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1 **Genomic analysis of European *Drosophila melanogaster* populations reveals**
2 **longitudinal structure, continent-wide selection, and previously unknown DNA viruses**

3
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57 **Abstract**

58 Genetic variation is the fuel of evolution, with standing genetic variation especially important for
59 short-term evolution and local adaptation. To date, studies of spatio-temporal patterns of genetic
60 variation in natural populations have been challenging, as comprehensive sampling is logistically
61 difficult, and sequencing of entire populations costly. Here, we address these issues using a
62 collaborative approach, sequencing 48 pooled population samples from 32 locations, and perform the
63 first continent-wide genomic analysis of genetic variation in European *Drosophila melanogaster*. Our
64 analyses uncover longitudinal population structure, provide evidence for continent-wide selective
65 sweeps, identify candidate genes for local climate adaptation, and document clines in chromosomal
66 inversion and transposable element frequencies. We also characterise variation among populations in
67 the composition of the fly microbiome, and identify five new DNA viruses in our samples.

68

69 **Introduction**

70 Understanding processes that influence genetic variation in natural populations is fundamental to
71 understanding the process of evolution (Dobzhansky 1970; Lewontin 1974; Kreitman 1983; Kimura
72 1984; Hudson *et al.* 1987; McDonald & Kreitman 1991; Adrian & Comeron 2013). Until recently,
73 technological constraints have limited studies of natural genetic variation to small regions of the
74 genome and small numbers of individuals. With the development of population genomics, we can
75 now analyse patterns of genetic variation for large numbers of individuals genome-wide, with samples
76 structured across space and time. As a result, we have new insight into the evolutionary dynamics of
77 genetic variation in natural populations (e.g., Hohenlohe *et al.* 2010; Cheng *et al.* 2012; Begun *et al.*
78 2007; Pool *et al.* 2012; Harpur *et al.* 2014; Zanini *et al.* 2015). But, despite this technological
79 progress, extensive large-scale sampling and genome sequencing of populations remains prohibitively
80 expensive and too labor-intensive for most individual research groups.

81 Here, we present the first comprehensive, continent-wide genomic analysis of genetic variation of
82 European *Drosophila melanogaster*, based on 48 pool-sequencing samples from 32 localities
83 collected in 2014 (fig. 1) by the European *Drosophila* Population Genomics Consortium (*DrosEU*;

84 <https://droseu.net>). *D. melanogaster* offers several advantages for genomic studies of evolution in
85 space and time. It boasts a relatively small genome, a broad geographic range, a multivoltine life
86 history which allows sampling across generations on short timescales, simple standard techniques for
87 collecting wild samples, and a well-developed context for population genomic analysis (e.g., Powell
88 1997; Keller 2007; Hales *et al.* 2015). Importantly, this species is studied by an extensive
89 international research community, with a long history of developing shared resources (Larracuenta &
90 Roberts 2015; Bilder & Irvine 2017; Haudry *et al.* 2020).

91 Our study complements and extends previous studies of genetic variation in *D. melanogaster*, both
92 from its native range in sub-Saharan Africa and from its world-wide expansion as a human
93 commensal. The expansion into Europe is thought to have occurred approximately 4,100 - 19,000
94 years ago and into North America and Australia in the last few centuries (e.g., Lachaise *et al.* 1988;
95 David & Capy 1988; Li & Stephan 2006; Keller 2007; Sprengelmeyer *et al.* 2018; Kapopoulou *et al.*
96 2018a; Arguello *et al.* 2019). The colonization of novel habitats and climate zones on multiple
97 continents makes *D. melanogaster* especially useful for studying parallel local adaptation, with
98 previous studies finding pervasive latitudinal clines in allele frequencies (e.g., Schmidt & Paaby 2008;
99 Turner *et al.* 2008; Kolaczowski *et al.* 2011; Fabian *et al.* 2012; Bergland *et al.* 2014; Machado *et al.*
100 2016; Kapun *et al.* 2016a), structural variants such as chromosomal inversions (reviewed in Kapun &
101 Flatt 2019), transposable elements (TEs) (Boussy *et al.* 1998; González *et al.* 2008; 2010), and
102 complex phenotypes (de Jong & Bochdanovits 2003; Schmidt & Paaby 2008; Schmidt *et al.* 2008;
103 Kapun *et al.* 2016b; Behrman *et al.* 2018), especially along the North American and Australian east
104 coasts. In addition to parallel local adaptation, these latitudinal clines are, however, also affected by
105 admixture with flies from Africa and Europe (Caracristi & Schlötterer 2003; Yukilevich & True
106 2008a; b; Duchon *et al.* 2013; Kao *et al.* 2015; Bergland *et al.* 2016).

107 In contrast, the population genomics of *D. melanogaster* on the European continent remains
108 largely unstudied (Božičević *et al.* 2016; Pool *et al.* 2016; Mateo *et al.* 2018). Because Eurasia was
109 the first continent colonized by *D. melanogaster* as they migrated out of Africa, we sought to
110 understand how this species has adapted to new habitats and climate zones in Europe, where it has

111 been established the longest (Lachaise *et al.* 1988; David & Capy 1988). We analyse our data at three
112 levels: (1) variation at single-nucleotide polymorphisms (SNPs) in nuclear and mitochondrial
113 (mtDNA) genomes ($\sim 5.5 \times 10^6$ SNPs in total); (2) structural variation, including TE insertions and
114 chromosomal inversion polymorphisms; and (3) variation in the microbiota associated with flies,
115 including bacteria, fungi, protists, and viruses.

116

117 **Results and Discussion**

118 As part of the *DrosEU* consortium, we collected 48 population samples of *D. melanogaster* from 32
119 geographical locations across Europe in 2014 (table 1; fig. 1). We performed pooled sequencing
120 (Pool-Seq) of all 48 samples, with an average autosomal coverage $\geq 50x$ (supplementary table S1,
121 Supplementary Material online). Of the 32 locations, 10 were sampled at least once in summer and
122 once in fall (fig. 1), allowing a preliminary analysis of seasonal change in allele frequencies on a
123 genome-wide scale.

124 A description of the basic patterns of genetic variation of these European *D. melanogaster*
125 population samples, based on SNPs, is provided in the supplement (see supplementary results,
126 supplementary table S1, Supplementary Material online). For each sample, we estimated genome-
127 wide levels of π , Watterson's θ and Tajima's D (corrected for pooling; Futschik & Schlötterer 2010;
128 Kofler *et al.* 2011). In brief, patterns of genetic variability and Tajima's D were largely consistent
129 with what has been previously observed on other continents (e.g., Fabian *et al.* 2012; Langley *et al.*
130 2012; Lack *et al.* 2015, 2016), and genetic diversity across the genome varies mainly with
131 recombination rate (Langley *et al.* 2012). We also found little spatio-temporal variation among
132 European populations in overall levels of sequence variability (table 2).

133 Below we focus on the identification of selective sweeps, previously unknown longitudinal
134 population structure across the European continent, patterns of local adaptation and clines, and
135 microbiota.

136

137 **Several genomic regions show signatures of continent-wide selective sweeps**

138 To identify genomic regions that have likely undergone selective sweeps in European populations of
139 *D. melanogaster*, we used *Pool-hmm* (Boitard *et al.* 2013; see supplementary table S2A,
140 Supplementary Material online), which identifies candidate sweep regions via distortions in the allele
141 frequency spectrum. We ran *Pool-hmm* independently for each sample and identified several genomic
142 regions that coincide with previously identified, well-supported sweeps in the proximity of *Hen1*
143 (Kolaczowski *et al.* 2011), *Cyp6g1* (Daborn *et al.* 2002), *wapl* (Beisswanger *et al.* 2006), and around
144 the chimeric gene *CR18217* (Rogers & Hartl 2012), among others (supplementary table S2B,
145 Supplementary Material online). These regions also showed local reductions in π and Tajima's *D*,
146 consistent with selective sweeps (fig. 2; fig. S1 and fig. S2; Supplementary Material online). The
147 putative sweep regions that we identified in the European populations included 145 of the 232 genes
148 previously identified using *Pool-hmm* in an Austrian population (Boitard *et al.* 2012; supplementary
149 table S2C, Supplementary Material online). We also identified other regions which have not
150 previously been described as targets of selective sweeps (supplementary table S2A, Supplementary
151 Material online). Of the regions analysed, 64 showed signatures of selection across all European
152 populations (supplementary table S2D, Supplementary Material online). Of these, 52 were located in
153 the 10% of regions with the lowest values of Tajima's *D* (SuperExactTest; $p < 0.001$). These may
154 represent continent-wide sweeps that predate the colonization of Europe (e.g., Beisswanger *et al.*
155 2006) or which have recently swept across the majority of European populations (supplementary table
156 S2D). Interestingly, 43 of the 64 genes (67%) that showed signatures of selection across all European
157 populations were located in regions with reduced Tajima's *D* in African populations, suggesting that
158 selective sweeps in these genes might predate the out-of-Africa expansion (Table S2D).

159 We then asked if there was any indication of selective sweeps particular to a certain habitat. To
160 this end, we classified the populations according to the Köppen-Geiger climate classification (Peel *et*
161 *al.* 2007) and identified several putative sweeps exclusive to arid, temperate and cold regions
162 (supplementary table S2A, Supplementary Material online). To shed light on potential phenotypes
163 affected by the potential sweeps we performed a gene ontology (GO) analysis. For temperate

164 climates, this analysis showed enrichment for functions such as ‘response to stimulus’, ‘transport’,
165 and ‘nervous system development’. For cold climates, it showed enrichment for ‘vitamin and co-
166 factor metabolic processes’ (supplementary table S2E, Supplementary Material online). There was no
167 enrichment of any GO category for sweeps associated with arid regions.

168 Thus, we identified several new candidate selective sweeps in European populations of *D.*
169 *melanogaster*, many of which occur in the majority of European populations and which merit future
170 study, using sequencing of individual flies and functional genetic experiments.

171

172 **European populations are structured along an east-west gradient**

173 We next investigated whether patterns of genetic differentiation might be due to demographic sub-
174 structuring. Overall, pairwise differentiation as measured by F_{ST} was relatively low, particularly for
175 the autosomes (autosomal F_{ST} 0.013–0.059; *X*-chromosome F_{ST} : 0.043–0.076; Mann-Whitney U test;
176 $p < 0.001$; supplementary table S1, Supplementary Material online). The *X* chromosome is expected
177 to have higher F_{ST} than the autosomes, given its relatively smaller effective population size (Mann-
178 Whitney U test; $p < 0.001$; Hutter *et al.* 2007). One population, from Sheffield (UK), was unusually
179 differentiated from the others (average pairwise $F_{ST} = 0.027$; $SE = 0.00043$ vs. $F_{ST} = 0.04$; $SE =$
180 0.00055 for comparisons without this population and with this population only; supplementary table
181 S1, Supplementary Material online). Including this sample in the analysis could potentially lead to
182 exaggerated patterns of geographic differentiation, as it is both highly differentiated and the furthest
183 west. We therefore excluded it from the following analyses of geographic differentiation, as this
184 approach is conservative. (For details see the Supplementary Material online; including or excluding
185 this population did not qualitatively change our results and their interpretation.)

