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1 Experimental evolution supports signatures of sexual selection in genomic
2 divergence.
3
4 R. Axel W. Wiberg*, Paris Veltsos, Rhonda R. Snook & Michael G. Ritchie*
5
6 **RAWW**: Centre for Biological Diversity, University of St Andrews, St Andrews, Fife,
7 Scotland KY16 9TH, UK. **Current address:** Zoological Institute, Department of
8 Environmental Sciences, University of Basel, Basel, 4051, Switzerland. Orcid 0000-0002-
9 8074-8670.
10 **PV**: Department of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS,
11 USA. Orcid 0000-0002-8872-6281
12 **RRS**: Department of Zoology, Stockholm University, Stockholm 106 91 Sweden. Orcid
13 0000-0003-1852-1448
14 **MGR**: Centre for Biological Diversity, University of St Andrews, St Andrews, Fife, Scotland
15 KY16 9TH, UK. Orcid 0000-0001-7913-8675.
16
17 * Corresponding authors
18
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28
29 **Abstract**

30 Comparative genomics has contributed to the growing evidence that sexual selection is
31 an important component of evolutionary divergence and speciation. Divergence by sexual se-
32 lection is implicated in faster rates of divergence of the X chromosome and of genes thought
33 to underlie sexually selected traits, including genes that are sex-biased in expression. How-
34 ever, accurately inferring the relative importance of complex and interacting forms of natural
35 selection, demography and neutral processes which occurred in the evolutionary past is chal-
36 lenging. Experimental evolution provides an opportunity to apply controlled treatments for
37 multiple generations and examine the consequent genomic divergence. Here we altered
38 sexual selection intensity, elevating sexual selection in polyandrous lines and eliminating it in
39 monogamous lines, and examined patterns of divergence in the genome of *Drosophila pseu-*
40 *doobscura* after more than 160 generations of experimental evolution. Divergence is not uni-
41 form across the genome but concentrated in "islands", many of which contain candidate
42 genes implicated in mating behaviours and other sexually selected phenotypes. These are
43 more often seen on the X chromosome, which shows divergence greater than neutral expecta-
44 tions. There are characteristic signatures of selection seen in these regions, with lower di-
45 versity and greater F_{ST} on the X chromosome than the autosomes, and differences in diversity
46 on the autosomes between selection regimes. Reduced Tajima's D implies that selective
47 sweeps have occurred within some of the divergent regions, despite considerable recombina-
48 tion. These changes are associated with both differential gene expression between the lines
49 and sex-biased gene expression within the lines. Our results are very similar to those thought
50 to implicate sexual selection in divergence in natural populations, and hence provide experi-
51 mental support for the likely role of sexual selection in driving such types of genetic diver-
52 gence, but also illustrate how variable outcomes can be for different genomic regions.

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53

54 **Impact Summary**

55 How does sexual selection contribute to the divergence of genomes? It is often thought that
56 sexual selection is a potent force in evolutionary divergence, but finding 'signatures' of
57 sexual selection in the genome is not straight-forward, and has been quite controversial re-
58 cently. Here we used experimental evolution to allow replicate populations of fruit fly to
59 evolve under relaxed or strengthened sexual selection for over 160 generations, then se-
60 quenced their genomes to see how they had diverged. The features we find are very similar to
61 those reported in populations of natural species thought to be under strong sexual selection.
62 We found that genomic divergence was concentrated in small patches of the genome rather
63 than widespread. These are more often seen on the X chromosome, which overall shows es-
64 pecially elevated divergence. There are also characteristic signatures of selection seen in
65 these regions, with lower genetic diversity suggesting that selection was strong in these re-
66 gions. The changes are associated with both differential gene expression between the lines
67 and sex-biased gene expression within the lines. Many of the patches of divergence also con-
68 tain candidate genes implicated in mating behaviours and other sexually selected phenotypes.
69 Our results provide experimental support for the likely role of sexual selection in driving such
70 types of genetic divergence.

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71

72 **Introduction**

73 The role of sexual selection in influencing evolutionary divergence and speciation is
74 unclear (Panhuis *et al.*, 2001; Ritchie, 2007; Maan & Seehausen, 2011; Servedio & Bough-
75 man, 2017). Associations between species diversity and proxies of sexual selection such as
76 sexual dimorphism or mating system variation often imply that sexual selection can acceler-
77 ate divergence, especially when acting alongside natural selection (Arnqvist *et al.*, 2000;
78 Gage *et al.*, 2002; Ellis & Oakley, 2016). However, different indicators of sexual selection
79 give contrasting results in such comparative studies, and a consensus is not clear (Kraaijeveld
80 *et al.*, 2011; Janicke *et al.*, 2018). One potentially compelling source of evidence that sexual
81 selection is involved in divergence is coming from the increasing number of comparative ge-
82 nomic studies available across a range of organisms. Many descriptions of genomes, includ-
83 ing those of species thought to have undergone strong sexual selection such as the Hawaiian
84 *Drosophila* or African cichlids, have found that genes associated with mating behaviour or
85 sensory perception potentially involved in sexual communication are often outliers in meas-
86 ures of divergence (e.g. Mattersdorfer *et al.*, 2012; Kang *et al.*, 2016). It has also been known
87 for some time that genes which diverge particularly rapidly and show stronger signatures of
88 positive divergent selection are often sex-biased in expression (Pröschel *et al.*, 2006; Ellegren
89 & Parsch, 2007; Zhang *et al.*, 2007). Sex-biased gene expression itself, especially male-
90 biased expression, evolves rapidly and this is associated with indicators of sexual selection
91 such as increased sexual dimorphism in birds (Harrison *et al.*, 2015; Wright *et al.*, 2019).
92 However, genes with sex-biased gene expression might experience more drift than unbiased
93 genes, either due to reduced pleiotropy (Gershoni & Pietrokovski, 2014; Allen *et al.*, 2018) or
94 because they experience only half the selection pressure of genes with unbiased expression
95 (Dapper & Wade, 2020). Additionally, divergence of sex chromosomes between species is
96 usually much greater than autosomes, sometimes dramatically so (Counterman *et al.*, 2004;
97 Ellegren *et al.*, 2012).

98 However, such patterns of divergence are not necessarily driven by elevated sexual se-
99 lection on these genes or genomic regions. Sex-biased gene expression is thought to evolve
100 due to sexually antagonistic selection on gene expression, which is an important factor in
101 sexual selection but can arise due to other types of conflict. Changes in sex-bias in gene ex-
102 pression are also complicated by additional factors including dosage compensation, turnover
103 of sex-biased expression and resolution of conflict via sex-linkage or sex-limited expression
104 (Mank *et al.*, 2010a; Wright *et al.*, 2019). The increased divergence of sex chromosomes is

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105 also potentially influenced by many factors, including a greater role of genetic drift due to a
106 smaller effective population size on X chromosomes compared to autosomes, dominance ef-
107 fects, and other consequences of sex-linkage such as dosage compensation (Vicoso & Char-
108 lesworth, 2006; Ellegren, 2009; Mank *et al.*, 2010b). Hemizygosity results in a lower effect-
109 ive population size (N_e) on the X (N_{eX}) than on autosomes (N_{eA}). Under random mating the
110 ratio of N_e is expected to be 3:4 and this should reduce neutral diversity and increase
111 between-species divergence by the same proportion (Vicoso & Charlesworth, 2006). Hemizy-
112 gosity should also result in an increased efficacy of selection for partially recessive beneficial
113 mutations on the X-chromosome, relative to autosomes, and against recessive deleterious
114 mutations on the X, relative to autosomes. Finally, because of the female-biased inheritance
115 patterns of X-linked loci (males transmit them only to daughters while females transmit them
116 to both daughters and sons), sex-limited selection as well as sexual selection will influence
117 their divergence (Mank *et al.*, 2010a; Corl & Ellegren, 2012; Wright *et al.*, 2015).

