

1 Evolution of gene expression variance during adaptation to high 2 temperature in *Drosophila*

3

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11

12 Abstract

13 Shifts in trait means are widely considered as evidence for adaptive responses, but the impact
14 on phenotypic variance remains largely unexplored. Here, we studied gene expression
15 variance of *Drosophila simulans* males before and after 100 generations of adaptation to a
16 novel hot laboratory environment. In each of the two independently evolved replicate
17 populations the variance of about 150 genes changed significantly (mostly reduction).
18 Although different genes were affected in both replicates, these genes are related to digestion
19 in the gut. This non-parallel selection response on the gene level in combination with a
20 convergent response at a higher phenotypic level reflects genetic redundancy, a characteristic
21 hallmark of polygenic adaptation. We propose that the constant and simple food source in the
22 laboratory resulted in selection for reduced variance in digestive genes. In natural populations
23 adaptation to diverse types of food may be beneficial, resulting in higher phenotypic
24 variance. This empirical evidence of phenotypic variance being the direct target of selection
25 during adaptation has important implications for strategies to identify selection signatures.

26

27 Introduction

28 Most studies of adaptation rely on shifts in trait mean as signal of selective response ¹⁻⁶. The
29 variance of the trait in a population, which is the prerequisite for an adaptive response ^{7,8}, has
30 received considerably less attention. As a result, our understanding of the evolution of
31 phenotypic variance is still rather limited. Probably most progress has been made in
32 quantitative genetics, describing the evolution of phenotypic variance in response to a sudden
33 shift in trait optimum ⁹⁻¹². For large populations and traits controlled by many unlinked loci

34 with equal effect, changes in trait optimum are not expected to affect the phenotypic variance
35 ^{13,14}. In contrast, a much more complex picture is expected when the effect sizes are not equal,
36 the population size is finite, or the traits have a simpler genetic basis ^{15–19}.

37

38 In addition to these indirect effects on phenotypic variance, it is also possible that not trait
39 mean, but the variance of a trait is the target of selection. For instance, stabilizing selection
40 may reduce the variance of a trait ²⁰. Canalization, one potential consequence of stabilizing
41 selection ²¹, describes the phenomenon that genetic and environmental perturbations can be
42 buffered and henceforth reduce the phenotypic variance. A classic textbook example for a
43 canalization factor is the heat shock protein Hsp70. Mutations of this chaperone gene result in
44 increased phenotypic variance due to the unmasking of genetic variation ²². Because
45 canalization differs between populations, it has been proposed that it may also evolve ^{23,24}.

46

47 We studied the evolution of gene expression variance after a shift in habitat and found that
48 the variance of most genes remained unaffected, even for genes with a significant change in
49 mean expression (Lai et al., 2021). Here, we focused on a small subset of genes, which
50 changed their phenotypic variance during 100 generations of adaptation. We propose that
51 selection operates directly on phenotypic variance. Because genes that evolved reduced
52 variance were enriched in the gut and selection in natural populations may favor a high
53 variance reflecting the diverse food sources, the constant and simple food source in the
54 laboratory favored a single phenotype, resulting in a loss of phenotypic variance.

55

56 **Results and discussion**

57 ***Rapid changes in gene expression variance during adaptation***

58 We measured the gene expression of ~20 whole body male individuals from two replicates of
59 hot-evolved and reconstituted ancestral populations (Lai et al., 2021). After adapting for 100
60 generations to the high temperature regime, the transcriptomic response of hot-evolved
61 populations was significantly diverged from their ancestors. Principle Component Analysis
62 indicated that PC1 explained 11.9% of the total variation and separated the hot-evolved
63 replicates from their ancestor which reflects the clear adaptive signatures to the novel, hot
64 temperature regime (Lai et al., 2021). The variances of the expression of each gene were
65 estimated and compared between the reconstituted ancestral populations and the two evolved
66 populations. The usage of different lot numbers during the RNA-Seq library preparation

67 (Supplementary file 1), allowed to contrast only ancestral and evolved samples generated
68 with the same lot number (See Materials and methods).

69

70 In both replicates, a small number of genes (166 and 148) significantly changed the
71 expression variance after 100 generations of adaptation (F-test, FDR < 0.05; Figure 1;
72 Supplementary file 2). Among the 166 genes with a significant change in variance in
73 replicate 1, the variance of 125 genes decreased while only 41 genes showed a variance
74 increase. This is a significant difference in the directionality of phenotypic variance evolution
75 ($\chi^2=42.51$, p-value < 7.0×10^{-11}). A similar difference was seen in replicate 2 ($\chi^2=14.30$, p-
76 value < 1.6×10^{-4}). 18 genes were shared between the two replicates. This suggests that the
77 genes with significant changes in variance may be subjected to similar evolutionary
78 processes.

79

80 ***Digestive genes in midgut rapidly decreased their transcriptional variance***

81 In order to characterize plausible processes that could explain the significant changes in gene
82 expression variance, we searched for gene ontology (GO) or tissue-specific expression
83 enrichment. In both replicates genes with increased variance had no consistent enrichment in
84 any biological processes or tissue-specific expression (Supplementary file 3 and 4). In
85 contrast, despite mostly different genes had decreased variance in the two replicates, in both
86 replicates a consistent enrichment for expression in the midgut was detected (Fisher's exact
87 test, FDR < 0.05, Figure 2, Supplementary file 3). GO enrichment analysis identified also
88 catabolism-related processes (e.g.: "organic substance catabolic process", "carbohydrate
89 metabolic process" and "organonitrogen compound catabolic process") in both replicates
90 (Supplementary file 4). In addition to the consistent enrichment in the midgut and catabolic
91 processes, we also observed an enrichment for digestive enzymes²⁵ (Fisher's exact test, odds
92 ratio = 4.21 and 3.53, p-value < 0.01), indicating that a different set of digestive genes in
93 midgut rapidly decreased their transcriptional variance in the two replicates during 100
94 generations of adaptation. The enrichment in midgut and digestive genes persisted when we
95 lowered the significance threshold of the F-test (FDR < 0.1, supplementary file 4), indicating
96 that our result does not depend on a specific cutoff to define the genes with reduced gene
97 expression variance.