186 Despite low overall levels of among-population differentiation, we found that European
187 populations exhibit clear evidence of geographic sub-structuring. For this analysis, we focused on
188 SNPs located within short introns, with a length ≤ 60 bp and which most likely reflect neutral
189 population structure (Haddrill *et al.* 2005; Singh *et al.* 2009; Parsch *et al.* 2010; Clemente & Vogl
190 2012; Lawrie *et al.* 2013). We restricted our analyses to polymorphisms in regions of high

191 recombination ($r > 3$ cM/Mb; Comeron *et al.* 2011) and to SNPs at least 1 Mb away from the
192 breakpoints of common inversions (and excluding the inversion bodies themselves), resulting in 4,034
193 SNPs used for demographic analysis. Focusing on high-recombination regions is important because
194 reduced rates of crossing over in low recombination regions might make the identification of putative
195 targets of selection difficult (cf. Kolaczowski *et al.* 2011; Fabian *et al.* 2012).

196 We found two signatures of geographic differentiation using these putatively neutral SNPs. First,
197 we identified a weak but significant correlation between pairwise F_{ST} and geographic distance,
198 consistent with isolation by distance (IBD; Mantel test; $p < 0.001$; $R^2=0.12$, max. $F_{ST} \sim 0.045$; fig.
199 3A). Second, a principal components analysis (PCA) on allele frequencies showed that the three most
200 important PC axes explain >25% of the total variance (PC1: 16.71%, PC2: 5.83%, PC3: 4.6%,
201 eigenvalues = 159.8, 55.7, and 44, respectively; fig 3B). The first axis, PC1, was strongly correlated
202 with longitude ($F_{1,42} = 118.08$, $p < 0.001$; table 2). Again, this pattern is consistent with IBD, as the
203 European continent extends further in longitude than latitude. We repeated the above PCA using
204 SNPs in four-fold degenerate sites, as these are also assumed to be relatively unaffected by selection
205 (Akashi 1995; Halligan & Keightley 2006; supplementary fig. S3, Supplementary Material online),
206 and found highly consistent results.

207 Because there was a significant spatial autocorrelation between samples (as indicated by Moran's
208 test on residuals from linear regressions with PC1; $p < 0.001$; table 2), we repeated the analysis with
209 an explicit spatial error model; the association between PC1 and longitude remained significant. To a
210 lesser extent PC2 was likewise correlated with longitude ($F_{1,42} = 7.15$, $p < 0.05$), but also with altitude
211 ($F_{1,42} = 11.77$, $p < 0.01$) and latitude ($F_{1,42} = 4.69$, $p < 0.05$; table 2). Similar to PC2, PC3 was strongly
212 correlated with altitude ($F_{1,42} = 19.91$, $p < 0.001$; table 2). We also examined these data for signatures
213 of genetic differentiation between samples collected at different times of the year. For the dataset as a
214 whole, no major PC axes were correlated with season, indicating that there were no strong differences
215 in allele frequencies shared between all our summer and fall samples ($p > 0.05$ for all analyses; table
216 2). For the 10 locations sampled in both summer and fall, we performed separate PC analyses for
217 summer and fall. Summer and fall values of PC1 (adjusted R^2 : 0.98; $p < 0.001$), PC2 (R^2 : 0.74; $p <$

218 0.001) and PC3 (R^2 : 0.81; $p < 0.001$) were strongly correlated across seasons. This indicates a high
219 degree of seasonal stability in local genetic variation.

220 Next, we attempted to determine if populations could be statistically classified into clusters of
221 similar populations. Using hierarchical model fitting based on the first four PC axes from the PCA
222 mentioned above, we found two distinct clusters (fig. 3B) separated along PC1, supporting the notion
223 of strong longitudinal differentiation among European populations. Similarly, model-based spatial
224 clustering also showed that populations were separated mainly by longitude (fig. 3C; using ConStruct,
225 with $K=3$ spatial layers chosen based on model selection procedure via cross-validation). We also
226 inferred levels of admixture among populations from this analysis, based on the relationship between
227 F_{ST} and migration rate (Wright *et al.* 1951) and using recent estimates of N_e in European populations
228 ($N_e \sim 3.1 \times 10^6$; Duchon *et al.* 2011; for pairwise migration rates see supplementary table S3,
229 Supplementary Material online). Within the Western European cluster and between the clusters, $4N_e m$
230 was similar ($4N_e m$ -WE = 43.76, $4N_e m$ -between = 45.97); in Eastern Europe, estimates of $4N_e m$
231 indicate significantly higher levels of admixture, despite the larger geographic range covered by these
232 samples ($4N_e m = 74.17$; Mann Whitney U-Test; $p < 0.001$). This result suggests that the longitudinal
233 differentiation in Europe might be partly driven by high levels of genetic exchange in Eastern Europe,
234 perhaps due to migration and recolonization after harsh winters in that region. However, these
235 estimates of gene flow must be interpreted with caution, as unknown demographic events can
236 confound estimates of migration rates from F_{ST} (Whitlock & MacCauley 1999).

237 In addition to restricted gene flow between geographic areas, local adaptation may explain
238 population sub-structure, even at neutral sites, if nearby and closely related populations are
239 responding to similar selective pressures. We investigated whether any of 19 climatic variables,
240 obtained from the WorldClim database (Hijmans *et al.* 2005), were associated with the genetic
241 structure in our samples. These climatic variables represent interpolated averages across 30 years of
242 observation at the geographic coordinates corresponding to our sampling locations. Since many of
243 these variables are highly intercorrelated, we analysed their joint effects on genetic variation, by using
244 PCA to summarize the information they capture. The first three climatic PC axes capture more than

245 77% of the variance in the 19 climatic variables (supplementary table S4, Supplementary Material
246 online). PC1 explained 36% of the variance and was strongly correlated ($r > 0.75$ or $r < -0.75$) with
247 climatic variables differentiating ‘hot and dry’ from ‘cold and wet’ climates (e.g., maximum
248 temperature of the warmest month, $r = 0.84$; mean temperature of warmest quarter, $r = 0.86$; annual
249 mean temperature, $r = 0.85$; precipitation during the warmest quarter, $r = -0.87$). Conversely, PC2
250 (27.3% of variance explained) distinguished climates with low and high differences between seasons
251 (e.g., isothermality, $r = 0.83$; temperature seasonality, $r = 0.88$; temperature annual range, $r = -0.78$;
252 precipitation in coldest quarter, $r = 0.79$). PC1 was strongly correlated with latitude (linear regression:
253 $R^2 = 0.48$, $p < 0.001$), whereas PC2 was strongly correlated with longitude ($R^2 = 0.58$, $p < 0.001$). PC2
254 was also correlated with latitude ($R^2 = 0.11$, $p < 0.05$) and with altitude ($R^2 = 0.12$, $p < 0.01$).

255 We next asked whether any of these climate PCs explained any of the genetic structure uncovered
256 above. Pairwise linear regressions of the first three PC axes based on allele frequencies of intronic
257 SNPs against the first three climatic PCs revealed that only one significant correlation after
258 Bonferroni correction: between climatic PC2 (‘seasonality’) vs. genetic PC1 (longitude; adjusted $\alpha =$
259 0.017 ; $R^2 = 0.49$, $P < 0.001$). This suggests that longitudinal differentiation along the European
260 continent might be partly driven by the transition from oceanic to continental climate, possibly
261 leading to local adaptation to gradual changes in temperature seasonality and the severity of winter
262 conditions.

263 Interestingly, the central European division into an eastern and a western clade of *D. melanogaster*
264 closely resembles known hybrid zones of organisms which form closely related pairs of sister taxa.
265 These biogeographic patterns have been associated with long-term reductions of gene flow between
266 eastern and western population during the last glacial maximum, followed by postglacial
267 recolonization of the continent from southern refugia (Hewitt 1999). However, in contrast to many of
268 these taxa, which often exhibit pronounced pre- and postzygotic isolation (Szymura & Barton 1986;
269 Haas & Brodin 2005; Macholán *et al.* 2008, Knief *et al.* 2019), we found low genome-wide
270 differentiation among eastern and western populations (average max. $F_{ST} \sim 0.045$), perhaps indicating
271 that the longitudinal division of European *D. melanogaster* is not the result of postglacial secondary

272 contact.

273

274 **Climatic predictors identify genomic signatures of local climate adaptation**

275 To further explore climatic patterns, and to identify signatures of local adaptation caused by climatic
276 differences among populations independent of neutral demographic effects, we tested for associations
277 of SNP alleles with climatic PC1 and PC2 using BayeScEnv (de Villemereuil & Gaggiotti 2015). The
278 total number of SNPs tested and the number of “top SNPs” (q -value < 0.05) are given in
279 supplementary table S5A (Supplementary Material online). A large proportion of the top SNPs were
280 intergenic (PC1: 33.5%; PC2: 32.2%) or intronic variants (PC1: 50.1%; PC2: 50.5%). Manhattan
281 plots of q -values for all SNPs are shown in fig. 4. These figures show some distinct “peaks” of highly
282 differentiated SNPs along with some broader regions of moderately differentiated SNPs (fig. 4). For
283 example, the circadian rhythm gene *timeout* and the ecdysone signalling genes *Eip74EF* and *Eip75B*
284 all lie near peaks associated with climatic PC1 (‘hot/dry’ vs. ‘cold/wet’; fig. 4, top panels). We note
285 that the corresponding genes have been identified in previous studies of clinal (latitudinal)
286 differentiation in North American *D. melanogaster* (Fabian *et al.* 2012; Machado *et al.* 2016). Indeed,
287 we found a significant overlap between genes associated with PC1 and PC2 (both of which are
288 correlated with latitude) in our study and candidate gene sets from these previous studies of latitudinal
289 clines (SuperExactTest; $p < 0.001$; Fabian *et al.* 2012; Machado *et al.* 2016). For example, out of
290 1,974 latitudinally varying loci along the North American east coast identified by Fabian *et al.* (2012),
291 we found 403 (20%) and 505 (26%) of them to also be associated with PC1 and PC2 in European
292 populations, respectively (table S5B-C). Moreover, the BayeScEnv analysis and *Pool-hmm* analysis
293 together identify four regions with both climatic associations and evidence for continent-wide
294 selective sweeps (supplementary table S5B-C, Supplementary Material online). Finally, four other
295 BayeScEnv candidate genes were previously identified as targets of selection in African and North
296 American populations based on significant McDonald-Kreitman tests (Langley *et al.* 2012; see
297 supplementary table S5B-C, Supplementary Material online).

298 We next asked whether any insights into the targets of local selection could be gleaned from

299 examining the functions of genes near the BayeScEnv peaks. We examined annotated features within
300 2kb of significantly associated SNPs (PC1: 3,545 SNPs near 2,078 annotated features; PC2: 5,572
301 SNPs near 2,717 annotated features; supplementary table S5B and C, Supplementary Material online).
302 First, we performed a GO term analysis with GOwinda (Kofler & Schlötterer 2012) to ask whether
303 SNPs associated with climatic PCs are enriched for any gene functions. For PC1, we found no GO
304 term enrichment. For PC2, we found enrichment for “cuticle development”, and “UDP-
305 glucosyltransferase activity”. Next, we performed functional annotation clustering with DAVID
306 (v6.8; Huang *et al.* 2009), and identified 37 and 47 clusters with an enrichment score > 1.3 for PC1
307 and PC2, respectively (supplementary table S5D-E, Supplementary Material online, Huang *et al.*
308 2009). PC1 was enriched for categories such as “sex differentiation” and “response to nicotine”,
309 whereas PC2 was enriched for functional categories such as “response to nicotine”, “integral
310 component of membrane”, and “sensory perception of chemical stimulus” (supplementary table S5D-
311 E, Supplementary Material online).