118 It is very difficult to infer the historical role of different evolutionary processes from
119 patterns of contemporary divergence between populations and species, because they can res-
120 ult in similar genomic signals (Butlin *et al.*, 2012). One way of directly addressing the role of
121 sexual selection or mating system variation in genomic divergence is to examine the genomic
122 consequences of experimental evolution under manipulated sexual selection regimes in the
123 laboratory. A great advantage of this approach is that there are potentially fewer confounding
124 variables involved than when making comparisons across species or natural populations.
125 However, a disadvantage is that the time scale over which divergence can be studied is typic-
126 ally much shorter than evolutionary time-scales in nature. Studies of experimental evolution
127 and speciation are in their infancy, and general conclusions are, as yet, difficult to draw
128 (White *et al.*, 2020). Enforcing monogamy in otherwise polyandrous species will lead to both
129 changes in the intensity of sexual selection and the balance of sexual conflict, as it effectively
130 eliminates sexual selection and sexually antagonistic selection. A classic example of such ma-
131 nipulation is where *D. melanogaster* were kept under enforced monogamy for about 50 gen-
132 erations (Holland & Rice, 1999). Females from the monogamy treatment had reduced longev-
133 ity compared to ancestral females, when exposed to ancestral males. This was expected be-
134 cause the reduction of conflict should favour less harmful males and females that are less res-
135 istant to male harm. Other experimental evolution studies under altered mating systems have
136 been performed in dung flies (Hosken *et al.*, 2001; Hosken & Ward, 2001; Martin & Hosken,
137 2003), different species of fruit flies (*D. melanogaster*; (Gerrard *et al.*, 2013; Hollis *et al.*,
138 2014; Innocenti *et al.*, 2014; Perry *et al.*, 2016); *D. pseudoobscura*; (Crudgington *et al.*,

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139 2005); *D. serrata;* (Chenoweth *et al.*, 2015) , seed beetles (McNamara *et al.*, 2020) and herm-
140 aphroditic flatworms (Janicke *et al.*, 2016). Though aspects of the treatments differ amongst
141 such experiments, some common patterns have emerged. Gene expression changes are seen,
142 especially of genes that are initially sex-biased, though the details can vary between studies
143 (Hollis *et al.*, 2014; Veltsos *et al.*, 2017). Moreover, gene expression changes can be more
144 pronounced for genes expressed in reproductive tissues (Innocenti *et al.*, 2014), and genes in-
145 volved in the post-mating physiological manipulation of female egg-laying and re-mating
146 rates (Perry *et al.*, 2016).

147 A feature emerging from genomic comparisons between diverging species is that details
148 of genomic architecture complicate the assessment of patterns of divergence across chromo-
149 somes. Whole chromosomal regions can show correlated responses due to reduced recombin-
150 ation and hitchhiking effects, especially in species with segregating inversions. Early studies
151 of species differences interpreted "islands" of divergence in the genome as resulting from di-
152 vergent selection on genes within these regions with gene flow homogenising the genetic
153 background (Turner *et al.*, 2005; Nosil *et al.*, 2009). More recently it has been appreciated
154 that chromosomal inversions and other regions of low recombination or diversity can accen-
155 tuate such clustered divergence (Noor & Bennett, 2009; Cruickshank & Hahn, 2014; Wolf &
156 Ellegren, 2016; Ravinet *et al.*, 2017). "Barrier loci", genomic regions under divergent selec-
157 tion that restrict gene flow (Butlin & Smadja, 2018), may occur within such clusters but the
158 lack of recombination makes them difficult to localise precisely. In experimental evolution
159 the amount of recombination will be determined by both genomic architecture and the num-
160 ber of generations completed during the study, which is often modest in studies of eukaryotes.
161 Also, in experimental evolution the lines can be kept effectively allopatric, so homogenising
162 gene flow in regions not experiencing selection should be absent. The genomic divergence
163 which occurs during experimental evolution is usually extensive, with widespread differences
164 dispersed throughout the genome (Kawecki *et al.*, 2012; Tobler *et al.*, 2014; Michalak *et al.*,
165 2019).

166 Here we directly test the influence of sexual selection on genomic divergence. We ex-
167 amine replicated experimentally evolved lines of *D. pseudoobscura* in which sexual selection
168 has been manipulated for over 160 generations. One set of 4 replicate lines were raised under
169 enforced monogamy (M lines), which should eliminate both sexual selection and conflict.
170 Another 4 replicates were reared under elevated polyandry (E lines), with 6 males per female.
171 Polyandry mediates the strength of both intra- and intersexual selection and sexual conflict
172 (Pizzari & Wedell, 2013) and elevated polyandry will increase both pre- and post- copulatory

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173 sexual selection via female choice and sperm competition beyond levels experienced in most
174 natural populations (Snook, 2014). Previous studies of these lines have found divergence in
175 some, but not all, of the types of traits predicted to diverge under sexual selection. Sperm
176 morphology and heteromorphism, and testis mass did not diverge, but E males had larger ac-
177 cessory glands and a greater mating capacity (Crudgington *et al.*, 2009), were more competit-
178 ive in mating encounters (Debelle *et al.*, 2016), and produced more attractive courtship song
179 than M males (Debelle *et al.*, 2017). Coevolutionary changes have occurred in female song
180 preferences (Debelle *et al.*, 2014). Sexually dimorphic cuticular hydrocarbons have also di-
181 verged between the lines (Hunt *et al.*, 2012).

182 Patterns of gene expression have also changed between the lines. E females show an in-
183 crease in expression of genes normally enriched in ovaries (Immonen *et al.*, 2014). Sex-
184 biased genes responded more strongly to the sexual selection treatment, but the direction of
185 gene expression changes differed between sexes, tissues, and according to courtship experi-
186 ence (Veltsos *et al.*, 2017). In most cases, the transcriptome was "feminised" under polyandry
187 (i.e. female-biased genes were up-regulated or male-biased genes down-regulated in E lines),
188 in a striking contrast to a similar study with *D. melanogaster* (Hollis *et al.*, 2014). Males
189 changed in patterns of gene expression in the testes and accessory glands, and changes in
190 gene expression in females following mating also diverged, especially in the female repro-
191 ductive tract (Veltsos et al. *in prep.*).

192 Here we examine genomic divergence between these lines using a pool-sequence ap-
193 proach (Schlötterer et al., 2014) after more than 160 generations of experimental evolution.
194 The relatively long time-scale of this study should reduce linkage effects on allele frequency
195 changes. We adopt a statistical approach that identifies alleles that have changed in frequency
196 consistently across the replicates, to help reduce the potentially confounding effects of drift or
197 replicate-specific selection (Wiberg et al. 2017). We find that divergent SNPs are not distrib-
198 uted randomly across the genome, but occur in distinct, obvious clusters. We examine what
199 genes are involved and find several with mutant phenotypes related to mating and courtship
200 behaviours. We found that the X chromosome has accumulated more divergence than the
201 autosomes and explore if divergence is associated with recombination rate or changes in gene
202 expression between the experimental lines.

203

204 **Methods**

205 *Experimental Evolution*

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206 A full description of the experimental evolution procedure is available elsewhere
207 (Crudgington *et al.*, 2005). Briefly, a population of *D. pseudoobscura* was established from
208 50 wild caught females, bred in the laboratory for four years then four independent
209 monogamy (M) and elevated polyandry (E) lines were established. M females were housed
210 with a single male and E females with 6 males, with females typically mating with two or
211 three males. The effective population size was maintained around 120 (Snook *et al.*, 2009) for
212 both treatments to try to minimise confounding effects of drift and treatment. At each
213 generation, offspring were collected and pooled together for each replicate line, and a random
214 sample used to constitute the next generation in the appropriate sex ratio, thus reflecting the
215 differential offspring production across families (Crudgington *et al.*, 2005; Crudgington *et al.*,
216 2009). Enforced monogamy is expected to eliminate sexual selection and sexual conflict
217 while elevated polyandry increases both pre- and postmating sexual selection and sexual
218 conflict beyond levels encountered in most natural populations and in the ancestral
219 population (Crudgington *et al.*, 2005; Bacigalupe *et al.*, 2007; Crudgington *et al.*, 2009).
220
221 *Sequencing and Mapping*
222 Sequencing was carried out after ca. 160 generations of selection (specifically, 164 for
223 replicate 1, 163 for replicate 2, 162 for replicate 3, and generation 160 for replicate 4). Two
224 pools of 40 females (one E and one M) were taken from each replicate line and genomic
225 DNA extracted using a standard Phenol-Chloroform extraction protocol. Each pool was
226 sequenced across two lanes on a Illumina HiSeq platform at the Center for Genomic Research
227 (CGR) at the University of Liverpool. Details of coverage are provided in the Supplementary
228 Material. Reads from each sequenced pool were mapped to the *D. pseudoobscura* reference
229 genome (FlyBase v3.1 February 2013) using BWA mem (v. 0.7.7; Li, 2013). Alignments
230 were filtered to remove duplicate reads, reads with a mapping quality < 30, and any reads
231 which were not properly paired, using samtools (v 1.3; Li *et al.*, 2009 following Schlotterer *et*
232 *al.*, 2014). Reads were locally re-aligned around indels using GATK (v3.7.0; McKenna *et al.*,
233 2010; DePristo *et al.*, 2011). The .bam files for each line were then merged using bamtools
234 (Barnett *et al.*, 2011) and the genome-wide coverage calculated from these merged files with
235 bedtools (v. 2.26; Quinlan & Hall, 2010). SNPs were called using a heuristic SNP calling
236 algorithm (PoolSNP; Kapun *et al.*, 2020). Sites were considered only if the total coverage at
237 the site was > 17 and < the 95th percentile for each contig or chromosome. An allele was only
238 called if the count for that allele across all pools was > 16 and the allele frequency across all

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239 pools was > 0.001. Nearly 2 million SNPs were called and used in downstream analyses (see
240 Supplementary Material).
241