98

99 ***Potential selection pressures for the reduction in expression variance in gut***

100 Given the consistent enrichment for the same tissues and GO categories in two independent
101 replicates, we conclude that random genetic drift is an unlikely explanation for the
102 pronounced reduction of expression variance in these genes. Rather, we propose that the
103 reduction in expression variance is a response to selection imposed by an altered
104 environmental factor. Based on functional enrichment, we consider two different hypotheses
105 that could explain the altered expression variance in the gut – microbiome and diet.

106
107 It is well-established that the microbiome has a pronounced effect on gene expression in the
108 gut, but without a strong taxon-specific signal ²⁶. To rule out that heterogeneity in
109 microbiome complexity explains the evolution of gene expression variance, we used all
110 remaining flies of the same common garden experiment from one evolved replicate and the
111 corresponding ancestral population (Supplementary file 1) to investigate the microbiome
112 diversity. The β -diversity, which quantifies the heterogeneity in microbiome complexity
113 within a population, was very similar for evolved and ancestral populations (Figure 3 and
114 Table 1). Despite the limitations of a very reduced sample size, our result is consistent with
115 previous studies ²⁷. Similarly, the microbiome composition cannot explain the reduced
116 variance, as we observed high heterogeneity in composition among individuals from the
117 ancestral and evolved populations (Fig. 3).

118
119 Alternatively, strong selection pressure on the phenotypic variance may have been imposed
120 by the monotonic laboratory food. Natural *Drosophila* populations are feeding from different
121 food sources in different microhabitats, that may require a broad gene expression diversity in
122 digestive genes. We reason that such gene expression heterogeneity may be either deleterious
123 in a simple laboratory environment or specific expression patterns may be optimal on the
124 laboratory food (Figure 4). Either scenario imposes a strong stabilizing selection on
125 phenotypic variance with no apparent directional effect on phenotypic mean in the novel
126 environment for the focal populations.

127
128 To illustrate our hypothesis, we simulated a quantitative trait experiencing strong stabilizing
129 selection over 200 generations and compared the dynamic of phenotypic variance with
130 neutrality. Our results showed substantial decrease in phenotypic variance when strong
131 stabilizing selection is imposed (Figure 5). This provides an illustrative support that the
132 strong stabilizing selection caused by monotonic lab food could alter the transcriptomic
133 variation in midgut digestion rapidly.

134

135 ***Genetic redundancy and its regulatory basis***

136 A particularly interesting observation was that both replicates had different sets of genes with
137 reduced variation, but both sets were enriched for genes expressed in the gut and the
138 digestive enzymes. Such genetic redundancy is a hallmark of polygenic adaptation²⁸ and
139 adaptation in replicated small populations provides an excellent opportunity to study it
140 (Figure 6a). While in large populations more parallel selection responses are expected,
141 genetic drift, in particular during the early stage, affects the selection outcome across
142 replicate populations of moderate size. With genetic drift, the expression variance of
143 digestion-related genes can be pushed in either direction. Henceforth, selection will favor
144 genes for which drift acts synergistically with selection, leading to a heterogeneous outcome
145 across replicates if there is sufficient polygenicity (i.e. more contributing loci than needed to
146 reach the new trait optimum).

147 While we demonstrated genetic redundancy for genes involved in digestive function, the
148 regulatory basis of the reduced variation is not yet clear. Gene expression can be regulated
149 either in *cis* or in *trans*. *Cis*-regulation implies that independent regulatory variants are
150 favored for each gene contributing to the selected phenotype (Figure 6b). It appears unlikely
151 that each of the genes is independently targeted by selection. Rather, a more parsimonious
152 explanation would be that several transcription factors (TFs) which cooperatively regulate
153 these genes are the target of selection and reduced the expression variance of downstream
154 genes. We explored this hypothesis and searched for *trans*-acting TF binding sites shared
155 among genes with decreased expression variance and high expression in the midgut. We
156 identified 18 and 8 TFs in replicate 1 and 2, some of which evolved their mean expression
157 (Supplementary file 5), but none evolved a significant change in expression variance. The
158 lack of significant variance evolution in these candidate targets of selection suggests a more
159 complex regulation of transcriptional variance. We consider it highly likely that the
160 expression of each redundant gene is in turn regulated by several trans-acting factors –
161 providing a second layer of possible genetic redundancy (Figure 6b). Clearly, more work is
162 needed to study the regulatory architecture of genetic redundancy, but the experimental
163 framework introduced here provides an excellent starting point.