312 We also asked whether the SNPs identified by BayeScEnv show consistent signatures of local
313 adaptation. Many associated genes (1,205) were also shared between PC1 and PC2. Some genes have
314 indeed been previously implicated in climatic and clinal adaptation, such as the circadian rhythm
315 genes *timeless*, *timeout*, and *clock*, the sexual differentiation gene *fruitless*, and the *couch potato*
316 locus which underlies the latitudinal cline in reproductive dormancy in North America (e.g., Tauber *et al.*
317 *al.* 2007; Schmidt *et al.* 2008; Fabian *et al.* 2012). Notably, these also include the major insulin
318 signaling genes *insulin-like receptor (InR)* and *forkhead box subgroup O (foxo)*, which have strong
319 genomic and experimental evidence implicating these loci in clinal, climatic adaptation along the
320 North America east coast (Paaby *et al.* 2010; Fabian *et al.* 2012; Paaby *et al.* 2014; Durmaz *et al.*
321 2019). Thus, European populations share multiple potential candidate targets of selection with North
322 American populations (cf. Fabian *et al.* 2012; Machado *et al.* 2016; also see Božičević *et al.* 2016).
323 We next turned to examining polymorphisms other than SNPs, i.e. mitochondrial haplotypes as well
324 as inversion and TE polymorphisms.

325

326 **Mitochondrial haplotypes also exhibit longitudinal population structure**

327 Mitochondrial haplotypes also showed evidence of longitudinal demographic structure in European
328 population. We identified two main alternative mitochondrial haplotypes in Europe, G1 and G2, each
329 with several sub-haplotypes (G1.1 and G1.2 and G2.1, G2.2 and G2.3). The two sub-types, G1.2 and
330 G2.1, are separated by 41 mutations (fig. 5A). The frequencies of the alternative G1 and G2 haplotype
331 varied among populations between 35.1% and 95.6% and between 4.4% and 64.9%, respectively (fig.
332 5B). Qualitatively, three types of European populations could be distinguished based on these
333 haplotypes: (1) central European populations, with a high frequency ($> 60\%$) of G1 haplotypes, (2)
334 Eastern European populations in summer, with a low frequency ($< 40\%$) of G1 haplotypes, and (3)
335 Iberian and Eastern European populations in fall, with a frequency of G1 haplotypes between 40-60%
336 (supplementary fig. S4, Supplementary Material online). Analyses of mitochondrial haplotypes from a
337 North American population (Cooper *et al.* 2015) as well as from worldwide samples (Wolff *et al.*
338 2016) also revealed high levels of haplotype diversity.

339 While there was no correlation between the frequency of G1 haplotypes and latitude, G1
340 haplotypes and longitude were weakly but significantly correlated ($r^2 = 0.10$; $p < 0.05$). We thus
341 divided the dataset into an eastern and a western sub-set along the 20° meridian, corresponding to the
342 division of two major climatic zones, temperate (oceanic) versus cold (continental) (Peel *et al.* 2007).
343 This split revealed a clear correlation ($r^2=0.5$; $p<0.001$) between longitude and the frequency of G1
344 haplotypes, explaining as much as 50% of the variation in the western group (supplementary fig. S4B,
345 Supplementary Material online). Similarly, in eastern populations, longitude and the frequency of G1
346 haplotypes were correlated ($r^2 = 0.2$; $p<0.001$), explaining approximately 20% of the variance
347 (supplementary fig. S4B, Supplementary Material online). Thus, these mitochondrial haplotypes
348 appear to follow a similar east-west population structure as observed for the nuclear SNPs described
349 above.

350

351 **The frequency of polymorphic TEs varies with longitude and altitude**

352 To examine the population genomics of structural variants, we first focused on transposable elements

353 (TEs). Similar to previous findings, the repetitive content of the 48 samples ranged from 16% to 21%
354 of the nuclear genome size (Quesneville *et al.* 2005; fig. 6). The vast majority of detected repeats
355 were TEs, mostly long terminal repeat elements (LTRs; range 7.55 % - 10.15 %) and long
356 interspersed nuclear elements (LINEs range 4.18 % - 5.52 %), along with a few DNA elements (range
357 1.16 % - 1.65 %) (supplementary table S6, Supplementary Material online). LTRs have been
358 previously described as being the most abundant TEs in the *D. melanogaster* genome (Kaminker *et al.*
359 2002; Bergman *et al.* 2006). Correspondingly, variation in the proportion of LTRs best explained
360 variation in total TE content (LINE+LTR+DNA) (Pearson's $r = 0.87$, $p < 0.01$, vs. DNA $r = 0.58$, $p =$
361 0.0117 , and LINE $r = 0.36$, $p < 0.01$ and supplementary fig. S5A, Supplementary Material online).

362 For each of the 1,630 TE insertion sites annotated in the *D. melanogaster* reference genome v.6.04,
363 we estimated the frequency at which a copy of the TE was present at that site using *T-lex2* (Fiston-
364 Lavier *et al.* 2015; see supplementary table S7, Supplementary Material online). On average, 56%
365 were fixed in all samples. The remaining polymorphic TEs mostly segregated at low frequency in all
366 samples (supplementary fig. S5B), potentially due to purifying selection (González *et al.* 2008; Petrov
367 *et al.* 2011; Kofler *et al.* 2012; Cridland *et al.* 2013; Blumenstiel *et al.* 2014). However, 246 were
368 present at intermediate frequencies (>10% and <95%) and located in regions of non-zero
369 recombination (Fiston-Lavier *et al.* 2010; Comeron *et al.* 2012; see supplementary table S7,
370 Supplementary Material online). Although some of these insertions might be segregating neutrally at
371 transposition-selection balance (Charlesworth *et al.* 1994; see supplementary fig. S5B, Supplementary
372 Material online), they are likely enriched for candidate adaptive mutations (Rech *et al.* 2019).

373 In each of the 48 samples, TE frequency and recombination rate were negatively correlated
374 genome-wide (Spearman rank sum test; $p < 0.01$), as has also been previously reported for *D.*
375 *melanogaster* (Bartolomé *et al.* 2002; Petrov *et al.* 2011; Kofler *et al.* 2012). This remains true when
376 fixed TE insertions were excluded (population frequency $\geq 95\%$) from the analysis, although it was
377 not statistically significant for some chromosomes and populations (supplementary table S8,
378 Supplementary Material online). In both cases, the correlation was stronger when broad-scale (Fiston-
379 Lavier *et al.* 2010) rather than fine-scale (Comeron *et al.* 2012) recombination rate estimates were

380 used, indicating that the former may best capture long-term population recombination patterns (see
381 supplementary materials and methods and supplementary table S8, Supplementary Material online).

382 We next tested whether variation in TE frequencies among samples was associated with spatially
383 or temporally varying factors. We focused on 111 TE insertions that segregated at intermediate
384 frequencies, were located in non-zero recombination regions, and that showed an interquartile range
385 (IQR) > 10 (see supplementary materials and methods, Supplementary Material online). Of these
386 insertions, 57 were significantly associated with a at least one variable of interest after multiple testing
387 correction (supplementary table S9A, Supplementary Material online): 13 were significantly
388 associated with longitude, 13 with altitude, five with latitude, three with season, and 23 insertions
389 with more than one of these variables (supplementary table S9A, Supplementary Material online).
390 These 57 TEs were mainly located inside genes (42 out of 57; Fisher's Exact Test, $p > 0.05$;
391 supplementary table S9A, Supplementary Material online).

392 The 57 TEs significantly associated with these environmental variables were enriched for two TE
393 families: the LTR 297 family with 11 copies, and the DNA *pogo* family with five copies (χ^2 -values
394 after Yate's correction < 0.05; supplementary table S9B, Supplementary Material online).
395 Interestingly, 17 of the 57 TEs coincided with previously identified adaptive candidate TEs,
396 suggesting that our dataset might be enriched for adaptive insertions (SuperExactTest, $p < 0.001$),
397 several of which exhibit spatial frequency clines that deviate from neutral expectation
398 (SuperExactTest, $p < 0.001$, supplementary table S9A, Supplementary Material online; cf.; Rech *et al.*
399 2019). Moreover, 18 of the 57 TEs also show significant correlations with either geographical or
400 temporal variables in North American populations (SuperExactTest, $p < 0.001$, supplementary table
401 S9A, Supplementary Material online; cf.; Lerat *et al.* 2019).

402

403 **Inversions exhibit latitudinal and longitudinal clines in Europe**

404 Polymorphic chromosomal inversions, another class of structural variants besides TEs, are well-
405 known to exhibit pronounced spatial (clinal) patterns in North American, Australian and other
406 populations, possibly due to spatially varying selection (reviewed in Kapun & Flatt 2019; also see

407 Mettler *et al.* 1977; Knibb *et al.* 1981; Leumeunier & Aulard 1992; Hoffmann & Weeks 2007; Fabian
408 *et al.* 2012; Kapun *et al.* 2014; Rane *et al.* 2015; Adrion *et al.* 2015; Kapun *et al.* 2016a). However, in
409 contrast to North America and Australia, inversion clines in Europe remain very poorly characterized
410 (Lemeunier & Aulard 1992; Kapun & Flatt 2019). We therefore sought to examine the presence and
411 frequency of six cosmopolitan inversions (*In(2L)t*, *In(2R)NS*, *In(3L)P*, *In(3R)C*, *In(3R)Mo*,
412 *In(3R)Payne*) in our European samples, using a panel of highly diagnostic inversion-specific marker
413 SNPs, identified through sequencing of cytologically determined karyotypes by Kapun *et al.* (2014)
414 (also see Kapun *et al.* 2016a). All 48 samples were polymorphic for one or more inversions (Figure
415 6). However, only *In(2L)t* segregated at substantial frequencies in most populations (average
416 frequency = 20.2%); all other inversions were either absent or rare (average frequencies: *In(2R)NS* =
417 6.2%, *In(3L)P* = 4%, *In(3R)C* = 3.1%, *In(3R)Mo* = 2.2%, *In(3R)Payne* = 5.7%) (cf. Kapun *et al.*
418 2016a; Kapun & Flatt 2019).

419 Despite their overall low frequencies, several inversions showed pronounced clinality, in
420 qualitative agreement with findings from other continents (Lemeunier & Aulard 1992; Kapun & Flatt
421 2019). For the analyses below, we tested for potentially confounding effects of significant residual
422 spatial autocorrelation among samples; all of these test were negative, except for *In(3R)C* (Moran's I
423 ≈ 0 , $p > 0.05$ for all tests; table 3). We observed significant latitudinal clines in Europe for *In(3L)P*,
424 *In(3R)C* and *In(3R)Payne* (binomial generalized linear model: Inversion frequency \sim Latitude +
425 Longitude + Altitude + Season; effect of Latitude: $p < 0.001$ for all; see table 3). Clines for *In(3L)P*
426 and *In(3R)Payne* were similar between Europe and North America (with frequencies for both
427 decreasing with latitude, $p < 0.05$; see supplementary table S10, Supplementary Material online).
428 However, all inversions differed in their frequency at the same latitude between North America and
429 Europe ($p < 0.001$ for the Latitude \times Continent interaction; supplementary table S10, Supplementary
430 Material online).

