiny
506 chromosome IV (i.e. more than 99% of the genome in total, hereafter referred to as
507 haplotypes) were sampled using “clone generator females” carrying a compound X(C(1)DX,
508 y, f), a Y chromosome from the LH_M base population, and a homozygous-viable translocation
509 of the two major autosomes (T(2:3)*rdgc st in ri p^p bw*). These chromosomal constructs and
510 the absence of molecular recombination in male *D. melanogaster* mediate the transmission of
511 the haplotypes from father to son. The males carrying a translocation and a wildtype
512 haplotype originally sampled from LH_M were crossed each generation to “clone generator
513 females”. In this way, these haplotypes were transmitted from father to son only, the grand-
514 maternal haplotypes being discarded every generation. Efforts were made to standardize the
515 effective population size between selected (ML) and control (C) populations by maintaining
516 the same number of haploid genomes in each. Finally, the same maintenance protocol was
517 used for C and ML populations, except that the C populations had normal transmission of

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

527

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

530

531 Generation of males and females expressing ML and C genotypes.

532

533 At generation 82 of experimental evolution, flies were collected to start a series of three
534 crosses necessary to generate the individuals for fitness measurements and wing morphology
535 analysis. Males from the ML selection treatment were first crossed to the clone generator
536 females described in the main text. The F1 males produced from this cross were then mated
537 to females that were homozygous for a balancer X chromosome (FM7) and translocation (T
538 (2 : 3)*rdgc st in ri pp bw*). F2 females that were heterozygous for the balancer X but
539 homozygous for the translocation were then back-crossed to the F1 males. The offspring of
540 this third cross were therefore males and females carrying one ML or C haplotype and the
541 translocation of chromosomes 2 and 3 used to evolve the ML populations.

542

543 SUPPLEMENTARY RESULTS

544

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

550

551 REFERENCES

552

553 Chippindale, A. K. and Rice, W. R. 2001. Y chromosome polymorphism is a strong
554 determinant of male fitness in *Drosophila melanogaster*. *Proc. Nat. Acad. Sci. USA* **98**: 5677-
555 5682.

556

557 Prasad, N. G., Bedhomme, S., Day, T., and Chippindale, A. K. 2007. An evolutionary cost of
558 separate genders revealed by male-limited expression. *Am. Nat.* **169**: 29-37.

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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	<i>P</i> -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.