242 *Genomic Analyses*
243 *Identifying Consistent Allele Frequency Differences*
244 Many evolve and resequence studies of *Drosophila* find that a multitude of SNPs have
245 diverged, perhaps tens of thousands (Michalak *et al.*, 2019). The number is inflated upwards
246 at least in part due to segregating inversions and other areas of low recombination, and
247 hitchhiking (Barghi & Schlotterer, 2019). In order to focus on the loci most likely to have
248 diverged due to the treatment, we only considered as candidate SNPs those which diverged
249 consistently across all 4 replicate pairs of lines. We identified these using quasibinomial
250 Generalised Linear Models, which are less prone than other statistical approaches to be
251 influenced by strong divergence in only some replicates (Wiberg *et al.*, 2017). The model
252 structure applied was;
253 $y \sim treatment + e$
254 where y is the allele frequency of the major allele (identified as the major allele across
255 all pools) within each sample, *treatment* is the experimental evolution treatment regime of
256 each sample, and e is a quasibinomially distributed error term. If any count within a
257 population was 0, +1 was added to all counts. P-values were converted to q-values using the
258 "qvalues" R package (v. 2.16.0; Storey & Tibshirani, 2003). A threshold of 0.05 was chosen
259 to control the false discovery rate (FDR), thus we define "top SNPs" as those which change
260 consistently across all replicates with q-value < 0.05 and the remainder are referred to as
261 "background" SNPs.
262

263 *Genetic Diversity and Differentiation*
264 We calculated genome-wide genetic diversity statistics (π and Tajima's D) for windows
265 of 50kb (with a 10kb overlap) using available python scripts (Kapun *et al.*, 2020). Similarly,
266 we computed pairwise F_{ST} estimates between E and M line pairs for each SNP using the R
267 package "poolfstat" (v. 0.0.1; Hivert *et al.*, 2018), averaged in windows of 50kb (with a 10kb
268 overlap between windows). Comparisons of parameters between selection regimes and
269 genomic regions were tested using non-parametric Wilcoxon tests. Additionally, we estimated
270 neutral expectations for F_{ST} expected from drift and differences in effective population sizes
271 on X chromosomes (F_X) as in (Machado *et al.*, 2016) using the equations of (Ramachandran
272 *et al.*, 2004) (equation 8 therein), F_X is given by:

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273

$$F_X = 1 - \left\{ \frac{\left(9\left(z+1\right)\left(1-F_A\right)\right)}{\left(8\left(2z+1\right)-\left(1-F_A\right)\left(7z-1\right)\right)} \right\}$$

274
275

276 where, z is the ratio of the number of breeding males to females and F_A is the observed F_{ST} on
277 autosomes. We assumed z to be either 1 or 6 to represent extreme possibilities based on the
278 mating system manipulation. For each E-M pairwise comparison, we calculated mean F_{ST}
279 across each chromosome type and converted to F_X. We used a bootstrapping approach to
280 obtain a random distribution of F_X for each replicate. For each of 1,000 bootstrap iterations
281 we sampled, with replacement, a number of windows equal to the total number across all
282 autosomes from the set of all windows, then we calculated mean F_{ST} across all sampled
283 windows and converted to F_X using the equation above. Additionally, we computed a value of
284 F_{ST} and Tajima's D for each annotated *D. pseudoobscura* gene by taking the mean value
285 across all 50kb windows that spanned a gene.

286

287 *Linkage Disequilibrium (LDx)*

288 Although haplotype information is not available from pool-seq data, short range linkage
289 information is available from paired reads. We used LDx (Feder *et al.*, 2012) to first compute
290 the r^2 of SNPs located on the same read pairs. We only used SNPs with a minor allele
291 frequency > 0.1, a minimum coverage of 10, a maximum read coverage of 400, and a phred
292 score > 20. Note that the empirical median insert size varied between 332-346 across
293 samples. We binned pairs of SNPs into distance classes and then computed mean r^2 per
294 distance class. We only used distance classes with a minimum of 5 SNPs. We estimated the
295 decay of r^2 as a function of distance by fitting a linear model of r^2 as a function of the log of
296 the distance between the SNPs. Thus, the slope measures the decay rate of linkage due to
297 recombination (Feder *et al.*, 2012), giving an indication of the distance over which LD is
298 present. In regions of low recombination one would expect high overall values of r^2 but a
299 weakly negative slope as LD is maintained over relatively longer regions of the genome.
300 Comparing the slope parameter across different genomic regions gives an indication of
301 differences in the recombination rate (or extent of selective sweeps). This was performed for
302 each chromosome, as well as for different regions on the 3rd chromosome (see below).

303

304 *Functional Genomics*

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305 To examine the function of genes near candidate SNPs we conducted enrichment
306 analyses. We used the *D. pseudoobscura* annotation and a dataset of regulatory long non-
307 coding RNAs (lncRNAs; Nyberg & Machado, 2016). We identified genes or lncRNAs within
308 a distance of 10kb up- or downstream of top SNPs with bedtools (Quinlan & Hall, 2010)
309 intersect (keeping any potential ties). Enhancer regions, transcription factor binding sites, and
310 other regulatory regions can occur up to 1 Mb up- or downstream from a target gene in other
311 species (e.g. Maston *et al.*, 2006; Chan *et al.*, 2010; Werner *et al.*, 2010; Pennacchio *et al.*,
312 2013) but typically lie within 2kb of a gene region in *D. melanogaster* (Arnosti, 2003), 10kb
313 thus represents a compromise. We submitted the implicated genes to ModPhEA (Weng &
314 Liao, 2017) for phenotypic enrichment analysis. We combined the phenotypic classes
315 “courtship behavior defective” (FBcv:0000399) and “mating rhythm defective”
316 (FBcv:0000401) into one phenotype group and also tested the phenotypic class “stress
317 response defective” (FBcv:0000408) for enrichment. We chose these classes *a priori* because
318 they were most likely to be involved in phenotypic differences between the treatments related
319 to mating or courtship behaviour and responses.
320 We also took advantage of gene expression data from the same experimental evolution
321 lines. Expression data is available from heads and abdomens of virgin and courted flies
322 (Veltsos *et al.*, 2017) and testes, accessory glands, ovaries and female reproductive tracts
323 from virgin flies, and ovaries and female reproductive tracts from mated females (Veltsos et
324 al., *in prep.*). Using these data we compiled a list of genes with differential expression
325 between E and M lines. For simplicity we considered a gene to be differentially expressed
326 between E and M lines if it shows significant differences in E/M contrasts in any of the
327 following data: combined virgin and courted head or abdomens of each sex (4 sets), virgin
328 individual reproductive tissues (4 sets), mated individual female reproductive tissues (2 sets).
329 Briefly, the analysis was conducted in edgeR v3.18.1 (Robinson *et al.*, 2010) running in R
330 v.3.4.0 (R Development Core Team, 2007). We used TMM normalization in edgeR and
331 measured dispersion using a negative binomial model from the genes within each contrast.
332 We employed a statistical definition for differential expression (FDR < 0.05; (Benjamini &
333 Hochberg, 1995) and did not require a minimum logFC threshold to consider a gene
334 differentially expressed as the effect of allometry should be minimal for samples from
335 specific organs (Montgomery & Mank, 2016), and the results are cross-checked with top
336 SNPs, making the analysis conservative. The associated scripts and final #number# gene set
337 are available in OSF1, OSF2, File S#.

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338 We used this list to ask if top SNPs co-localised with genes that are differentially
339 expressed between the lines and if these also show different levels of diversity (Tajima's D)
340 or differentiation (F_{ST}) between E and M lines. We used a resampling approach, sampling
341 genes (without replacement) from the *D. pseudoobscura* annotation, to determine the amount
342 of overlap with the DE genes that is expected by chance. For each sample, we picked a set of
343 428 genes from the annotation, which is the same size as the set of genes near top SNPs (see
344 Results). We then calculated the proportion of these genes that also occur in the DE gene sets
345 and repeated this procedure 1,000 times to build a distribution of expected overlap between
346 re-sampled gene-sets and the DE gene sets. If the empirical set of genes near top SNPs had a
347 proportional overlap >= the 95th percentile of the re-sampled distribution it was deemed a
348 "significant" overlap.

349 Using the values of Tajima's D and F_{ST} computed for each gene (see above) we also
350 asked whether there was any evidence of different levels of diversity or divergence between
351 DE genes in any set (N = 3,173) and non-DE genes (N = 13,583). For Tajima's D we contrast
352 DE and non-DE genes separately for each chromosome type (autosomes, X-chromosome left
353 arm, X-chromosome right arm), and each experimental evolution treatment (E and M; 6
354 contrasts in total), using Wilcoxon rank sum tests. For F_{ST} we contrast DE genes and non-DE
355 genes separately for each chromosome type (3 contrasts), testing for differences with
356 Wilcoxon rank sum tests. In both cases, the mean value for non-DE genes was used as a
357 single value against which to compare DE genes, which reduces the effect of the enormous
358 sample size for the non-DE genes on the significance of the test.