431 Latitudinal inversion clines previously observed along the North American and Australian east
432 coasts (supplementary fig. S6 and supplementary table S10, Supplementary Material online; Kapun *et*
433 *al.* 2016a) have been attributed to spatially varying selection, especially in the case of *In(3R)Payne*

434 (Durmaz *et al.* 2018; Anderson *et al.* 2005; Umina *et al.* 2005; Kennington *et al.* 2006; Rako *et al.*
435 2006; Kapun *et al.* 2016a,b; Kapun & Flatt 2019). Similar to patterns in North America (Kapun *et al.*
436 2016a), we observed that clinality of the three inversion polymorphisms was markedly stronger than
437 for putatively neutral SNPs in short introns (see supplementary table S11, Supplementary Material
438 online), suggesting that these polymorphisms are maintained non-neutrally. Together, these findings
439 suggest that latitudinal inversion clines in Europe are shaped by spatially varying selection, as they
440 are in North America (Kapun *et al.* 2016a; Kapun & Flatt 2019).

441 We also detected longitudinal clines for *In(2L)t* and *In(2R)NS*, with both polymorphisms
442 decreasing in frequency from east to west (see table 3; $p < 0.01$; also cf. Kapun & Flatt 2019).
443 Longitudinal clines for these two inversions have also been found in North America (cf. Kapun &
444 Flatt 2019). One of these inversions, *In(2L)t*, also changed in frequency with altitude (table 3; $p <$
445 0.001). These longitudinal and altitudinal inversion clines did, however, not deviate from neutral
446 expectation (supplementary table S11, Supplementary Material online).

447

448 **European *Drosophila* microbiomes contain Entomophthora, trypanosomatids and previously** 449 **unknown DNA viruses**

450 The microbiota can affect life history traits, immunity, hormonal physiology, and metabolic
451 homeostasis of their fly hosts (e.g., Trinder *et al.* 2017; Martino *et al.* 2017) and might thus reveal
452 interesting patterns of local adaptation. We therefore examined the bacterial, fungal, protist, and viral
453 microbiota sequence content of our samples. To do this, we characterised the taxonomic origin of the
454 non-*Drosophila* reads in our dataset using MGRAST, which identifies and counts short protein motifs
455 ('features') within reads (Meyer *et al.* 2008). We examined 262 million reads in total. Of these, most
456 were assigned to *Wolbachia* (mean 53.7%; fig. 7; supplementary table S1), a well-known
457 endosymbiont of *Drosophila* (Werren *et al.* 2008). The abundance of *Wolbachia* protein features
458 relative to other microbial protein features (relative abundance) varied strongly between samples,
459 ranging from 8.8% in a sample from Ukraine to almost 100% in samples from Spain, Portugal,
460 Turkey and Russia (supplementary table S12, Supplementary Material online). Similarly, *Wolbachia*

461 loads varied 100-fold between samples, as estimated from the ratio of *Wolbachia* protein features to
462 *Drosophila* protein features (supplementary table S12, Supplementary Material online). In contrast to
463 a previous study (Kriesner *et al.* 2016), there was no evidence for clinality of *Wolbachia* loads ($p =$
464 0.13, longitude; $p = 0.41$, latitude; Kendall's rank correlation). However, these authors measured
465 infection frequencies while we measured *Wolbachia* loads in pooled samples. Because the frequency
466 of infection does not necessarily correlate with microbial loads measured in pooled samples, we might
467 not have been able to detect such a signal in our data.

468 Acetic acid bacteria of the genera *Gluconobacter*, *Gluconacetobacter*, and *Acetobacter* were the
469 second largest group, with an average relative abundance of 34.4% among microbial protein features.
470 Furthermore, we found evidence for the presence of several genera of Enterobacteria (*Serratia*,
471 *Yersinia*, *Klebsiella*, *Pantoea*, *Escherichia*, *Enterobacter*, *Salmonella*, and *Pectobacterium*). *Serratia*
472 occurs only at low frequencies or is absent from most of our samples, but reaches a very high relative
473 abundance among microbial protein features in the Nicosia (Cyprus) summer collection (54.5%). This
474 high relative abundance was accompanied by an 80x increase in *Serratia* bacterial load.

475 We also detected several eukaryotic microorganisms, although they were less abundant than the
476 bacteria. We found trypanosomatids, previously reported to be associated with *Drosophila* in other
477 studies (Wilfert *et al.* 2011; Chandler & James 2013; Hamilton *et al.* 2015), in 16 of our samples, on
478 average representing 15% of all microbial protein features identified in these samples.

479 Fungal protein features make up <3% of all but three samples (from Finland, Austria and Turkey;
480 supplementary table S12, Supplementary Material online). This is somewhat surprising because
481 yeasts are commonly found on rotting fruit, the main food substrate of *D. melanogaster*, and co-occur
482 with flies (Barata *et al.* 2012; Chandler *et al.* 2012). This result suggests that, although yeasts can
483 attract flies and play a role in food choice (Becher *et al.* 2012; Buser *et al.* 2014), they might not be
484 highly prevalent in or on *D. melanogaster* bodies. One reason might be that they are actively digested
485 and thus not part of the microbiome. We also found the fungal pathogen *Entomophthora muscae* in 14
486 samples, making up 0.18% of the reads (Elya *et al.* 2018).

487 Our data also allowed us to identify DNA viruses. Only one DNA virus has been previously

488 described for *D. melanogaster* (*Kallithea* virus; Webster *et al.* 2015; Palmer *et al.* 2018) and only two
489 additional ones from other Drosophilid species (*Drosophila innubila* Nudivirus [Unckless 2011],
490 Invertebrate Iridovirus 31 in *D. obscura* and *D. immigrans* [Webster *et al.* 2016]). In our data set,
491 approximately two million reads came from *Kallithea* nudivirus (Webster *et al.* 2015), allowing us to
492 assemble the first complete *Kallithea* genome (>300-fold coverage in the Ukrainian sample
493 UA_Kha_14_46; Genbank accession KX130344).

494 We also found reads from five additional DNA viruses that were previously unknown
495 (supplementary table S13, Supplementary Material online). First, around 1,000 reads come from a
496 novel nudivirus closely related to both *Kallithea* virus and to *Drosophila innubila* nudivirus (Unckless
497 2011) in sample DK_Kar_14_41 from Karensminde, Denmark supplementary table S13,
498 Supplementary Material online). As the reads from this virus were insufficient to assemble the
499 genome, we identified a publicly available dataset (SRR3939042: 27 male *D. melanogaster* from
500 Esparto, California; Machado *et al.* 2016) with sufficient reads to complete the genome (provisionally
501 named “*Esparto* Virus”; KY608910). Second, we also identified two novel Densoviruses
502 (*Parvoviridae*). The first is a relative of *Culex pipiens* densovirus, provisionally named “*Viltain*
503 virus”, found at 94-fold coverage in sample FR_Vil_14_07 (Viltain; KX648535). The second is
504 “*Linvill Road* virus”, a relative of *Dendrolimus punctatus* densovirus, represented by only 300 reads
505 here, but with high coverage in dataset SRR2396966 from a North American sample of *D. simulans*,
506 permitting assembly (KX648536; Machado *et al.* 2016). Third, we detected a novel member of the
507 *Bidnaviridae* family, “*Vesanto* virus”, a bidensovirus related to *Bombyx mori* densovirus 3 with
508 approximately 900-fold coverage in sample FI_Ves_14_38 (Vesanto; KX648533 and KX648534).
509 Finally, in one sample (UA_Yal_14_16), we detected a substantial number of reads from an
510 Entomopox-like virus, which we were unable to fully assemble (supplementary table S13,
511 Supplementary Material online).

512 Using a detection threshold of >0.1% of the *Drosophila* genome copy number, the most commonly
513 detected viruses were *Kallithea* virus (30/48 of the pools) and *Vesanto* virus (25/48), followed by
514 *Linvill Road* virus (7/48) and *Viltain* virus (5/48), with *Esparto* virus and the entomopox-like virus

515 being the rarest (2/48 and 1/48, respectively). Because *Wolbachia* can protect *Drosophila* from
516 viruses (Teixeira et al., 2008), we hypothesized that *Wolbachia* loads might correlate negatively with
517 viral loads, but found no evidence of such a correlation ($p = 0.83$ Kallithea virus; $p = 0.76$ Esparto
518 virus; $p = 0.52$ Viltain virus; $p = 0.96$ Vesanto 1 virus; $p = 0.93$ Vesanto 2 virus; $p = 0.5$ Linvill Road
519 virus; Kendall's rank correlation). Perhaps this is because the *Kallithea* virus, the most prevalent virus
520 in our data set, is not expected to be affected by *Wolbachia* (Palmer et al., 2018). Similarly, Shi et al.
521 (2018) found no link between *Wolbachia* and the prevalence or abundance of RNA viruses in data
522 from individual flies.

523 The variation in bacterial microbiomes across space and time reported here is analysed in more
524 detail in Wang *et al.* (2020); this study suggests that some of this variation is structured
525 geographically (cf. Walters *et al.* 2020). Thus, microbiome composition might contribute to
526 phenotypic differences and local adaptation among populations (Haselkorn *et al.* 2009; Richardson *et*
527 *al.* 2012; Staubach *et al.* 2013; Kriesner *et al.* 2016; Wang and Staubach 2018).

528

529 **Conclusions**

530 Here, we have comprehensively sampled and sequenced European populations of *D. melanogaster* for
531 the first time (fig. 1). We find that European *D. melanogaster* populations are longitudinally
532 differentiated for putatively neutral SNPs, mitochondrial haplotypes as well as for inversion and TE
533 insertion polymorphisms. Potentially adaptive polymorphisms also show this pattern, possibly driven
534 by the transition from oceanic to continental climate along the longitudinal axis of Europe. We note
535 that this longitudinal differentiation qualitatively resembles the one observed for human populations
536 in Europe (e.g., Cavalli-Sforza 1966; Xiao et al. 2004; Francalacci & Sanna 2008; Novembre *et al.*
537 2008). Given that *D. melanogaster* is a human commensal (Keller 2007, Arguello *et al.* 2019), it is
538 thus tempting to speculate that the demographic history of European populations might have been
539 influenced by past human migration. Outside Europe, east-west structure has been previously found in
540 sub-Saharan Africa populations of *D. melanogaster*, with the split between eastern and western
541 African populations having occurred ~70 kya (Michalakis & Veuille 1996; Aulard *et al.* 2002;

542 Kapopoulou *et al.* 2018b), a period that coincides with a wave of human migration from eastern into
543 western Africa (Nielsen *et al.* 2017). However, in contrast to the pronounced pattern observed in
544 Europe, African east-west structure is relatively weak, explaining only ~2.7% of variation, and is
545 primarily due to an inversion whose frequency varies longitudinally. In contrast, our demographic
546 analyses are based on SNPs located in >1 Mb distance from the breakpoints of the most common
547 inversions and excluding the inversion bodies, making it unlikely that the longitudinal pattern we
548 observe is driven by inversions.