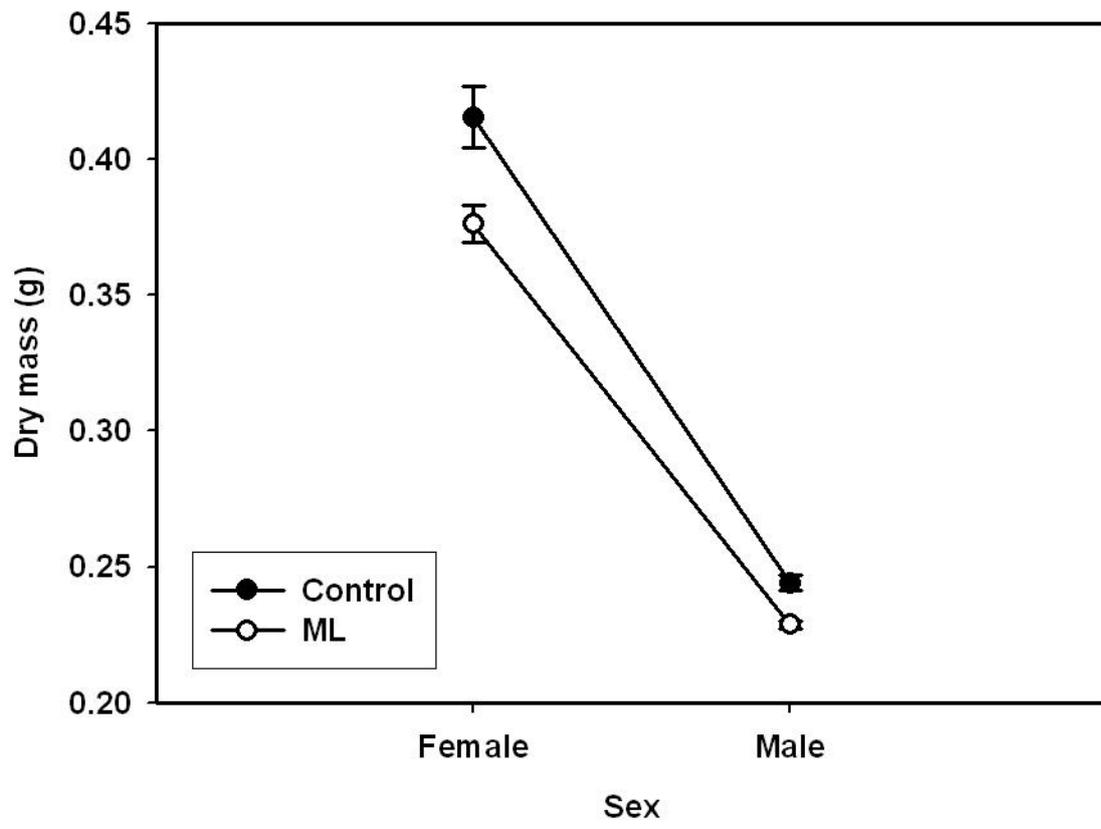


Figure S1A

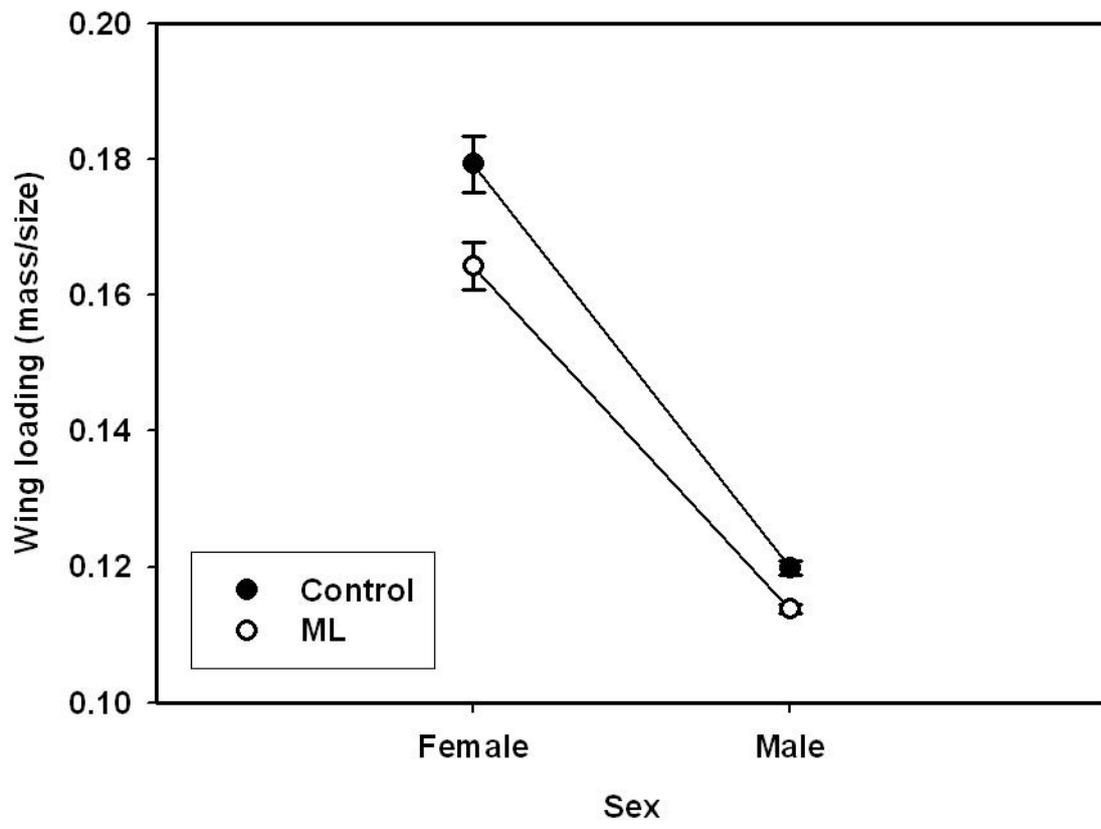


Figure S1B