164

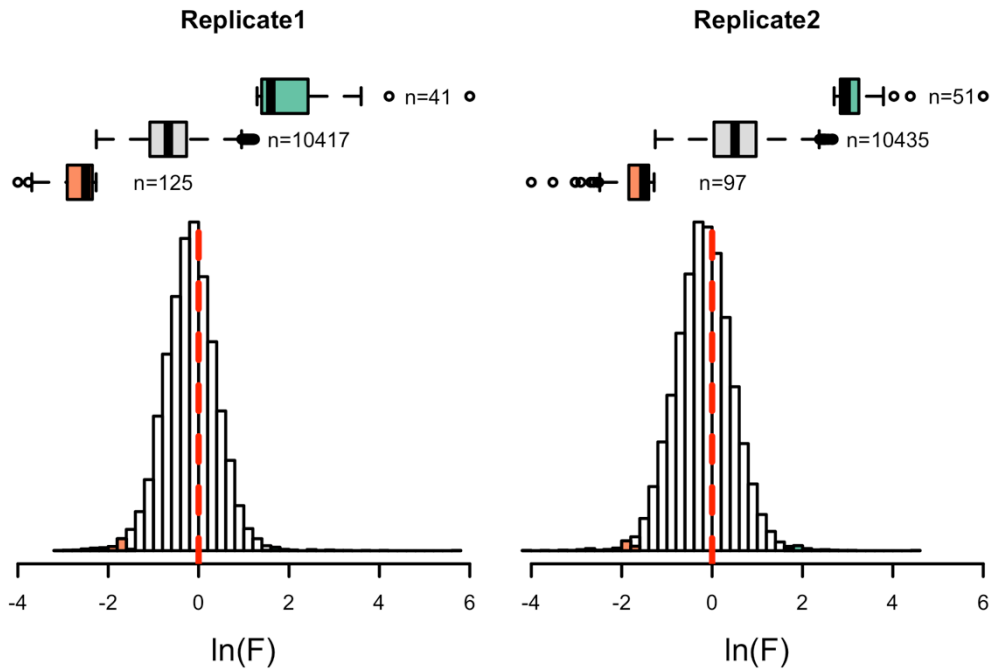
165 **Concluding remarks**

166 Previous studies on adaptive phenotypic evolution mainly focused on “population means”, to
167 explain adaptation to different environments. Nevertheless, selection altering “phenotypic

168 variance within a population” (e.g.: stabilizing selection, disruptive selection...) has been
169 largely neglected. To our knowledge our study provides the first empirical evidence that
170 phenotypic variance can be the direct target of selection during adaptation. This has
171 important consequences for future research on phenotypic evolution, rather than searching for
172 changes in mean phenotype as a response to selection, it may also be important to consider
173 that phenotypic variance may be subject to selection and can contribute to our understanding
174 of adaptation processes in natural and experimental populations.

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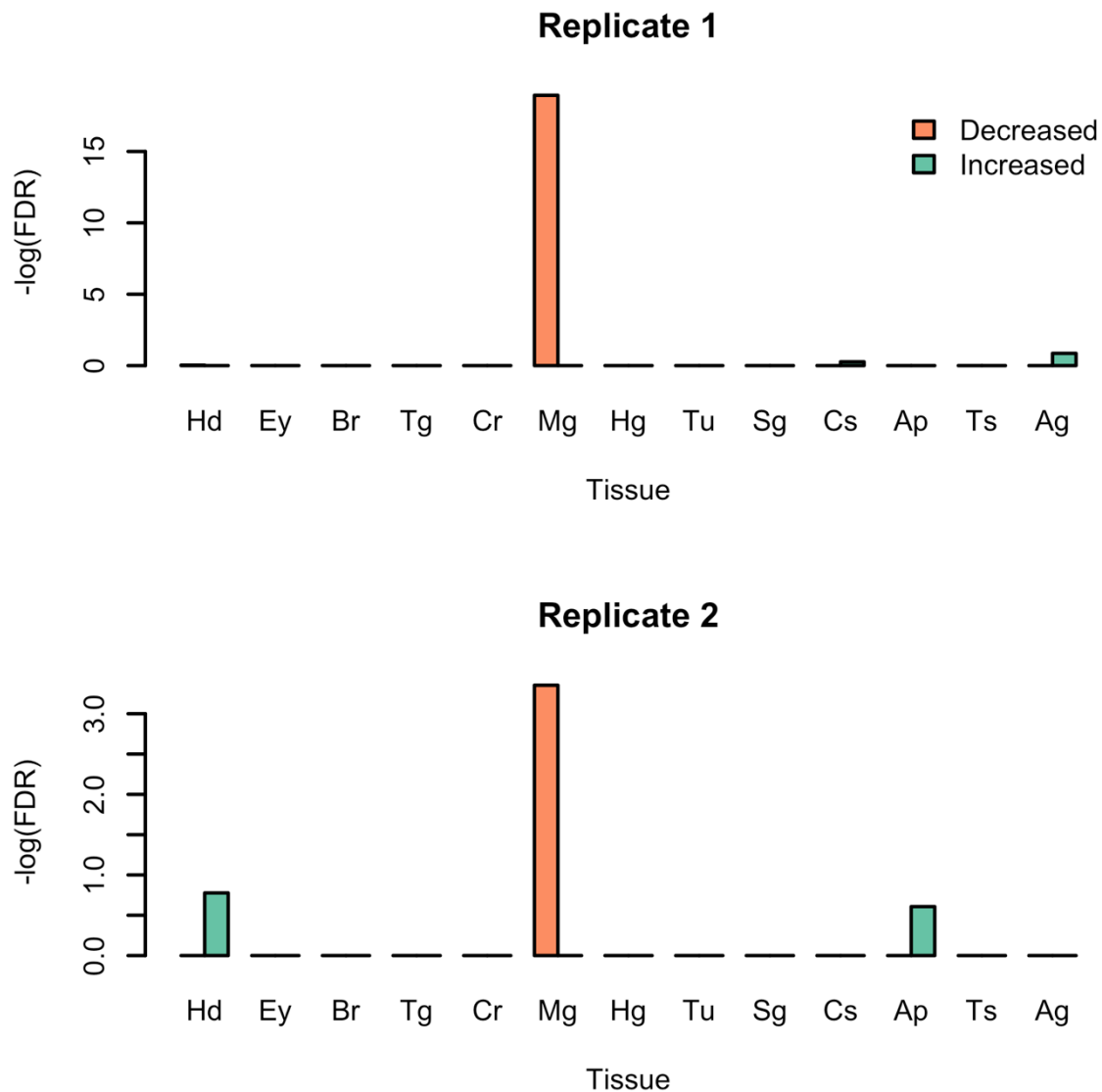
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177

178 **Figure 1. Evolution of gene expression variance.** The distribution of the change in gene
179 expression variances ($\ln(F)$) during the evolution experiment in the 1st (left panel) and 2nd
180 (right panel) replicate. Boxes in salmon indicate the genes with decreased variance in both
181 replicates ($n=125$ and 97) and boxes in green represent genes with increased variance ($n=41$
182 and 51). Boxes in grey include the other genes without significant change in variance
183 ($n=10417$ and 10435).