549 Our extensive sampling was feasible only due to synergistic collaboration among many research
550 groups. Our efforts in Europe are paralleled in North America by the *Dros-RTEC* consortium
551 (Machado *et al.* 2019), with whom we are collaborating to compare population genomic data across
552 continents. Together, we have sampled both continents annually since 2014; we aim to continue to
553 sample and sequence European and North American *Drosophila* populations with increasing spatio-
554 temporal resolution in future years. With these efforts, we hope to provide a rich community resource
555 for biologists interested in molecular population genetics and adaptation genomics.

556

557 **Materials and methods**

558 A detailed description of the materials and methods is provided in the supplementary materials and
559 methods (see Supplementary Material online); here we give a brief overview of the dataset and the
560 basic methods used. The 2014 *DrosEU* dataset represents the most comprehensive spatio-temporal
561 sampling of European *D. melanogaster* populations to date (fig. 1; supplementary table S1,
562 Supplementary Material online). It comprises 48 samples of *D. melanogaster* collected from 32
563 geographical locations across Europe at different time points in 2014 through a joint effort of 18
564 research groups. Collections were mostly performed with baited traps using a standardized protocol
565 (see supplementary materials and methods, Supplementary Material online). From each collection, we
566 pooled 33–40 wild-caught males. We used males as they are more easily distinguishable
567 morphologically from similar species than females. Despite our precautions, we identified a low level
568 of *D. simulans* contamination in our sequences; we computationally filtered these sequences from the

569 data prior to further analysis (see Supplementary Material online). To sequence these samples, we
570 extracted DNA and barcoded each sample, and sequenced the ~40 flies per sample as a pool (Pool-
571 Seq; Schlötterer *et al.* 2014), as paired-end fragments on a *Illumina NextSeq 500* sequencer at the
572 Genomics Core Facility of Pompeu Fabra University. Samples were multiplexed in 5 batches of 10
573 samples, except for one batch of 8 samples (supplementary table S1, Supplementary Material online).
574 Each multiplexed batch was sequenced on 4 lanes at ~50x raw coverage per sample. The read length
575 was 151 bp, with a median insert size of 348 bp (range 209-454 bp). Our genomic dataset is available
576 under NCBI Bioproject accession PRJNA388788. Sequences were processed and mapped to the *D.*
577 *melanogaster* reference genome (v.6.12) and reference sequences from common commensals and
578 pathogens. Our bioinformatic pipeline is available at https://github.com/capoony/DrosEU_pipeline.
579 To call SNPs, we developed custom software (*PoolSNP*; see supplementary material and methods;
580 <https://github.com/capoony/PoolSNP>), using stringent heuristic parameters. In addition, we obtained
581 genome sequences from African flies from the *Drosophila* Genome Nexus (DGN;
582 <http://www.johnpool.net/genomes.html>; see supplementary table S14 for SRA accession numbers).
583 We used data from 14 individuals from Rwanda and 40 from Siavonga (Zambia). We mapped these
584 data to the *D. melanogaster* reference genome using the same pipeline as for our own data above, and
585 built consensus sequences for each haploid sample by only considering alleles with > 0.9 allele
586 frequencies. We converted consensus sequences to *VCF* and used *VCFtools* (Danecek *et al.* 2011) for
587 downstream analyses. Additional steps in the mapping and variant calling pipeline and further
588 downstream analyses of the data are detailed in the supplementary materials and methods
589 (Supplementary Materials online).

590

591 **Supplementary Materials**

592 Supplementary materials and methods, supplementary results and supplementary figs. S1–S13 and
593 supplementary tables S1–S18 are available at Molecular Biology and Evolution online
594 (<http://www.mbe.oxfordjournals.org/>).

595

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609

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953 **Tables**

954 **Table 1. Sample information for all populations in the *DrosEU* dataset.** Origin, collection date, season and sample size (number of chromosomes: *n*) of
 955 the 48 samples in the *DrosEU* 2014 data set. Additional information can be found in supplementary table S1 (Supplementary Material online).

ID	Country	Location	Coll. Date	r ID	Numbe	Lat	Lon (°)	Alt	Season	<i>n</i>	Coll. name
					(°)	(m)					
AT_Mau_14_01	Austria	Mauternbach	2014-07-20	1	48.38	15.56	572	S	80	Andrea J. Betancourt	
AT_Mau_14_02	Austria	Mauternbach	2014-10-19	2	48.38	15.56	572	F	80	Andrea J. Betancourt	
TR_Yes_14_03	Turkey	Yesiloz	2014-08-31	3	40.23	32.26	680	S	80	Banu Sebnem Onder	
TR_Yes_14_04	Turkey	Yesiloz	2014-10-23	4	40.23	32.26	680	F	80	Banu Sebnem Onder	
											Catherine Montchamp-
FR_Vil_14_05	France	Viltain	2014-08-18	5	48.75	2.16	153	S	80	Moreau	
											Catherine Montchamp-
FR_Vil_14_07	France	Viltain	2014-10-27	7	48.75	2.16	153	F	80	Moreau	
FR_Got_14_08	France	Gotheron	2014-07-08	8	44.98	4.93	181	S	80	Cristina Vieira	
	United										
UK_She_14_09	Kingdom	Sheffield	2014-08-25	9	53.39	-1.52	100	S	80	Damiano Porcelli	

United												
UK_Sou_14_10	Kingdom	South Queensferry	2014-07-14	10	55.97	-3.35	19	S	80	Darren Obbard		
CY_Nic_14_11	Cyprus	Nicosia	2014-08-10	11	35.07	33.32	263	S	80	Eliza Argyridou		
United												
UK_Mar_14_12	Kingdom	Market Harborough	2014-10-20	12	52.48	-0.92	80	F	80	Eran Tauber		
United												
UK_Lut_14_13	Kingdom	Lutterworth	2014-10-20	13	52.43	-1.10	126	F	80	Eran Tauber		
DE_Bro_14_14	Germany	Broggingen	2014-06-26	14	48.22	7.82	173	S	80	Fabian Staubach		
DE_Bro_14_15	Germany	Broggingen	2014-10-15	15	48.22	7.82	173	F	80	Fabian Staubach		
UA_Yal_14_16	Ukraine	Yalta	2014-06-20	16	44.50	34.17	72	S	80	Iryna Kozeretstska		
UA_Yal_14_18	Ukraine	Yalta	2014-08-27	18	44.50	34.17	72	S	80	Iryna Kozeretstska		
UA_Ode_14_19	Ukraine	Odesa	2014-07-03	19	46.44	30.77	54	S	80	Iryna Kozeretstska		
UA_Ode_14_20	Ukraine	Odesa	2014-07-22	20	46.44	30.77	54	S	80	Iryna Kozeretstska		
UA_Ode_14_21	Ukraine	Odesa	2014-08-29	21	46.44	30.77	54	S	80	Iryna Kozeretstska		
UA_Ode_14_22	Ukraine	Odesa	2014-10-10	22	46.44	30.77	54	F	80	Iryna Kozeretstska		
UA_Kyi_14_23	Ukraine	Kyiv	2014-08-09	23	50.34	30.49	179	S	80	Iryna Kozeretstska		
UA_Kyi_14_24	Ukraine	Kyiv	2014-09-08	24	50.34	30.49	179	F	80	Iryna Kozeretstska		

UA_Var_14_25	Ukraine	Varva	2014-08-18	25	50.48	32.71	125	S	80	Oleksandra Protsenko
UA_Pyr_14_26	Ukraine	Pyriatyn	2014-08-20	26	50.25	32.52	114	S	80	Oleksandra Protsenko
UA_Dro_14_27	Ukraine	Drogobych	2014-08-24	27	49.33	23.50	275	S	80	Iryna Kozeretcka
UA_Cho_14_28	Ukraine	Chornobyl	2014-09-13	28	51.37	30.14	121	F	80	Iryna Kozeretcka
UA_Cho_14_29	Ukraine	Chornobyl Yaniv	2014-09-13	29	51.39	30.07	121	F	80	Iryna Kozeretcka
SE_Lun_14_30	Sweden	Lund	2014-07-31	30	55.69	13.20	51	S	80	Jessica Abbott
DE_Mun_14_31	Germany	Munich	2014-06-19	31	48.18	11.61	520	S	80	John Parsch
DE_Mun_14_32	Germany	Munich	2014-09-03	32	48.18	11.61	520	F	80	John Parsch
PT_Rec_14_33	Portugal	Recarei	2014-09-26	33	41.15	-8.41	175	F	80	Jorge Vieira
ES_Gim_14_34	Spain	Gimnells (Lleida)	2014-10-20	34	41.62	0.62	173	F	80	Lain Guio
ES_Gim_14_35	Spain	Gimnells (Lleida)	2014-08-13	35	41.62	0.62	173	S	80	Lain Guio
FI_Aka_14_36	Finland	Akaa	2014-07-25	36	61.10	23.52	88	S	80	Maaria Kankare
FI_Aka_14_37	Finland	Akaa	2014-08-27	37	61.10	23.52	88	S	80	Maaria Kankare
FI_Ves_14_38	Finland	Vesanto	2014-07-26	38	62.55	26.24	121	S	66	Maaria Kankare
DK_Kar_14_39	Denmark	Karensminde	2014-09-01	39	55.95	10.21	15	F	80	Mads Fristrup Schou
DK_Kar_14_41	Denmark	Karensminde	2014-11-25	41	55.95	10.21	15	F	80	Mads Fristrup Schou
CH_Cha_14_42	Switzerland	Chalet à Gobet	2014-07-24	42	46.57	6.70	872	S	80	Martin Kapun

CH_Cha_14_43	Switzerland	Chalet à Gobet	2014-10-05	43	46.57	6.70	872	F	80	Martin Kapun
AT_See_14_44	Austria	Seeboden	2014-08-17	44	46.81	13.51	591	S	80	Martin Kapun
UA_Kha_14_45	Ukraine	Kharkiv	2014-07-26	45	49.82	36.05	141	S	80	Svitlana Serga
UA_Kha_14_46	Ukraine	Kharkiv	2014-09-14	46	49.82	36.05	141	F	80	Svitlana Serga
		Chornobyl								
UA_Cho_14_47	Ukraine	Applegarden	2014-09-13	47	51.27	30.22	121	F	80	Svitlana Serga
UA_Cho_14_48	Ukraine	Chornobyl Polisske	2014-09-13	48	51.28	29.39	121	F	70	Svitlana Serga
UA_Kyi_14_49	Ukraine	Kyiv	2014-10-11	49	50.34	30.49	179	F	80	Svitlana Serga
UA_Uma_14_50	Ukraine	Uman	2014-10-01	50	48.75	30.21	214	F	80	Svitlana Serga
RU_Val_14_51	Russia	Valday	2014-08-17	51	57.98	33.24	217	S	80	Elena Pasyukova

957 **Table 2. Clinality of genetic variation and population structure.** Effects of geographic variables and/or seasonality on genome-wide average levels of
958 diversity (π , θ and Tajima's D ; top rows) and on the first three axes of a PCA based on allele frequencies at neutrally evolving sites (bottom rows). The values
959 represent F -ratios from general linear models. Bold type indicates F -ratios that are significant after Bonferroni correction (adjusted $\alpha=0.0055$). Asterisks in
960 parentheses indicate significance when accounting for spatial autocorrelation by spatial error models. These models were only calculated when Moran's I test,
961 as shown in the last column, was significant. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
962