359 Finally, we also asked whether the changes in sex-biased expression (data from Veltsos
360 *et al.*, 2017) between E and M treatments (ΔSB_{EM}) was related to diversity (Tajima's D)
361 within either E or M lines. Sex-bias in expression was assessed for two tissues, head and
362 abdomen, in both courted or virgin data combined. Within each tissue, sex-bias was
363 computed as the $\log_2$(fold change) in expression between males and females in E and M lines
364 separately, after which ΔSB_{EM} is calculated as $\log_2(FC)_E - \log_2(FC)_M$. Thus, positive values of
365 ΔSB_{EM} correspond to greater male-bias in expression in the E lines, while negative values
366 correspond to greater male-bias in the M lines. ΔSB_{EM} was then related to values of Tajima's
367 D in either E ($TajD_E$) or M ($TajD_M$) lines. For each tissue (head and abdomen) we performed
368 an ANCOVA with chromosome (autosome, X-chromosome right arm, and X-chromosome
369 left arm) as a co-factor, as well as mean Tajima's D across E lines and mean Tajima's D
370 across M lines as co-variates. We also included the interactions between Tajima's D and
371 chromosome. The full model is:

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372

373 $$\Delta SB_{EM} \sim chromosome + TajD_E + TajD_M + TajD_E{:}chromosome + TajD_M{:}chromosome$$

374

375 We further extracted the 30bp up- and down-stream of each SNP from the reference
376 genome using gffread from the Cufflinks package (v2.2.1; (Trapnell *et al.*, 2010) and tested
377 for an enrichment of TF binding site motifs around top SNPs with the AME routine from the
378 MEME package (v. 4.10.2; (McLeay & Bailey, 2010). GO term enrichment analysis was
379 performed with GOwinda (v. 1.12; (Kofler & Schlotterer, 2012). We considered SNPs to be
380 associated with genes if they occurred within 10kb up or downstream of an annotated gene.
381 An empirical p-value distribution was produced from 1 million simulated SNP sets.
382 All statistical analyses were made with R (v. 3.6.3; R Development Core Team 2020)
383 except where otherwise stated. Figures were drawn using the “ggplot2” package (v. 2.2.1;
384 (Wickham, 2009) and associated packages (table S1).

385

386 **Results**

387 *Consistent Allele Frequency Differences*

388 In total, 480 SNPs show significant consistent allele frequency differences due to the
389 experimental evolution treatment (hereafter the “top SNPs”). These occur on all of the main
390 chromosomes but many show striking co-occurrence into a few clusters of highly
391 differentiated SNPs (figure 1A). The distribution of the top SNPs across the genome is not
392 random, with a significant excess on the 3rd chromosome and both arms of the X chromosome
393 (table S3). In particular, a large cluster of differentiated SNPs are observed at the end of the
394 right arm of chromosome 3 (figure 1A). Other large clusters occur on both arms of the X
395 chromosome (figure 1A). If all top SNPs within 50kb of others are grouped into clusters, this
396 produces 70 distinct clusters throughout the genome (figure 1A). The majority of SNPs
397 (72.9%) occur in only 6 clusters with > 10 SNPs.

398 Such clustered divergence is often seen in comparisons between natural species
399 (Ravinet *et al.*, 2017) but rarely in experimental evolution (e.g. Kauranen *et al.*, 2019). We
400 considered 10 random permutations of the treatment labels among SNP sets and observed far
401 fewer SNPs with q-values < 0.05 than in the original dataset. We are therefore confident that
402 our approach reliably identifies SNPs with consistently different allele frequencies between
403 the treatments. We also tested if the divergence was more clustered than random samples
404 between the lines using a permutation test (for full details of the randomisation tests see the
405 Supplementary Material). We also examined if variation in coverage might be associated with

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406 calling clustered divergence. We compared coverage within these clusters to 100 random
407 genomic regions with a similar distribution in size shows that, although there is a minor
408 difference in coverage between peaks with top SNPs, the variation in coverage across
409 samples is far greater, we therefore conclude that difference in coverage around top SNPs and
410 the rest of the genome cannot explain the patterns (figure S1).
411 The clusters do not correspond to known inversions in *D. pseudoobscura.* In particular,
412 the large cluster on chromosome 3 containing many (N = 199, 41.5%) top SNPs does not
413 correspond to the most common inversions that have shaped the evolution of this
414 chromosome in the wild (Wallace et al., 2011; Wallace et al., 2013). Allele frequencies in E
415 and M lines for the top 100 SNPs are shown in figure S2. More than half of these (57%) are
416 fixed differences in all replicates. Across all the top SNPs, 12% are fixed differences between
417 the E and M lines in all replicates, with all of these occurring on the X chromosomes
418
419 *Genetic diversity*
420 We identified a set of candidate SNPs which varies consistently in allele frequency in
421 response to experimental treatment. Such patterns are strongly suggestive of the action of
422 selection. We therefore also assessed the levels of genetic diversity throughout the genome
423 and in regions surrounding these candidates. On a broad scale, Tajima's D does not vary
424 much across chromosomes (figure S3). Strikingly, Tajima's D is substantially lower on
425 chromosome 3, though the interaction effect of chromosome and treatment is not statistically
426 significant ($F_{4,30}$ = 0.59, p = 0.68). Strongly localised selective sweeps, should locally reduce
427 Tajima's D. Within E lines, Tajima's D is actually on average slightly higher within the
428 clusters containing top SNPs (-0.03) than outside these clusters (-0.05; Wilcoxon signed rank
429 test: V = 17623, p-value = 0.04). Within M lines there is no statistically significant difference
430 between clusters (-0.07) and outside clusters (-0.06; V = 13390, p-value = 0.3). However,
431 patterns of Tajima's D are very variable. The most differentiated region on chromosome 3
432 shows reduced Tajimas's D within the E treatment compared to the M treatment (figure 1B),
433 as would be expected following selective sweeps. Similar patterns are seen for some peaks on
434 the X chromosome (figure S4). In a few cases, there are reductions of Tajima's D associated
435 with regions containing top SNPs within M lines compared to E lines (figure 1B and figure
436 S4). However, many of these regions are quite small and consequently estimates of Tajima's
437 D may be unreliable (figure S3).
438 Nucleotide diversity across the chromosomes was estimated as π (figure S5). Diversity
439 is lower overall in E lines than in M lines (figure 2A). Diversity varies significantly across

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440 chromosomes in both E and M lines (figure 2A; $F_{4,30} = 29.3$, $p < 0.001$), but the interaction
441 with treatment is not significant ($F_{4,30} = 0.98$, $p = 0.44$). Lowest diversity (in both treatments)
442 is seen on the more differentiated chromosomes (X and 3; figure 2A). Median π is marginally
443 non-significantly lower within the clusters of M (V = 12471, p = 0.05), but not E (V = 13843,
444 p = 0.19), lines. The ratio of diversity between the sex chromosome and autosomes is lower
445 in E lines than in M lines, though this is variable across replicates (figure 2B). Overall, it
446 seems like there is greater evidence for selective sweeps in E lines, especially for the X.
447 Comparisons of genomic divergence are often based on patterns of F_{ST}. Although
448 obviously not independent of changes in allele frequency, we also examined the patterns of
449 F_{ST} seen between the E and M lines for comparison with published studies and to examine the
450 X / autosome divergence in more detail. F_{ST} is generally higher on the X chromosome than on
451 autosomes (figure 3B), even after accounting for the expected greater effects of drift on the X
452 over the autosomes (see Methods for the equations; figure 3B). Hence the X:A ratio of F_{ST} is
453 always > 1 (figure 3C). These results hold regardless of the value of z (see Methods for the
454 equations). F_{ST} was higher within peak regions than outside peak regions (0.64 vs. 0.59;
455 Wilcoxon signed rank test: V = 15309, p-value < 0.001, Figure 3D), as expected as allele
456 frequencies differ most within the clusters. It should be noted that the above measures of
457 differentiation and genetic diversity are often variable and precise estimates depend on the
458 number of SNPs detected, the coverage, and number of replicate lines. Accordingly, we
459 emphasise that while broad-scale patterns are likely to be robust, values for any one genomic
460 region or gene should be taken with appropriate caution.
461
462 *Linkage Disequilibrium*
463 Background selection or selective sweeps could lead to clustered genomic divergence,
464 often with low diversity, especially in regions of low recombination such as telomeric
465 regions. We examined patterns of linkage disequilibrium in the clusters and if this varied with
466 treatment. Throughout the genome, the decay rate (*a* parameter) of LD is generally shallower
467 (i.e. less negative) in the E treatment (figure 4A). This is seen for chromosome 3 as well as
468 both arms of the X chromosome (figure 4A). A lower decay rate is indicative of more LD,
469 due to less recombination and/or a potential for greater hitchhiking under positive selection.
470 Contrary to predictions, we found a steeper rate of decay (less LD) within the differentiated
471 region of chromosome 3 than outside it, especially in E lines (figure 4B and C). Although
472 statistically significant ($F_{(2,13)} = 4.6$, $p < 0.001$), these differences are slight. The most striking
473 pattern overall is greater overall LD on chromosome 3.