184

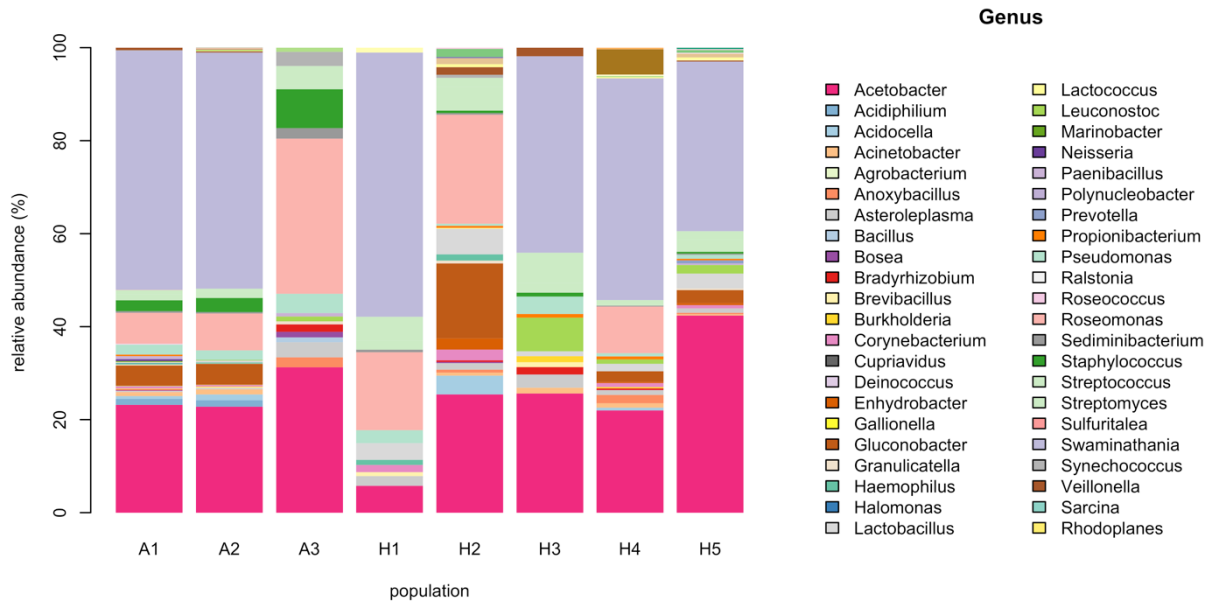


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186 **Figure 2. Tissue enrichment of genes with significant changes in expression variance.**

187 The bars indicates the significance ($-\ln(\text{FDR})$) of enrichment for genes with significant
188 variance changes (orange: genes with decreased variance; green: genes with increased
189 variance) among genes with tissue-specific gene expression pattern (Br-brain, Hd-head, Cr-
190 crop, Mg-midgut, Hg-hindgut, Tb-malpighian tubule, Tg-thoracoabdominal ganglion, Cs-
191 carcass, Sg-salivary gland, Fb-fat body, Ey-eye and Hr-heart). In both replicates a highly
192 significant enrichment can be found in the midgut for genes with reduced expression
193 variance.