<i>Factor</i>	<i>Latitude</i>	<i>Longitude</i>	<i>Altitude</i>	<i>Season</i>	<i>Moran's I</i>
$\pi_{(X)}$	4.11*	1.62	15.23***	1.65	0.86
$\pi_{(Aut)}$	0.91	2.54	27.18***	0.16	-0.86
$\theta_{(X)}$	2.65	1.31	15.54***	2.22	0.24
$\theta_{(Aut)}$	0.48	1.44	13.66***	0.37	-1.13
$D_{(X)}$	0.02	0.38	5.93*	3.26	-2.08
$D_{(Aut)}$	0.09	0.76	5.33*	0.71	-1.45
PC1	0.63	118.08*** (***)	3.64	0.75	4.2***
PC2	4.69*	7.15*	11.77**	1.68	-0.32
PC3	0.39	0.23	19.91***	0.28	1.38

963

964 **Table 3. Climaticity and/or seasonality of chromosomal inversions.** The values represent *F*-ratios from binomial generalized linear models to account for
965 frequency data. Bold type indicates deviance values that were significant after Bonferroni correction (adjusted $\alpha=0.0071$). Asterisks in parentheses indicate
966 significance when accounting for spatial autocorrelation by spatial error models. These models were only calculated when Moran's *I* test, as shown in the last
967 column, was significant. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

968

<i>Factor</i>	<i>Latitude</i>	<i>Longitude</i>	<i>Altitude</i>	<i>Season</i>	<i>Moran's I</i>
<i>ln(2L)_t</i>	2.2	10.09**	43.94***	0.89	-0.92
<i>ln(2R)_{NS}</i>	0.25	14.43***	2.88	2.43	1.25
<i>ln(3L)_P</i>	21.78***	2.82	0.62	3.6	-1.61
<i>ln(3R)_C</i>	18.5*** (***)	0.75	1.42	0.04	2.79**
<i>ln(3R)_{Mo}</i>	0.3	0.09	0.35	0.03	-0.9
<i>ln(3R)_{Payne}</i>	43.47***	0.66	1.69	1.55	-0.89

969

970 **FIGURE LEGENDS**

971

972 **Fig. 1. The geographic distribution of population samples.** Locations of all samples in the 2014
973 *DrosEU* data set. The color of the circles indicates the sampling season for each location: ten of the
974 32 locations were sampled at least twice, once in summer and once in fall (see table 1 and
975 supplementary table S1, Supplementary Material online). Note that some of the 12 Ukrainian
976 locations overlap in the map.

977

978 **Fig. 2. Candidate signals of selective sweeps in European populations.** The central panel shows
979 the distribution of Tajima's D in 50 kb sliding windows with 40 kb overlap, with red and green
980 dashed lines indicating Tajima's $D = 0$ and -1 , respectively. The top panel shows a detail of a genomic
981 region on chromosomal arm *2R* in the vicinity of *Cyp6g1* and *Hen1* (highlighted in red), genes
982 reportedly involved in pesticide resistance. This strong sweep signal is characterized by an excess of
983 low-frequency SNP variants and overall negative Tajima's D in all samples. Colored solid lines depict
984 Tajima's D for each sample (see supplementary fig. S2 for color codes, Supplementary Material
985 online); the black dashed line shows Tajima's D averaged across all samples. The bottom panel shows
986 a region on *3L* previously identified as a potential target of selection, which shows a similar strong
987 sweep signature. Notably, both regions show strongly reduced genetic variation (supplementary fig.
988 S1, Supplementary Material online).

989

990 **Fig. 3. Genetic differentiation among European populations.** (A) Average F_{ST} among populations
991 at putatively neutral sites. The centre plot shows the distribution of F_{ST} values for all 1,128 pairwise
992 population comparisons, with the F_{ST} values for each comparison obtained from the mean across all
993 4,034 SNPs used in the analysis. Plots on the left and the right show population pairs in the lower
994 (blue) and upper (red) 5% tails of the F_{ST} distribution. (B) PCA analysis of allele frequencies at the
995 same SNPs reveals population sub-structuring in Europe. Hierarchical model fitting using the first
996 four PCs showed that the populations fell into two clusters (indicated by red and blue), with cluster
997 assignment of each population subsequently estimated by k -means clustering. (C) Admixture

998 proportions for each population inferred by model-based clustering with *ConStruct* are highlighted as
999 pie charts (left plot) or Structure plots (centre). The optimal number of 3 spatial layers (K) was
1000 inferred by cross-validation (right plot).

1001

1002 **Fig. 4. Manhattan plots of SNPs with q -values < 0.05 in BayeScEnv association tests with PC1 or**
1003 **PC2 of bioclimatic variables.** Vertical lines denote the breakpoints of common inversions. The gene
1004 names highlight some candidate genes found in our study and which have previously been identified
1005 as varying clinally by Fabian *et al.* (2012) and Machado *et al.* (2016) along the North American east
1006 coast. Note that q -values of 0 (which are infinite on a log-scale) are plotted at the top of each figure,
1007 above the grey dash-dotted horizontal lines in order to separate them from the other candidates with q -
1008 values > 0 . These zero values are unlikely to be spurious as the densities of these infinite values tend
1009 to line up with peaks of $\log_{10}(q)$ below the dashed line, suggesting that they represent highly
1010 significant continuations of these peaks.

1011

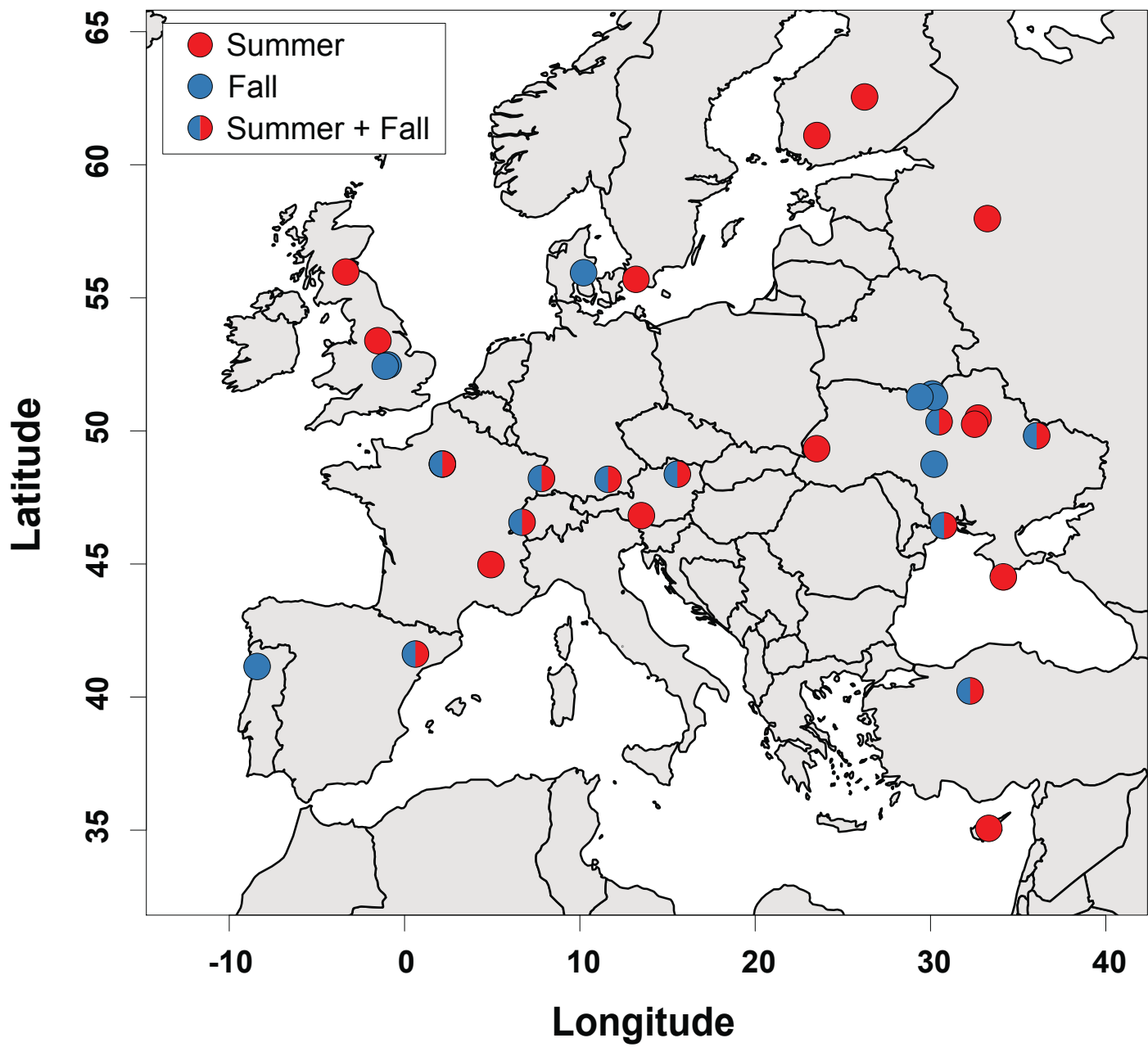
1012 **Fig. 5. Mitochondrial haplotypes.** (A) TCS network showing the relationship of 5 common
1013 mitochondrial haplotypes; (B) estimated frequency of each mitochondrial haplotype in 48 European
1014 samples.