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474

475 *Gene functions and expression variation*

476 Out of the 480 top SNPs, 201 (42%) lie within a gene model (i.e. either in an intron or
477 within an exon; the remaining are intergenic. The top SNPs are not significantly enriched in
478 any GO terms after correcting for multiple testing, even at a 10% FDR (table S4). Similarly,
479 there is no enrichment of genes with annotations for mating behaviour or stress response
480 phenotypic classes. However, several genes within 10kb of a top SNP are potentially
481 interesting candidate genes for traits evolving under sexual selection based on described
482 functions (table S4). For example, the genes *Odorant-binding protein 47a* (*Obp47*),
483 *pickpocket 6* (*ppk6*), and *Accessory gland protein 53C14c* (*Acp53C14c*) all occur within 10kb
484 of a top SNP and are genes potentially underlying sexually selected behaviours or traits. Two
485 of these genes (*ACP53C14c* and *Obp47a*) are within the region of highly differentiated SNPs
486 on the 3rd chromosomes, which also includes several additional accessory gland proteins
487 (*Acp53Ea, Acp53C14b, Acp53C14a*), and other genes (table S4), all of which are thought to
488 influence mating and courtship behaviours or phenotypes based on known functions of
489 similar genes in *D. melanogaster*.

490 Previous studies have shown that there is divergence in gene expression patterns
491 between E and M lines (Immonen *et al.*, 2014; Veltsos *et al.*, 2017; Veltsos et al., *in prep.*).
492 We therefore asked if these expression differences were associated with the top SNPs. Genes
493 within 10kb (N = 428) of the top SNPs show a significantly greater overlap with genes that
494 are differentially expressed (DE) in ovaries and testes between E and M lines than expected
495 by chance (figure S6 and table S3). This pattern also holds for genes within 1Mb (N = 7,045;
496 figure S7). Also, there is evidence that F_{ST} between E and M lines is higher for genes that are
497 DE between the lines, especially for X-linked genes (figure 5A; Wilcoxon rank sum tests,
498 Autosomes - V = 1026000, p = 0.03; X-chromosome right arm - V = 89067, p = 0.005; X-
499 chromosome left arm – V = 59623, p = 0.04). There is no evidence that Tajima's D is
500 different between DE and non-DE genes (Wilcoxon rank sum test; all $p > 0.05$; figure 5B).
501 There is some evidence that the degree to which sex-biased expression of a gene changes
502 between E and M lines is associated with Tajima's D in M lines, but only on the X-
503 chromosome and only within abdominal tissues (figure 5C). Specifically, as the change in
504 sex-bias becomes more negative (i.e. more female-biased expression in M lines), Tajima's D
505 also becomes more negative (interaction of Tajima's D in M lines and chromosome type:
506 $F_{(11189,11191)} = 4.4$, p = 0.01).

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507 The regions immediately up- or down-stream of top SNPs are not enriched for TF
508 binding motifs or lncRNAs, after correction for multiple testing, so there were no obvious
509 differences between treatments in regions expected to influence gene expression variation.
510

511 **Discussion**

512 There is much debate about the influence of sexual selection and sexually antagonistic
513 selection on patterns of genomic variation (Mank, 2017; Sayadi *et al.*, 2019) and how this
514 may influence divergence between species (Wolf & Ellegren, 2016). Sex-biased gene
515 expression, especially male-bias, evolves quickly and is related to phenotypic sexual
516 dimorphism (Wright *et al.*, 2019). Outliers in genome scans often implicate sexual selection
517 as a diversifying force (Andres *et al.*, 2008; Blankers *et al.*, 2018). Sexual antagonism may be
518 associated with genomic signatures of selective sweeps or balancing selection (Cheng &
519 Kirkpatrick, 2016; Wright *et al.*, 2019) and may be promoted by strong sexual selection
520 (Connallon & Clark, 2012; 2013; Dutoit *et al.*, 2018; Ruzicka *et al.*, 2019). However,
521 inferences of the sources of selection on natural variation in genomic divergence are usually
522 indirect and ambiguous, because multiple forces act in concert to produce variation seen at
523 the genomic level in nature. Here we used experimental evolution to alter sexual selection
524 intensity, elevating sexual selection in polyandrous lines and eliminating it in monogamous
525 lines, and examined patterns of divergence in the genome after more than 160 generations of
526 experimental evolution.

527 Many of the results we found recapitulate patterns seen in natural populations and
528 between species. Divergence is not uniform across the genome but clustered in "islands" of
529 divergence, some of which contain candidate genes for an involvement in mating success.
530 These clusters are more often seen on the X chromosome, which is a "hot spot" for
531 divergence. There are signatures of selection within the islands of divergence, with
532 marginally lower diversity (π) within clusters than the rest of the genome, but only in M lines.
533 F_{ST} between E and M lines is greater within clusters, and is also greater on the X than
534 autosomes, and differences in diversity are seen in the autosomes between selection regimes.
535 Low Tajima's D implies selective sweeps have occurred, but only within some of the
536 divergent regions. These patterns of diversity and divergence are associated with changes in
537 both differential gene expression between the lines and sex-biased genes. Overall, F_{ST}
538 between the lines is high in all replicates, probably due to low overall effective population
539 sizes, though effective population sizes are similar between E and M lines (Snook *et al.*,
540 2009).

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541 The concept of "islands" of divergence originated from comparisons of genomic
542 divergence between species (Nosil *et al.*, 2009; Ravinet *et al.*, 2017). These are usually
543 thought to have arisen due to the combination of strong selection on barrier loci and genetic
544 hitchhiking within genomic regions, with background gene flow reducing divergence outside
545 of the islands. Here we find distinct clustered divergence akin to the islands seen in natural
546 systems. Our system is effectively allopatric, so there was no background gene flow
547 counteracting divergence outside of these clusters, which therefore must have arisen due to
548 strong localised divergent selection across all replicates. Although *D. pseudoobscura* has
549 relatively well-characterised inversion polymorphisms (Sturtevant & Dobzhansky, 1936;
550 Dobzhansky & Sturtevant, 1938; Wallace *et al.*, 2011), the clusters we describe do not
551 correspond to the most common inversions known for this species, which are often very
552 large. Our short-read sequencing approach allowed some examination of LD and there was
553 no suggestion of reduced recombination within the clusters. In fact, the large peak at the right
554 end of chromosome 3 (figure 4) surprisingly seems to be within a region of high
555 recombination (which is often suppressed at telomeric regions). Interestingly, recombination
556 is higher within this peak than the chromosome-wide rate, but also differs between the
557 treatments, being greater in the M lines. Perhaps selection against recombination was reduced
558 in monogamous individuals because of epistatic interactions in the region which were
559 important in sexual selection or sexual conflict. There was no obvious difference in LD in the
560 other clusters but their smaller size and hence "noisier" estimates makes robust inferences
561 from pool-seq data difficult. Indeed, the estimates of LD within the cluster on chromosome 3
562 also rely on relatively few SNPs at longer ranges compared to the rest of the chromosome, so
563 inferences need to be taken with caution.

564 The lack of background gene flow or stronger linkage disequilibrium within the clusters
565 suggests that they have arisen primarily through localised strong selection that is consistent
566 across all replicates. In support of this, we see lower Tajima's D in some of the larger clusters.
567 However, these patterns are very variable with lower Tajima's D in different clusters for the E
568 and M lines. Thus, overall, there is no significant difference in Tajima's D between E and M
569 lines. Systematic differences in Ne between E and M lines might be expected to lead to
570 consistent differences in Tajima's D. One might predict lower N_e in M lines due to fewer
571 mating individuals and, correspondingly, lower Tajima's D in M lines, though the
572 experimental design tried to minimise this and previous studies found no evidence of such a
573 reduction in Ne (Snook *et al.,* 2009.

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574 The genes contained within the clusters are not enriched for genes of particular
575 functional categories, however, they do include strong candidate genes for an involvement in
576 mating system evolution. For example, the large region on chromosome 3 contains numerous
577 accessory gland proteins. In *D. melanogaster* these are well known to influence male
578 reproductive success, exert antagonistic effects on female fecundity and lifespan, and play a
579 role in sperm competitive success (Chapman *et al.*, 1995; Ram & Wolfner, 2007). Some of
580 the evolutionary response in E lines is antagonistic, because M females have a lower
581 fecundity when mated with E males. Moreover, when mated to E males, the reproductive
582 schedule of M females is manipulated to the males benefit (Crudgington *et al.*, 2010).
583 Accessory gland proteins show accelerated coding sequence and expression evolution across
584 species (Swanson & Vacquier, 2002; Begun & Lindfors, 2005). Other genes within the
585 clusters are involved in sexual chemical communication, which is also often implicated in
586 outlier analyses in genome comparisons between species (Smadja & Butlin, 2009). For
587 example, mutants of members of the pickpocket family in *D. melanogaster* show aberrant
588 male mating success because of their involvement in the detection of female pheromones
589 (Thistle *et al.*, 2012; Toda *et al.*, 2012). E males, subject to both intra- and intersexual
590 selection, have diverged in aspects of courtship behaviour, such as time until initiation of
591 courtship, have a higher intensity courtship song and have a higher competitive mating
592 success than M males (Debelle *et al.*, 2016; Debelle *et al.*, 2017).