194



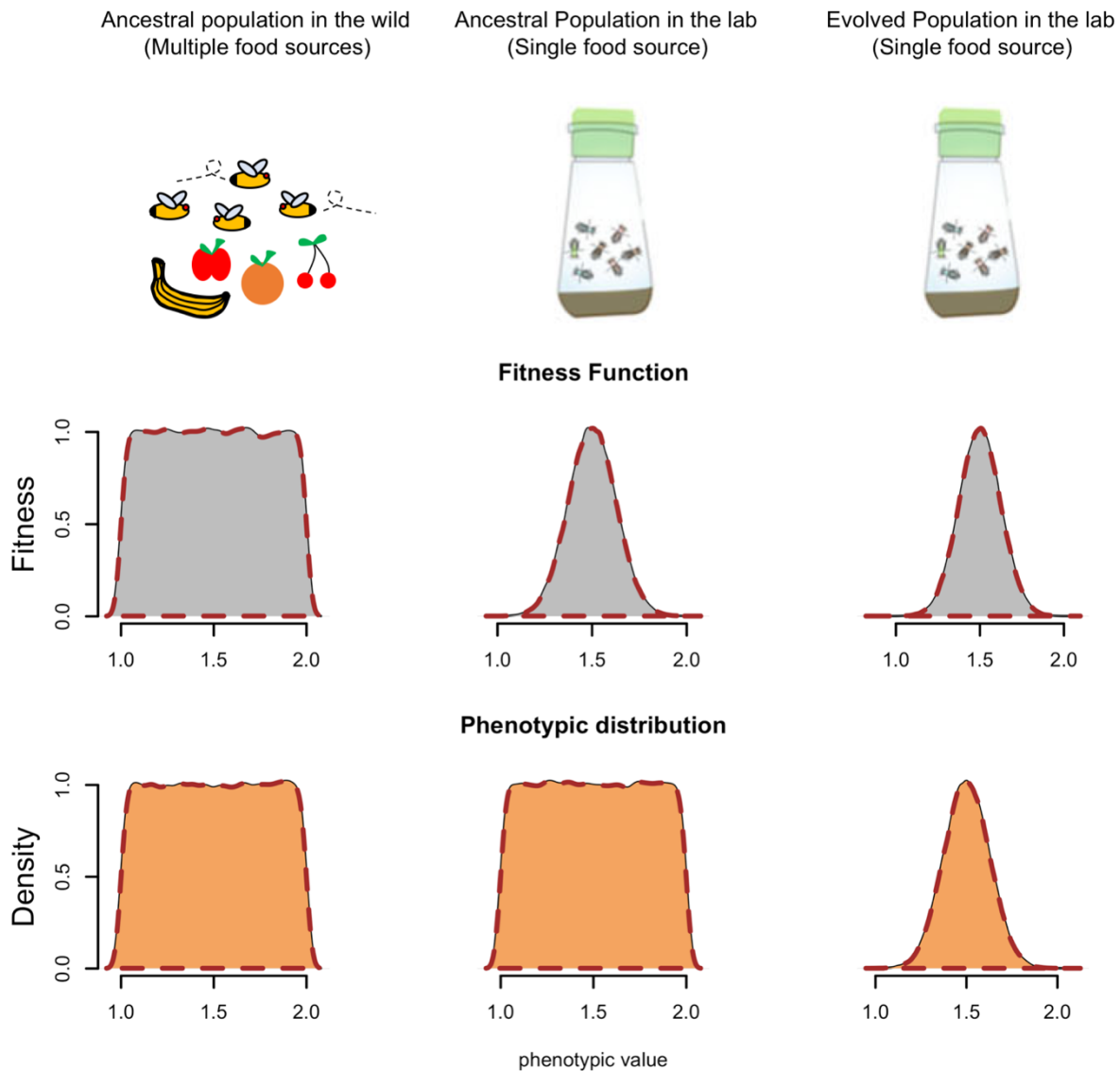
195

196 **Figure 3. Microbiome composition in ancestral and evolved flies.**

197 Microbiome composition on the genus level for three individuals from the ancestral

198 population (A1-A3) and five individuals from a hot-evolved population (H1-H5).

199



200

201 **Figure 4. Hypothesis of a simpler lab food selecting for decreased gene expression**

202 **variance in the midgut.** A proposed model for potential selection for decreased expression

203 variance in midgut imposed by the drastic change in food supply. Food sources change

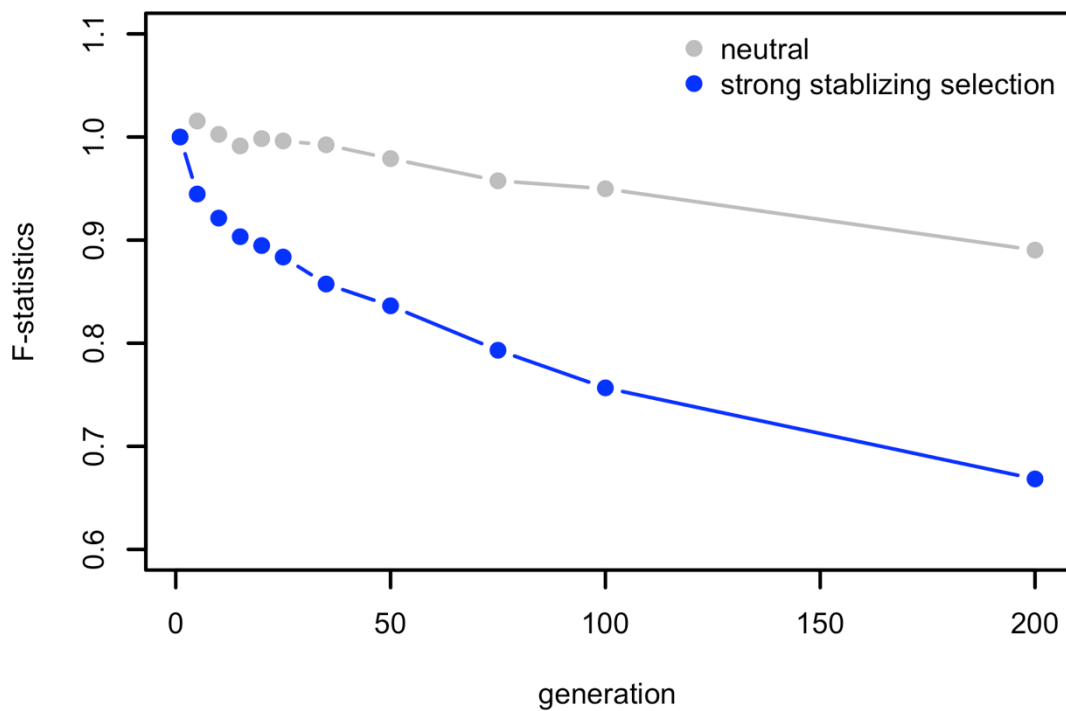
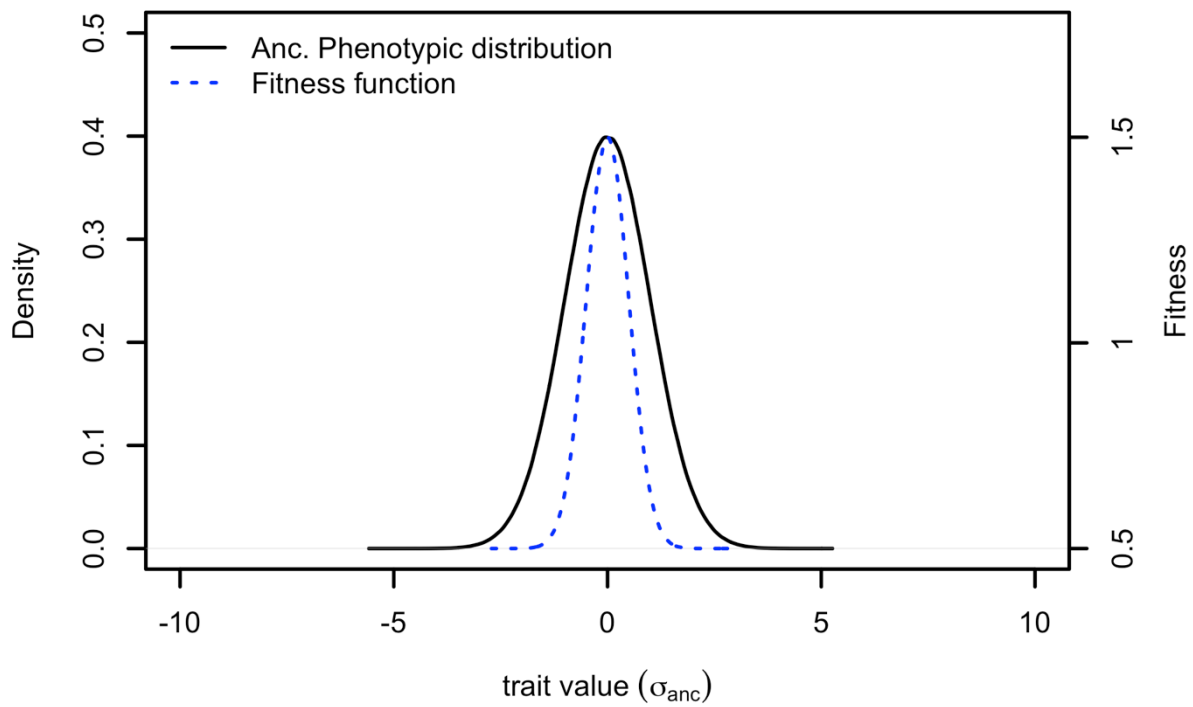
204 dramatically when we bring these files from the wild into the lab. The distributions of fitness