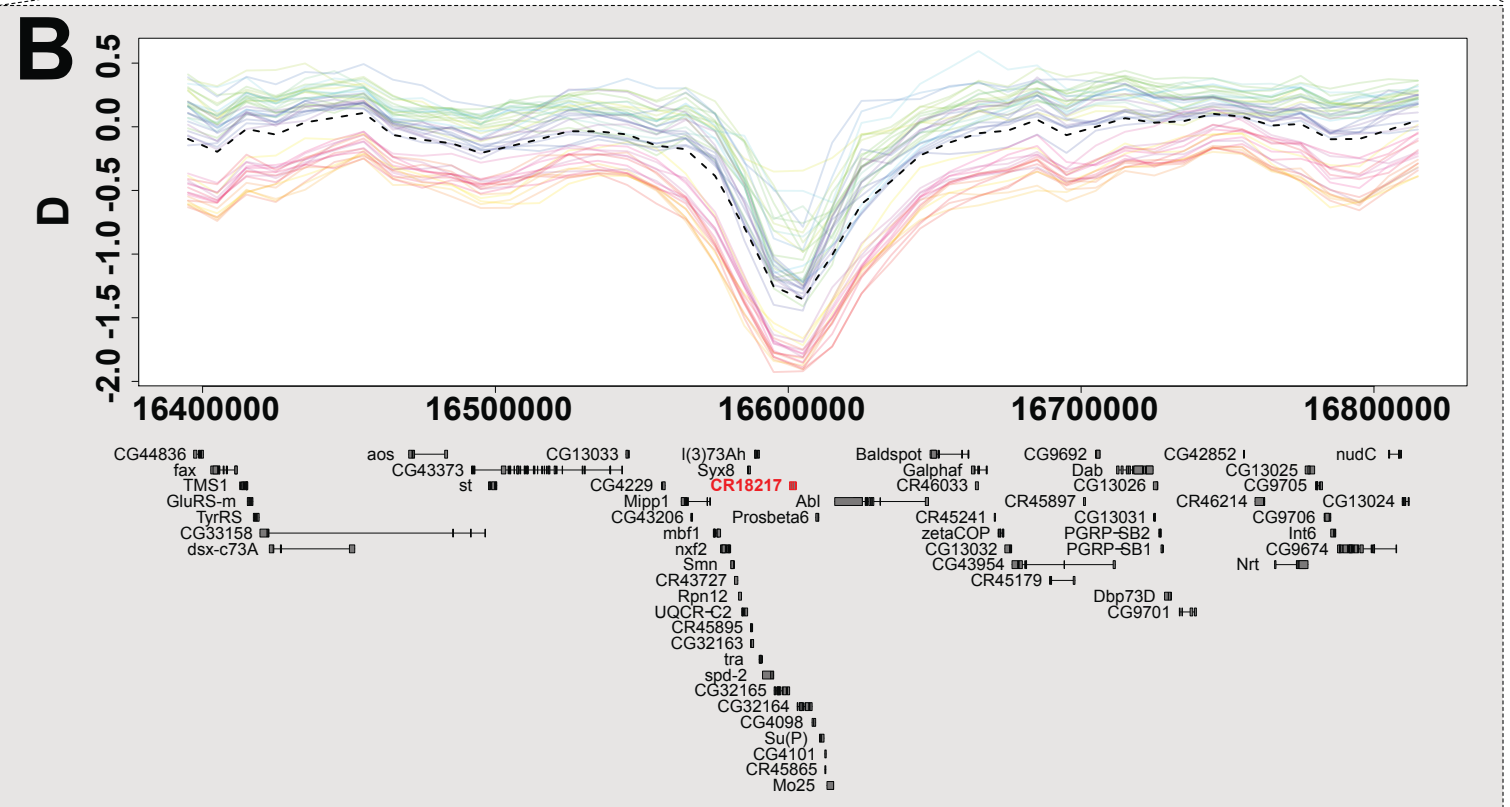
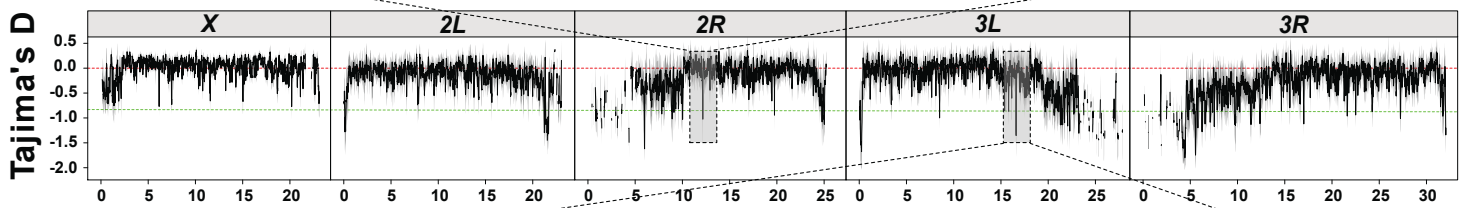
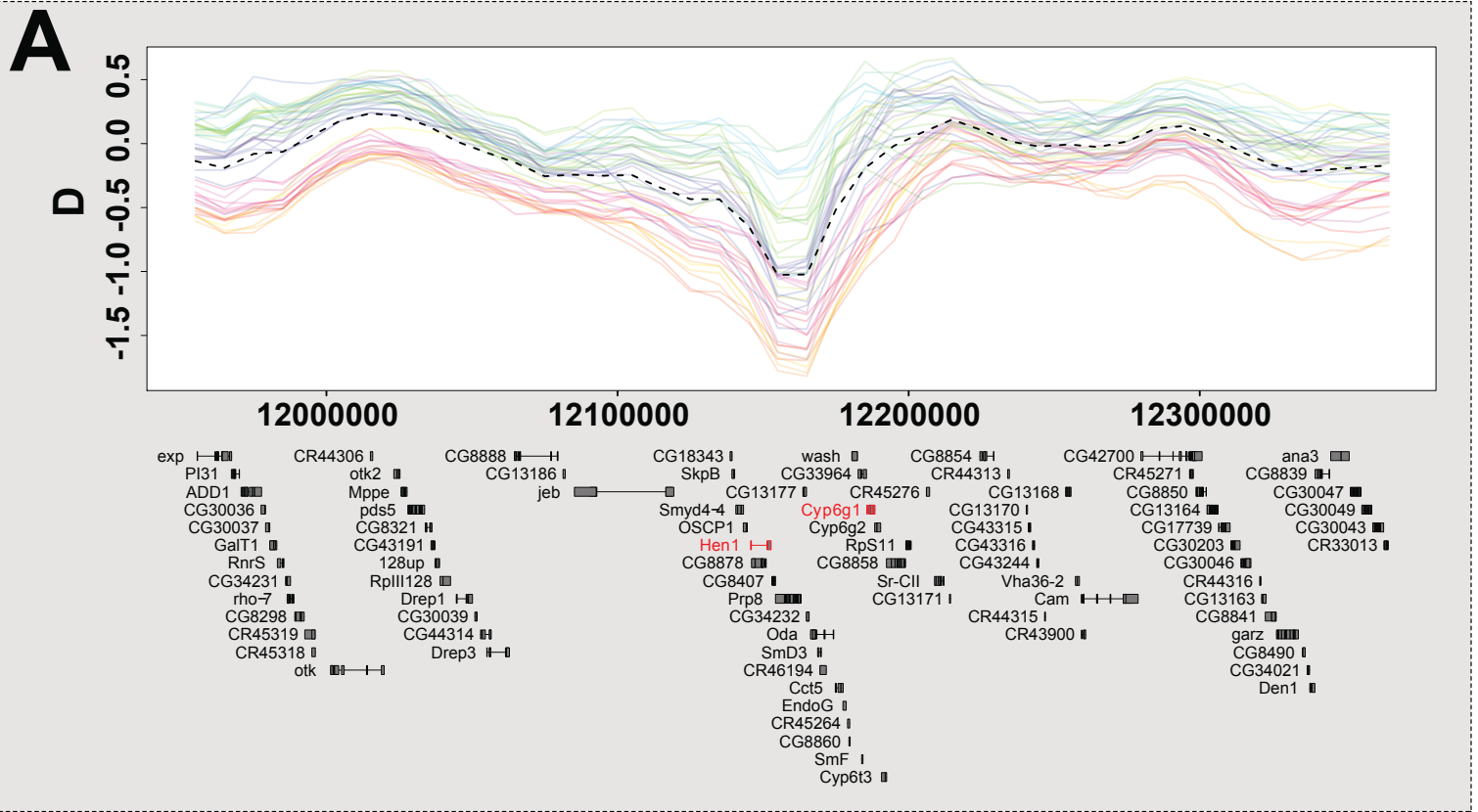
1015

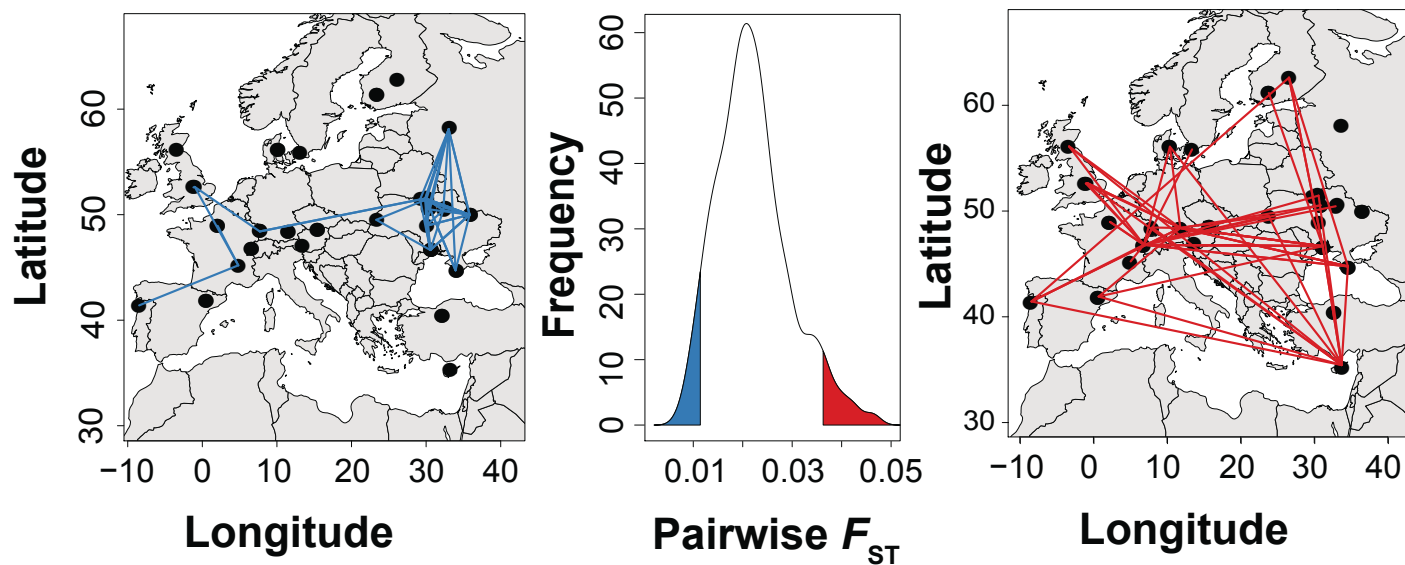
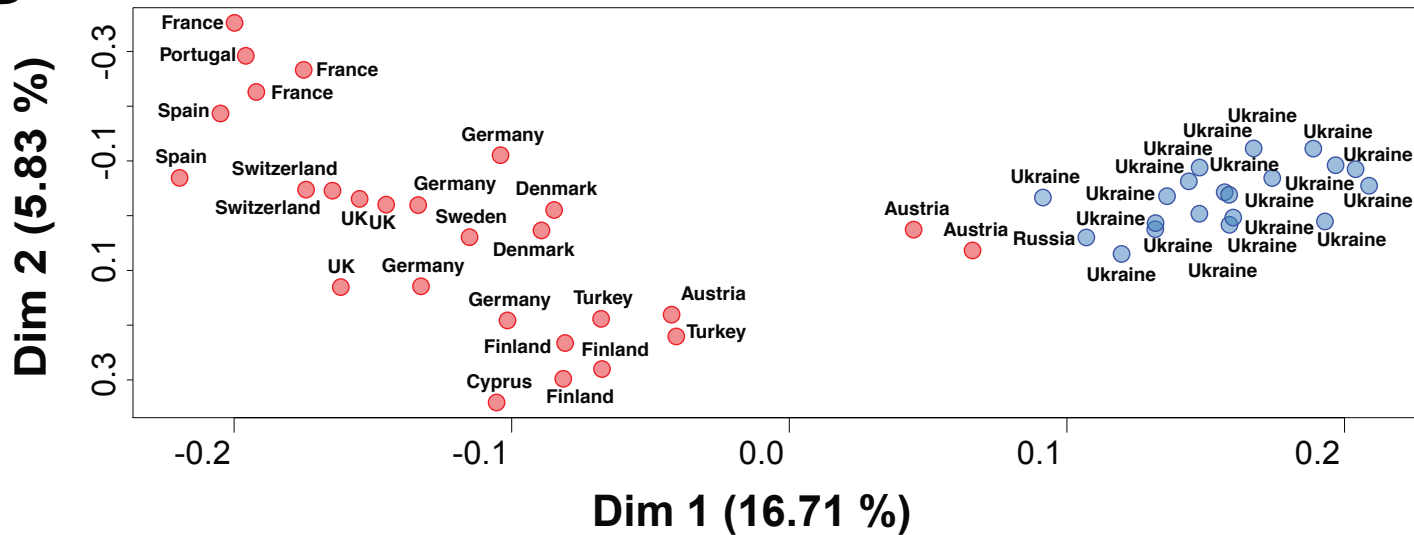
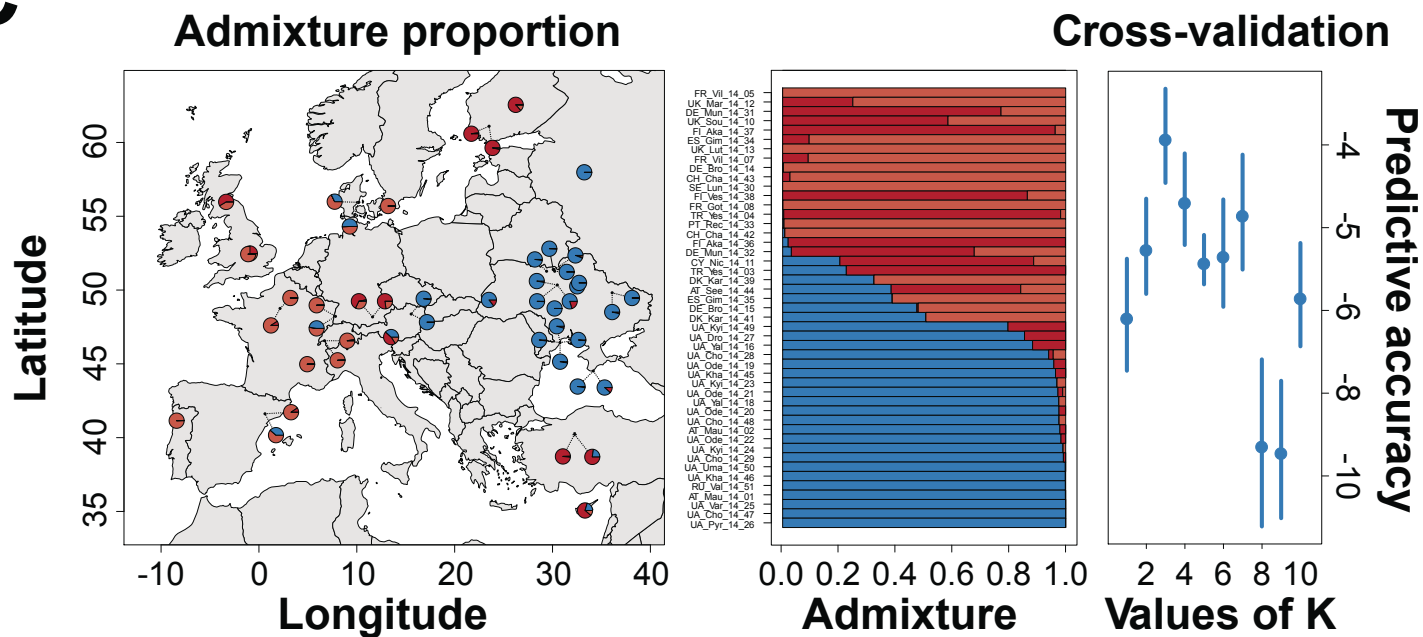
1016 **Fig. 6. Geographic patterns of structural variants.** The upper panel shows stacked bar plots with
1017 the relative abundances of transposable elements (TEs) in all 48 population samples. The proportion
1018 of each repeat class was estimated from sampled reads with dnaPipeTE (2 samples per run, 0.1X
1019 coverage per sample). The lower panel shows stacked bar plots depicting absolute frequencies of six
1020 cosmopolitan inversions in all 48 population samples.