593 If strong selection has driven this clustered genomic divergence, an interesting question
594 is whether the responses to selection are stronger in the E or M lines. Imposing monogamy on
595 a naturally polyandrous species probably leads to relaxed selection on many genes involved
596 in intra-or intersexual competition. Therefore, the response is likely to involve changes in
597 both the intensity and direction of selection on some loci. Thus, perhaps the variation in
598 signals of selection we see in Tajima's D and changes in LD are to be expected. Overall, we
599 see stronger reductions in diversity in E lines, perhaps suggesting that directional selection
600 was stronger when sexual selection was strengthened.

601 One pattern very commonly seen in studies of natural populations and species is more
602 rapid divergence of the X chromosome (Vicoso & Charlesworth, 2006). We also see this here,
603 the X having a higher prevalence of divergent clustered regions and consequently higher F_{ST}
604 between the lines. Remarkably, all SNPs with fixed differences between the lines occurred on
605 the X. Faster X evolution can occur for many reasons, including greater genetic drift due to
606 its smaller effective population size, and beneficial recessive alleles on the X are more
607 responsive to selection due to male hemizygosity (Meisel & Connallon, 2013). We calculated

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608 expected X/A divergence ratios under a range of plausible sex ratios and the observed X/A
609 divergence exceeded all of them, suggesting the accelerated X divergence is not due to drift
610 effects alone, selection or a combination of effects are likely involved. Genes under sexual
611 selection are potentially more likely to be sex-linked, due to antagonistic, or sex-limited
612 selection (Reinhold, 1998; Kirkpatrick & Hall, 2004). Sexually selected or antagonistic loci
613 are perhaps also more likely to show dominance effects (Grieshop & Arnqvist, 2018).

614 Previously we found that gene expression differences have evolved between the lines,
615 especially in sex-biased genes (Veltsos *et al.*, 2017). Here we show that there is significant
616 overlap between differentially expressed genes and the regions of genomic divergence of the
617 lines found here. Thus, the expression divergence is associated with the broad patterns of
618 genomic divergence. Also, F_{ST} is greater for the differentially expressed genes, once again
619 recapitulating patterns from natural systems (sex-biased genes here are not more likely to be
620 sex-linked, so this is independent of the large X effect seen). We find no overall difference in
621 Tajima's D between DE and non-DE loci.

622 Links between genomic parameters and sex-biased gene expression variation have been
623 a somewhat contentious source of evidence of sexual selection, especially antagonistic forms
624 of sexual selection (Kasimatis *et al.*, 2019; Cheng & Kirkpatrick, 2020; Mank *et al.*, 2020).
625 Genes that are male-biased in expression show accelerated divergence between species and
626 sex-biased gene expression shows rapid evolution and turnover (Pröschel *et al.*, 2006;
627 Harrison *et al.*, 2015). Whether sex-biased expression is expected to be related to sex-specific
628 F_{ST} or signatures of balancing selection such as Tajima's D is open to debate, partly because
629 of the potential resolution of antagonistic selection by the strengthening of sex-biased
630 expression. However, there is one very intriguing pattern in our data where the magnitude of
631 change in sex-biased gene expression is related to Tajima's D. As ΔSB increases (i.e. more
632 male-biased expression in E lines) Tajima's D in these lines becomes more negative. This
633 pattern is potentially consistent with more resolved sexual conflict in the M lines, because
634 males in M lines are released from sexual selection, and selection driving female-beneficial
635 alleles to high frequency could result in sweeps and/or reduced balancing selection. However,
636 perhaps analyses over the course of the experimental evolution study would be required to
637 convincingly demonstrate associations between changes in sex-bias and potential measures of
638 balancing selection.

639 In conclusion, we have examined genomic divergence following >160 generations of
640 experimental evolution under altered mating systems. We find that genomic divergence
641 between the experimental lines is highly clustered in the genome, much greater on the X and

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642 is associated with changes in gene expression between the experimental lines. Associations
643 with LD and population genetic parameters indicative of selective sweeps or balancing
644 selection are also observed, but are very variable. This raises the possibility that selection has
645 been strong in both M and E lines, but differs in nature (relaxed in M, directional in E),
646 complicating predictions of responses. Overall, our main results support those seen in natural
647 populations, providing an elegant demonstration of the power of experimental evolution to
648 aid the interpretation of complex patterns of natural variation.
649

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659

660 **Author contributions**

661 RAWW performed the data analysis. PV contributed data. The experiment was designed by
662 MGR and RRS. All authors contributed to writing the MS.
663

664 **Data Accessibility**

665 Raw reads have been deposited in the short read archive (SRA) of NCBI under the BioProject
666 PRJNA661678
667
668

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669 **Figures and Figure Legends**

670

671 **Figure 1. A)** Manhattan plot of log10(q-values) for each SNP from a quasibinomial GLM
672 with treatment as a predictor. Red points denote SNPs with a q-value < 0.05 and the
673 horizontal red dashed line indicates the q < 0.05 cutoff. Grey bars give the locations and span
674 of the 70 divergent regions (see text). **B)** Mean (± SE) Tajima's D across replicates for the
675 three most divergent regions (see text), red points denote SNPs with a q-value < 0.05, all
676 have been plotted at the same value on the y-axis for convenience.

677

678 **Figure 2. A)** Levels of genetic diversity (π) on each chromosome in E and M lines. π is
679 estimated in overlapping windows of 50kb, then averaged across the chromosomes. Boxplots
680 show the distribution of π on each chromosome across replicate experimental evolution lines.
681 **B)** The X chromosome to autosome ratio of π in the replicates of E and M lines and overall.

682

683 **Figure 3. A)** F_{ST} between E and M treatment lines on the main chromosome arms for each
684 replicate. F_{ST} is calculated for each SNP then averaged within overlapping 50kb windows on
685 each chromosomal segment. **B)** The X:autosome ratio of F_{ST} within each replicate line. The
686 error bars are bootstrap 95% confidence intervals. **C)** Observed F_{ST} on the autosomes (black)
687 and on the X chromosome (dark grey) as well as the expected F_{ST} on the X chromosomes
688 assuming a value of $z = 6$ (light grey) (see Methods), error bars represent bootstrap 95%
689 confidence intervals. **D)** The difference in FST between windows within "peaks" of top SNPs
690 and windows outside of these peaks.

691

692 **Figure 4. A)** Slope coefficients from the model $r^2 \sim a + \log(bp)$ where *bp* is the distance
693 between pairs of SNPs and r^2 is the average measure of LD between SNPs. Distributions are
694 shown for average values of each of the main chromosomes as well as X chromosomes
695 across replicates in E and M lines. **B)** Decay in LD as a function of distance between SNPs
696 with the chromosome 3 peak region (see figure 3) and outside the peak region for E and M
697 lines. **C)** The distribution of slope parameters for SNPs within the chromosome 3 peak and
698 outside the peak region.

699

700 **Figure 5. A)** F_{ST} at DE vs. non-DE genes for different chromosome types. Asterisks indicate
701 significant differences **B)** Tajima's D at DE vs. non-DE genes for different chromosome

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702 types. **C)** relationship between change in sex-bias between E and M lines and Tajima's D in
703 M lines.
704
705 Figures S1 – S7 and Tables S1 – S3 can be found in the Supplementary Material

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1016

1017

Figure 1

A

log10(q-value)

2
3
4.1
4.2
4.3
4.4
4.5
XL.1a
XL.1e
XL.3a
XL.3b
XR.3a
XR.5
XR.6
XR.8

0 5 10 15 20 25 30

Position (Mb)