205 landscapes and expression value of the genes encoding digestive enzymes may change as the

206 food sources switched. After 100 generations on a single food source, the genes encoding

207 digestive enzymes decreased their expression variance.

208



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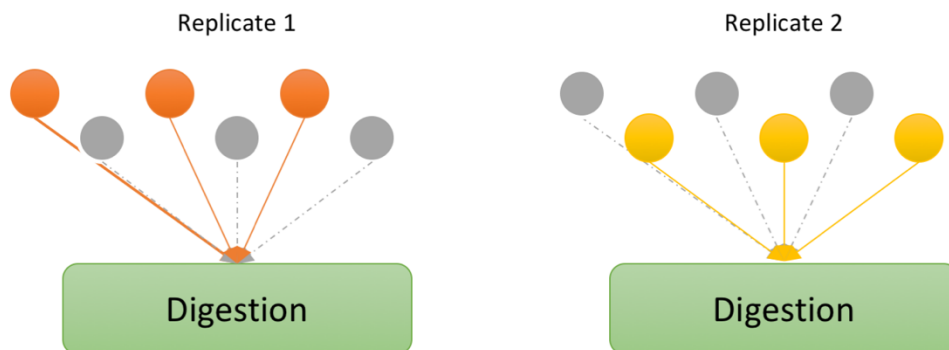
210 **Figure 5. Reduction in variance by strong stabilizing selection.**

211 **a.** Computer simulations of a scenario where the shift to a simpler environment results in
212 stronger stabilizing selection. The ancestral phenotypic distribution of quantitative trait under
213 stabilizing selection before the population was introduced to the simple environment (black).

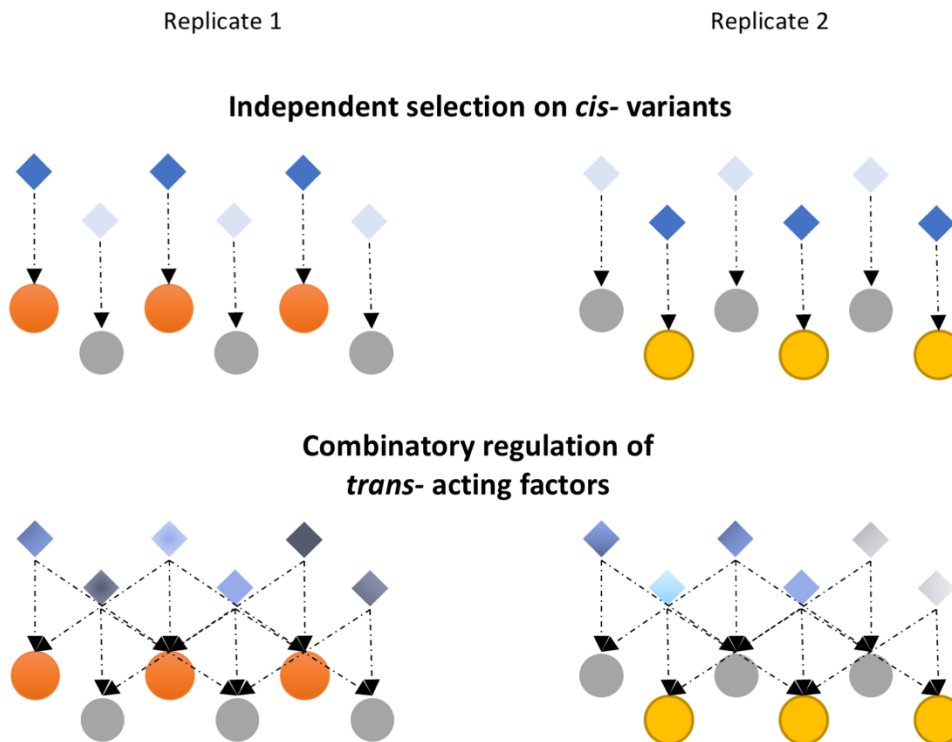
214 The fitness function after the habitat shift is shown in blue. The variance of the fitness
215 function is set to 0.5 standard deviation of the ancestral trait distribution. **b.** The changes in
216 phenotypic variance under strong stabilizing selection (blue) and neutrality (grey). The
217 change in phenotypic variance (F) is calculated as the ratio between the evolved and ancestral
218 phenotypic variance at each generation ($\sigma_{gen.}^2/\sigma_0^2$) for each scenario. For each scenario, 1000
219 runs of simulations have been performed.
220