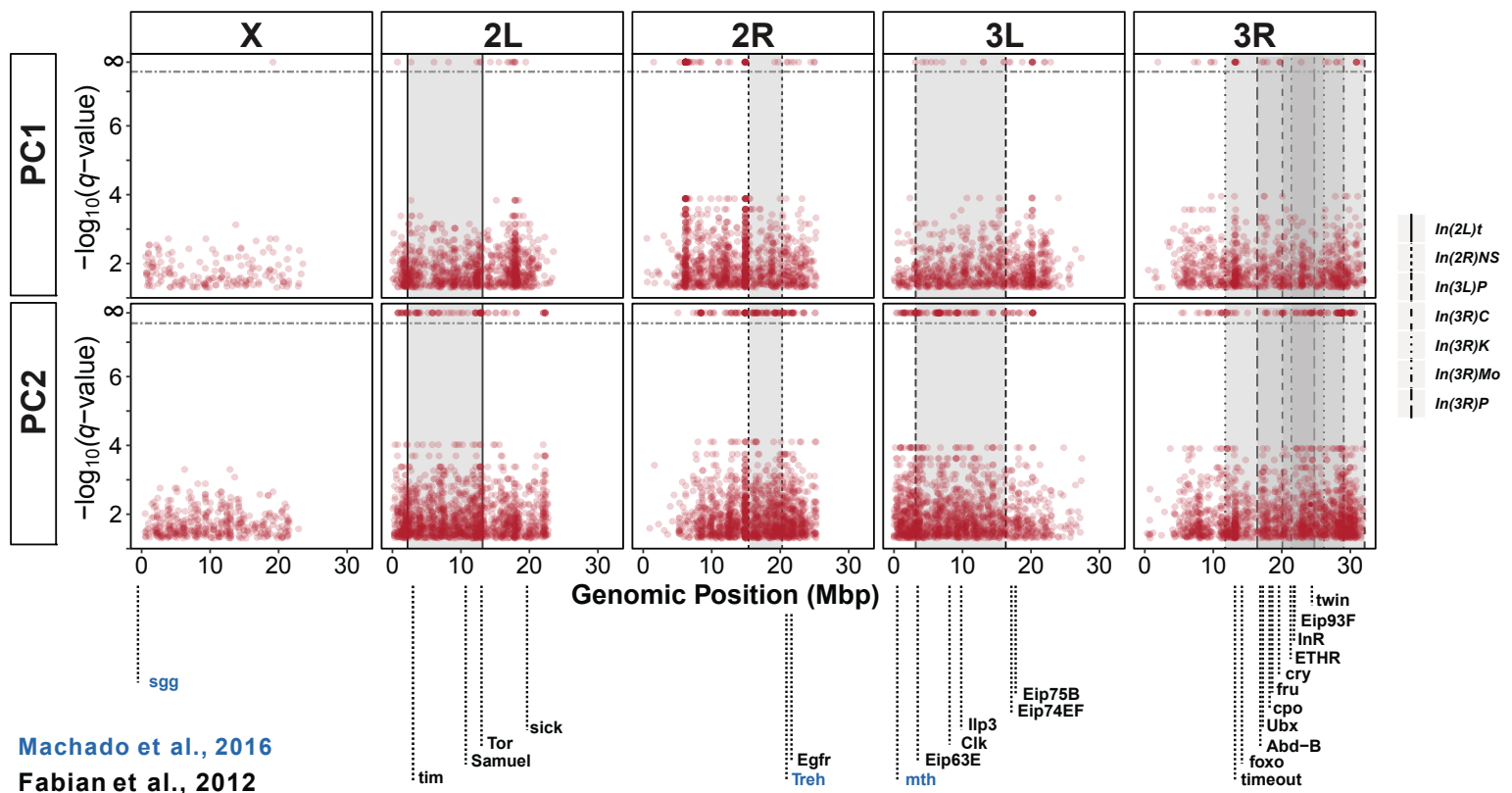
1021

1022 **Fig. 7. Microbiota associated with *Drosophila*.** Relative abundance of *Drosophila*-associated
1023 microbes as assessed by MGRAST classified shotgun sequences. Microbes had to reach at least 3%
1024 relative abundance in one of the samples to be represented



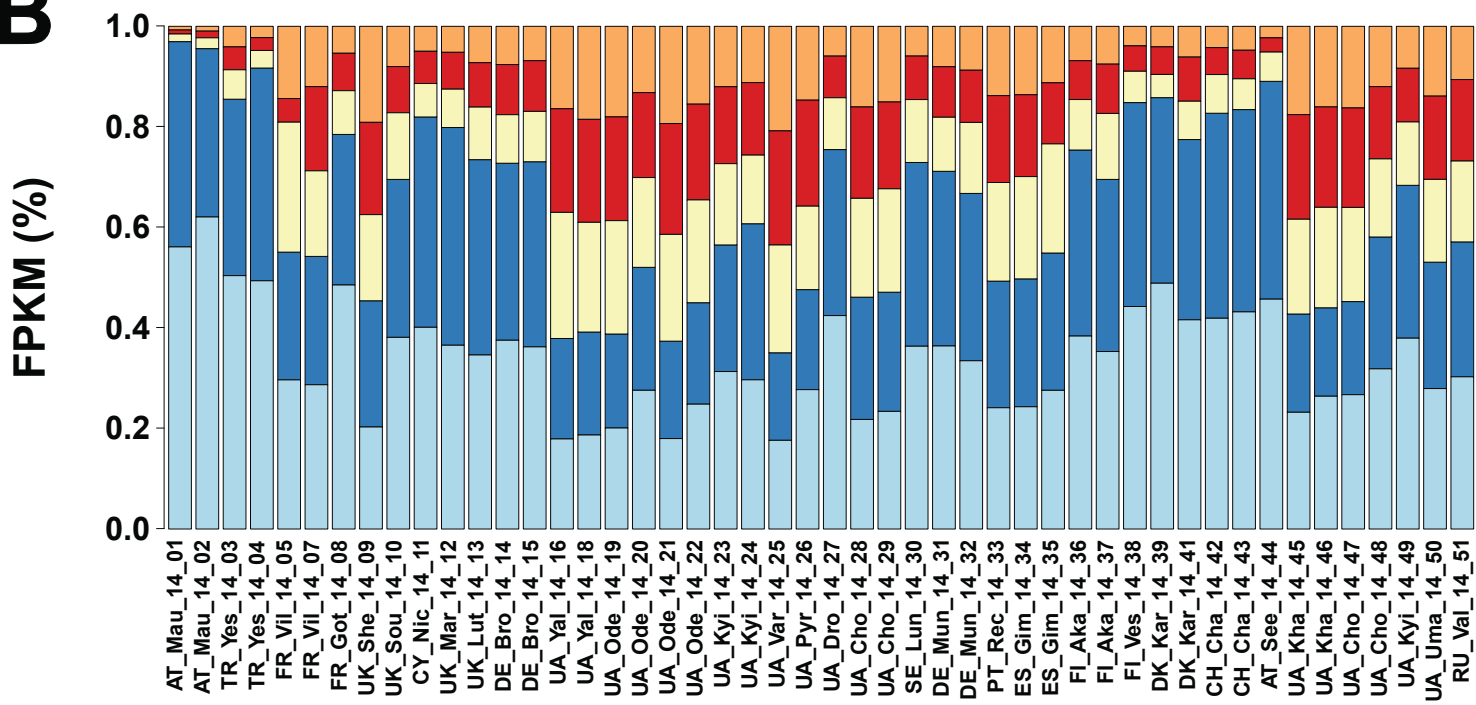


A**B****C**



Machado et al., 2016

Fabian et al., 2012

A**B**

Relative Abundance