B

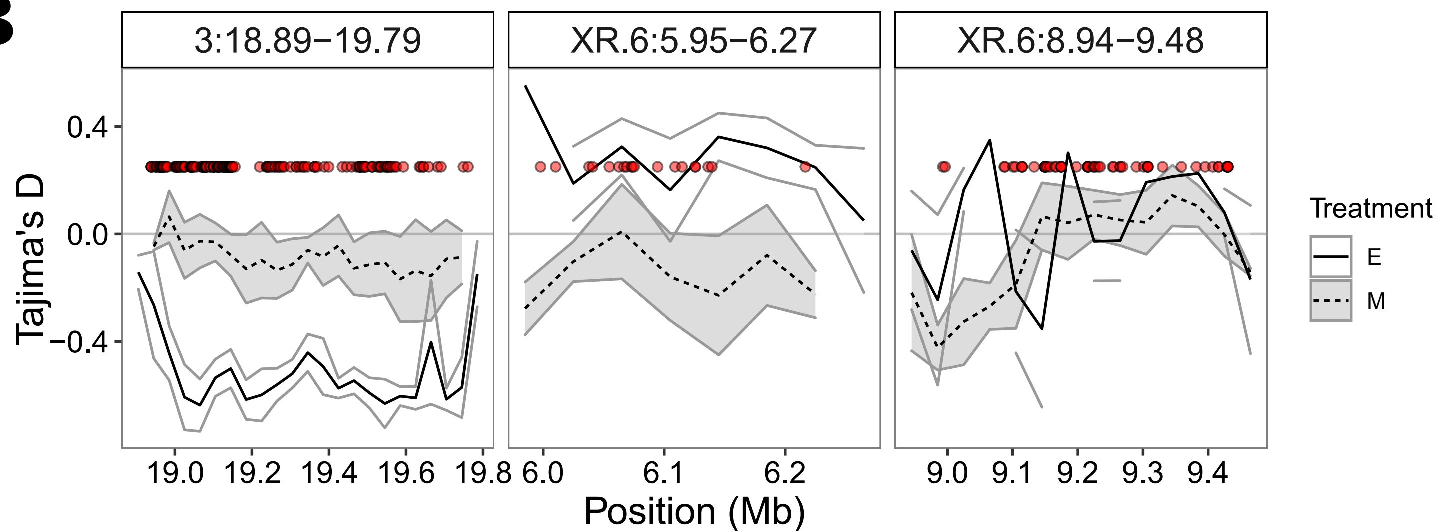


Figure 2

A

0.005
0.004
0.003
0.002
0.001
π
2
3
4
XL
XR
Chromosome
Treatment
E
M

B

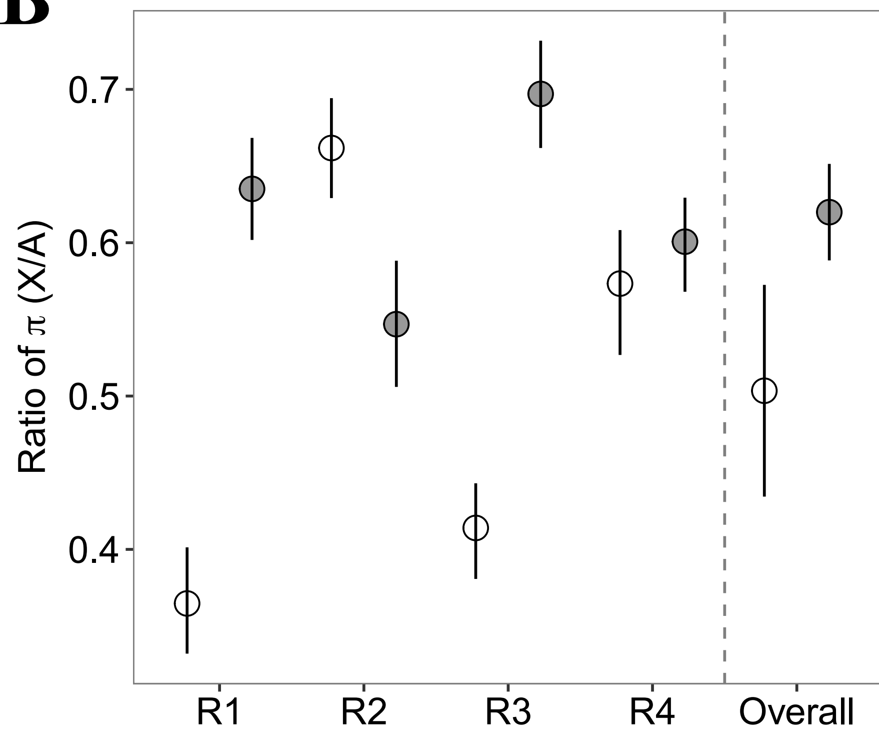
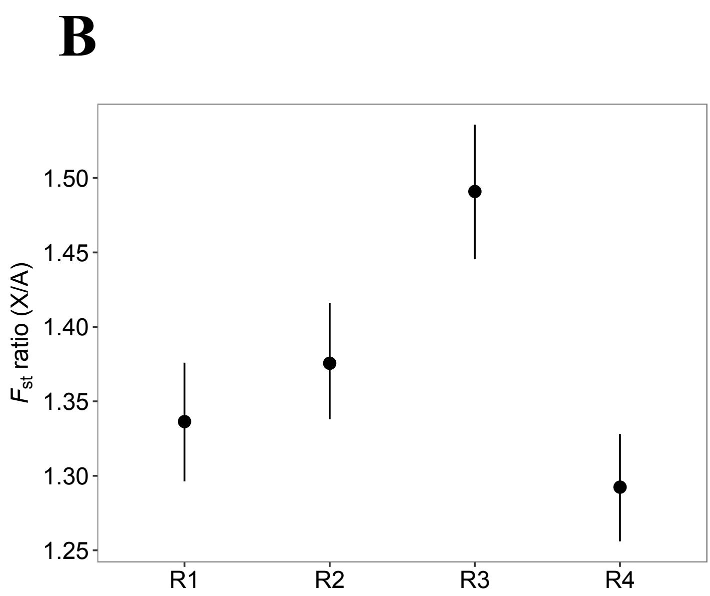
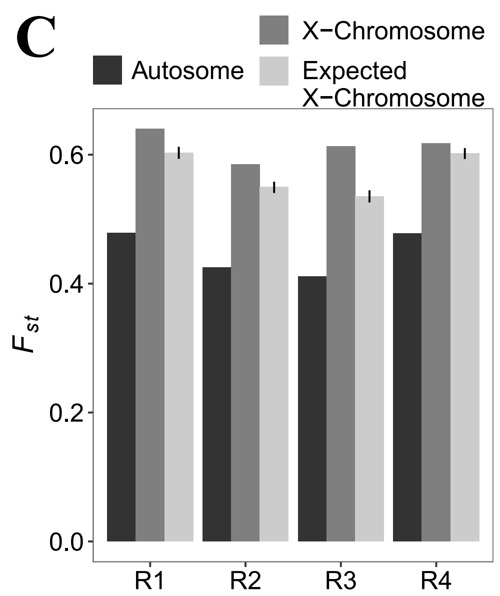