221

a. Genetic redundancy



b. Regulatory architecture



222

223

224 **Figure 6. Schematic illustration of genetic redundancy at gene level with two possible**

225 **regulatory architectures explaining the reduction in expression variance. a. Genetic**

226 **redundancy: six genes contribute to digestion (higher-level phenotype) and the new**

227 **phenotypic optimum could be reached by expression changes of three genes. Stochastic**

228 effects result in different gene sets (orange/yellow) responding to selection in the two
229 replicates (replicate1/replicate2). **b.** Two hypotheses about a regulatory architecture that
230 allows for the rapid decrease in variance of digestion-related genes. Either selection acts
231 independently on the *cis*-regulatory variants of each gene or combinatorial changes of several
232 TFs reduce the expression variance.

233

234

235

236 **Table 1. Microbiome diversity in the reconstituted ancestral and hot-evolved population**
237 **based on 16S-rRNA amplicon sequencing**

	Ancestral	Evolved
<i>α-diversity</i>	23	16.7
<i>β-diversity</i>	1.83	1.8
<i>γ-diversity</i>	42	30

238

239 **Materials and methods**

240 Experimental evolution

241 The setup of populations and evolution experiment have been described by ²⁹. Briefly, ten
242 outbred populations seeded from 202 isofemale lines were exposed to a laboratory
243 experiment at 28/18 °C with 12hr light/12hr dark photoperiod for more than 100 generations.
244 Each replicate consisted of 1000 to 1250 adults at each generation.

245

246 Common garden experiment

247 The collection of samples from the evolution experiment for RNA-Seq was preceded by two
248 generations of common garden (CGE). The common garden experiment was performed at
249 generation 103 of the evolution in the hot environment and this CGE has been described in
250 ^{5,29-31}. In brief, an ancestral population was reconstituted by pooling five mated females from
251 184 founder isofemale lines ³². Two replicates of the reconstituted ancestral population and
252 two independently evolved populations at generation 103 were reared for two generations
253 with egg-density control (400 eggs/bottle) at the same temperature regime as in the evolution
254 experiment. Freshly eclosed flies were transferred onto new food for mating. Sexes were
255 separated under CO₂ anesthesia at day 3 after eclosure, left to recover from CO₂ for two
256 days, and at the age of five days whole-body mated flies of each sex were snap-frozen at 2pm
257 in liquid nitrogen and stored at -80°C until RNA extraction. In this study, more than 30
258 individual male flies from two reconstituted ancestral populations (replicate no. 27 and no.
259 28) and two evolved populations (replicate no. 4 and no. 9) were subjected to RNA-Seq.

260

261 RNA extraction and library preparation

262 Whole bodies of individual male flies were removed from the -80°C freezer and immediately
263 homogenized in Qiazol lysis reagent (Qiagen, Hilden, Germany). The homogenate was
264 treated with DNase I followed by addition of chloroform, centrifugation and mixture of the
265 upper phase with 70% ethanol as described for the Qiagen RNeasy Universal Plus Mini Kit.
266 The mixture was subsequently loaded onto a RNeasy MinElute Spin column as provided by
267 the RNeasy Plus Micro Kit (Qiagen, Hilden, Germany), and all washing steps were
268 performed according to the instructions for that kit. All resulting total RNA was used to
269 prepare stranded mRNA libraries on the Neoprep Library Prep System (Illumina, San Diego,
270 USA) following the manufacturer's protocol: Neoprep runs were performed using software
271 version 1.1.0.8 and protocol version 1.1.7.6 with default settings for 15 PCR cycles and an
272 insert size of 200bp. All libraries for individuals of ancestral replicate no. 27 and evolved

273 replicate no. 4 were prepared with library cards of lot no. 20180170; all libraries for
274 individuals of ancestral replicate no. 28 and evolved replicate no. 9 were prepared with
275 library cards of lot no. 20178099. 50bp single-end reads were sequenced on an Illumina
276 HiSeq 2500. All sequencing data will be available in European Nucleotide Archive (ENA)
277 under the accession number PRJEB37011 upon publication.

278

279 RNA-Seq data processing and quality check

280 All RNA-Seq reads were trimmed using ReadTools (Gómez-Sánchez and Schlötterer, 2018)
281 with quality score of 20 and aligned to *Drosophila simulans* reference genome³³ using
282 GSNAP³⁴ with parameter setting -k 15 -N 1 -m 0.08. Exon-aligned reads were piped into
283 Rsubread³⁵ to calculate read counts of each gene, and raw read counts of each gene were
284 normalized with the TMM method implemented in edgeR³⁶. Samples with severe 3'- bias
285 were removed based on visual inspection of the gene-body coverage plot^{37,38}. We observed
286 some outlier individuals and suspected that the freezing process may have led to detachment
287 of body parts, such as eyes or heads, in these individuals. We compared gene expression
288 between such outliers and all other samples and performed tissue enrichment analysis for
289 genes with at least 2-fold lower expression in the outlier samples. Samples with evidence of
290 tissue detachment were excluded. After filtering, each population remained approximately 20
291 individuals (Supplementary file 1). Only genes with at least 1 count per million base (CPM)
292 were included in the analyses to avoid extremely lowly expressed genes.

293

294 RNA-Seq data analysis

295 For all RNA-Seq data we only compared samples which were prepared with library cards
296 from the same lot number to avoid batch effects (Replicate 1: evolved replicate 4 vs.
297 reconstituted ancestral population replicate 27; Replicate 2: evolved replicate 9 vs.
298 reconstituted ancestral population replicate 28).

299 For the analysis of expression variance evolution, we applied natural log transformation³⁹ to
300 eliminate the strong mean-variance dependency in RNA-Seq data due to the nature of the
301 negative binomial distribution (Lai et al., 2021). The variance of the expression of each gene
302 (lnCPM) was estimated in each population. The change of gene expression variance was
303 determined with the F test between the variance within the ancestral population and the
304 variance within the evolved population of each gene. P-value adjustment was performed
305 using the Benjamini-Hochberg false discovery rate (FDR) correction.

306

307 Gene ontology and tissue enrichment analysis

308 We used ClueGO⁴⁰ to perform gene ontology (GO) enrichment analyses of the candidate
309 genes have significant change on variance. To understand in which tissues the genes of
310 interest are expressed, we made use of tissue-specific expression profiles of adult males of
311 *Drosophila melanogaster* on flyatlas2⁴¹. This data set includes 13 tissues in male flies.
312 Genes that are expressed 2-fold higher in a given tissue than in the whole body are identified.
313 Fisher's exact test was performed to test if the genes of interest are enriched for genes highly
314 expressed in one tissue. P-value adjustment was performed using the Benjamini-Hochberg
315 false discovery rate (FDR) correction.

316

317 Microbiome diversity in ancestral and evolved populations

318 To explore the heterogeneity in gut microbiome, we performed 16S-rRNA amplicon
319 sequencing on three remaining individual males of the ancestral and evolved populations
320 from the same common garden experiment (Supplementary file 1).

321 We used primers designed to amplify the V3-V4 hypervariable regions of the 16S rRNA
322 gene. The primers had an overhang to match Nextera Index primers (Forward primer: 5'-
323 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-CCTACGGGNGGCWGCAG-3',
324 Reverse primer: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-
325 GACTACHVGGGTATCTAATCC-3'). PCR products were amplified with 30 cycles at an
326 annealing temperature of 65°C, purified using AMPure XP beads (Beckman Coulter,
327 Carlsbad, CA) and subjected to a second PCR to introduce dual index sequences using
328 Nextera XT Index Kits (Illumina, San Diego, CA). In the second PCR, we used 6 cycles and
329 an annealing temperature of 55°C, and both PCRs were carried out in 5µl total volume using
330 the NEBNext Ultra II Q5 Mastermix (New England Biolabs, Ipswich, MA). The final
331 amplicons were again purified, quantified using the Qubit HS assay kit (Invitrogen, Carlsbad,
332 CA), and 125 bp paired-end reads were sequenced on an Illumina HiSeq 2500.

333 The 16S-rRNA sequence data were trimmed using ReadTools⁴² with quality score of 20.
334 Unpaired reads were removed. Owing to the variation in sequencing depths between samples,
335 all samples were down-sampled to the lowest depth (66,625 pairs/sample, Supplementary file
336 1). Each bam file was converted into a fastq.gz file and analyzed with Kraken2⁴³ following
337 the recommended parameters and the estimation of genus abundance was corrected by
338 Bracken⁴⁴.

339 Genus abundance of the microbiome community in each sample was obtained. With the
340 filtration (read number < 5), extremely lowly abundant genera were excluded. β -diversity⁴⁵
341 was then calculated to evaluate the heterogeneity of the microbiome complexity among the
342 three individuals from the same population.

$$343 \quad \beta - \text{diversity} = \frac{\gamma - \text{diversity}}{\alpha - \text{diversity}}$$

344 where γ -diversity is the genera species richness in a population and α -diversity is the mean
345 richness within an individual.

346

347 Simulation study

348 We performed forward simulations with MimicrEE2⁴⁶ using the *qff* mode to illustrate the
349 influence of the genetic architecture on the evolution of phenotypic variance during the
350 adaptation to a new trait optimum. With 189 founder haplotypes²⁹, we simulated quantitative
351 traits under the control of 20 numbers of loci with an effective population size of 300. For
352 each trait, we assume an additive model and the negative correlation ($r=-0.7$) between the
353 effect size ($\alpha \sim \Gamma(100,15)$) and starting frequency (Barghi et al., 2019). We used *correlate()*
354 function implemented in “fabricatr” R package⁴⁷ to generate the effect sizes with negative
355 correlation ($r=-0.7$) with starting frequency. The sum of effect sizes of each trait was
356 normalized to 1. We assumed heritability $h^2 = 0.6$. To simulate strong stabilizing selection
357 without trait optimum shift, we provided the fitness function: $N(\bar{x}_A, 0.5\sqrt{V_A})$, where \bar{x}_A is the
358 ancestral phenotypic mean and $\sqrt{V_A}$ is the ancestral genetic variance. For the neutrality case,
359 we assumed the same fitness for each individual. For each trait under each scenario, the
360 phenotypic variance was calculated at different generations and normalized to the ancestral
361 phenotypic variance at generation 1 to investigate the dynamic of phenotypic variance during
362 the evolution.

363

364 Transcription factor enrichment analysis

365 Transcription factor enrichment analysis among the genes with significant decreased variance
366 in the midgut was done with Rcistarget (version 1.0.2)⁴⁸. First, enrichment of cis-regulatory
367 elements (CREs) 5kb upstream and intronic sequences of the genes of interest
368 (Supplementary file 5) was identified. The motif-search database used here was based on the
369 latest motif ranking files of *Drosophila* species (“dm6-5kb-upstream-full-tx-
370 11species.mc8nr.feather”). Parameter setting used in this analysis is as following:

371 nesThreshold = 5 and aucMaxRank = 0.05. The predicted transcription factors (TFs) were
372 considered as candidate TFs regulating the genes of interest.

373

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381

382 **Author contribution**

383 W.Y.L and C.S. conceived the study. W.Y.L performed the data analysis. W.Y.L. and C.S.
384 wrote the manuscript.

385

386 **Competing interests**

387 The authors declare no competing interests.

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389

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