Figure 3





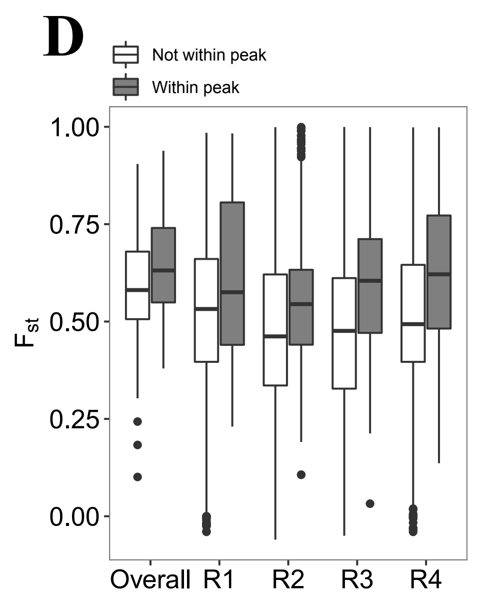


Figure 4

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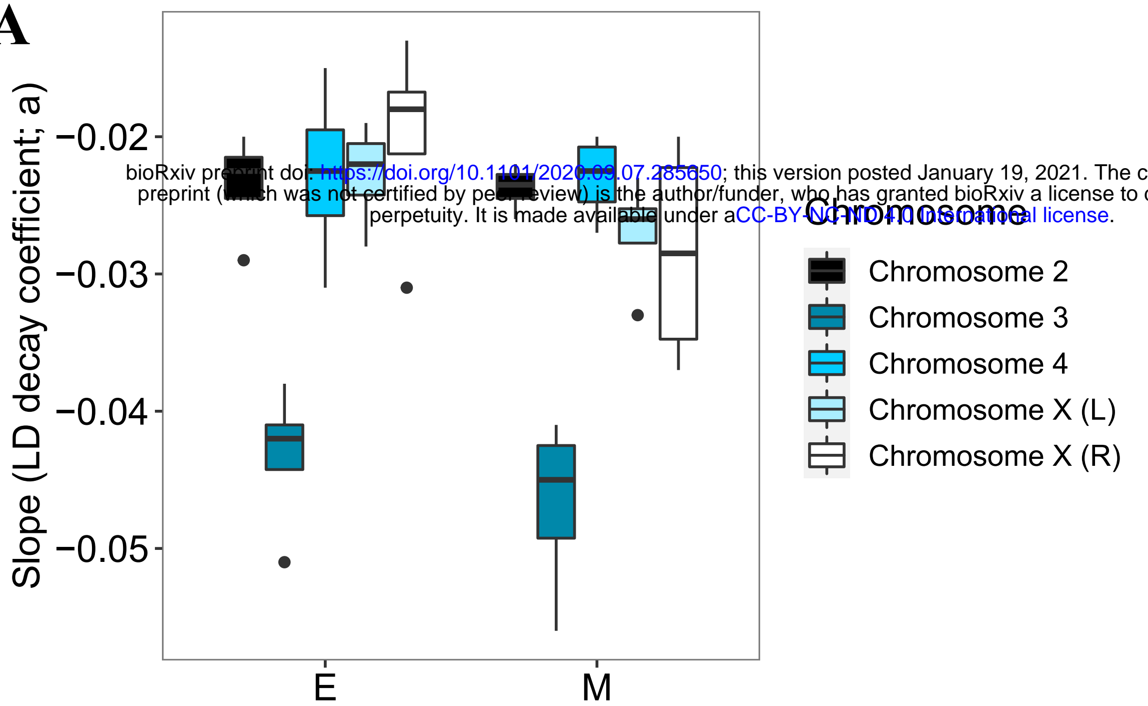
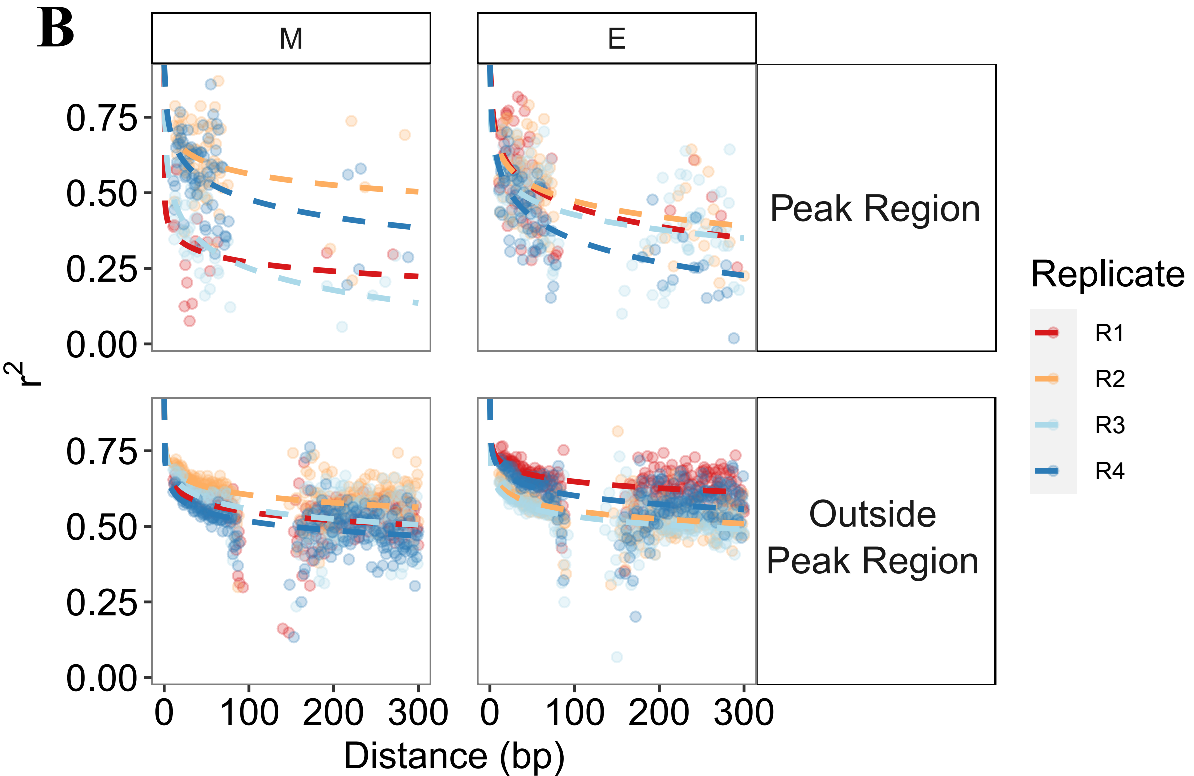




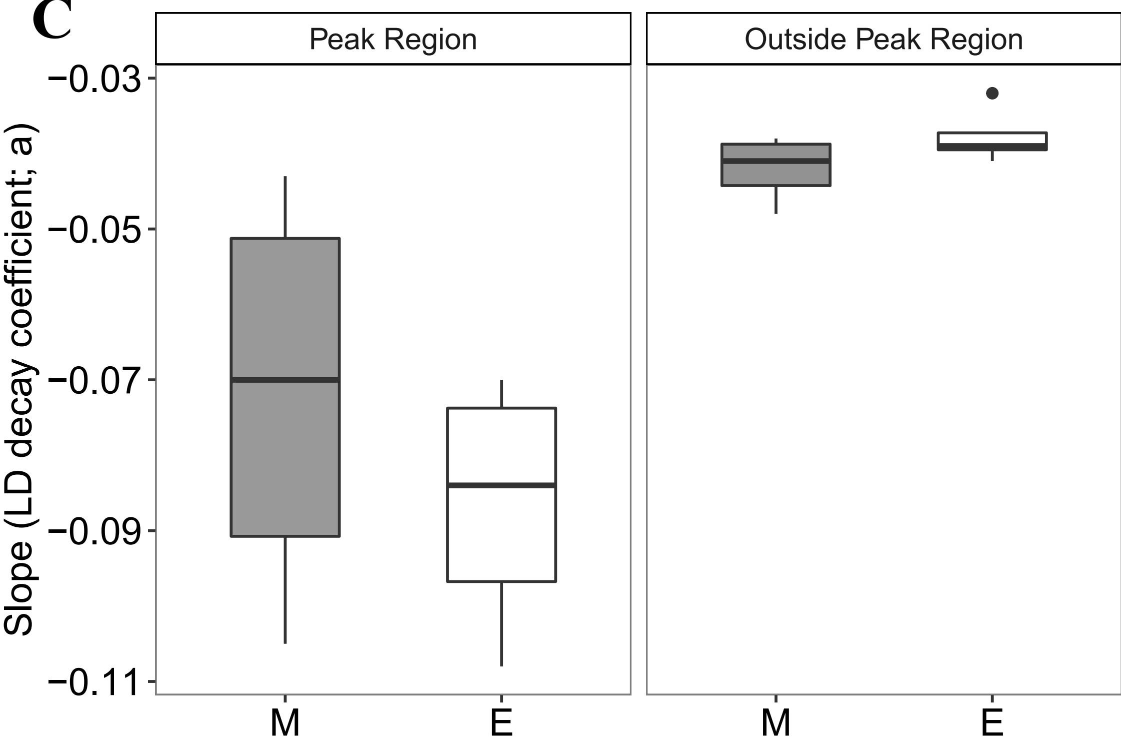
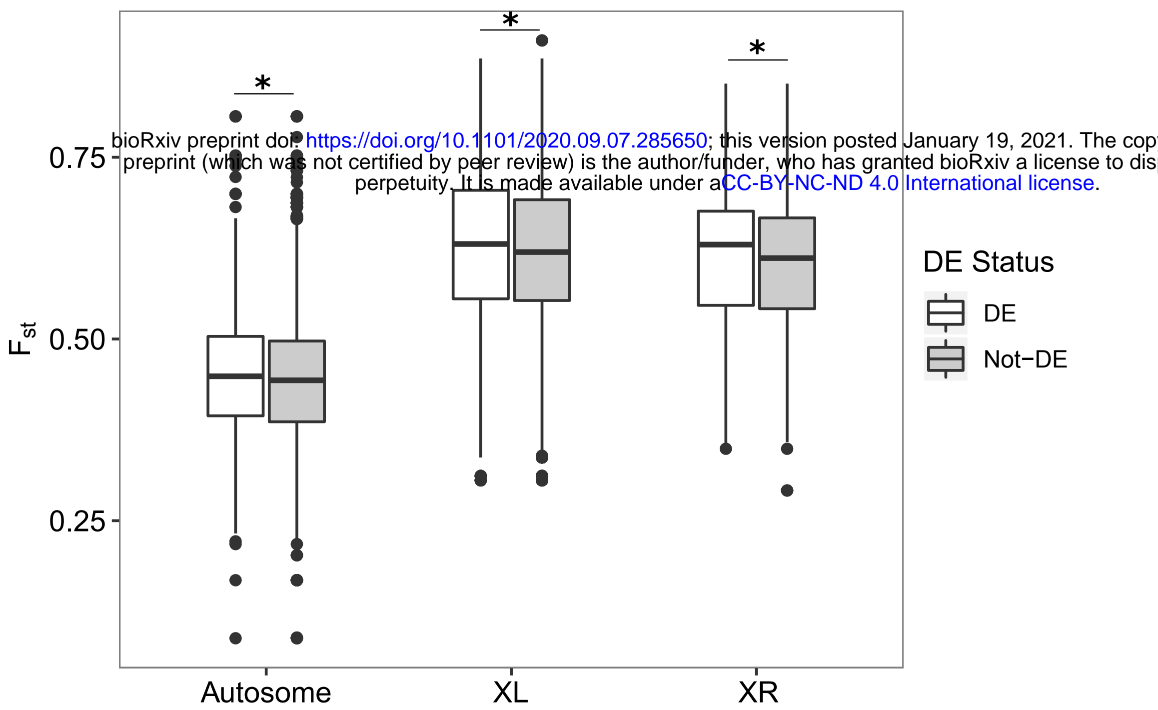


Figure 5



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