

1 **GLOBAL WARMING AND CHROMOSOMAL INVERSION**

2 **ADAPTATION IN ISOLATED ISLANDS: *DROSOPHILA***

3 ***SUBOBSCURA* POPULATIONS FROM MADEIRA**

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24 **Abstract**

25 Global warming is an environmental phenomenon to which species must adapt to
26 survive. The chromosomal inversion polymorphism of *Drosophila subobscura*
27 constitutes a genomic architecture that provides **this species with** an adaptive capacity.
28 Until now, the impact of global warming on this polymorphism has been studied in
29 populations located either **on continental mainland** or on islands **not far from a**
30 **continent**. In this **context**, gene flow could be a relevant mechanism allowing the
31 movement of thermally adapted inversions between populations. **We sampled and**
32 **studied chromosomal polymorphism on Madeira, a small island in the Atlantic Ocean.**
33 **We compared our findings** with those reported **in the same location approximately four**
34 **and five decades** ago. Moreover, we studied whether global warming **has occurred on**
35 this island by analyzing mean, maximum and minimum temperatures **over a fifty-five-**
36 **year period. All atmospheric parameters have increased significantly, consistent with**
37 **climate change expectations. Frequencies and CTI** (Chromosomal Thermal Index)
38 values of thermal adapted inversions remained **quite** stable over years. Furthermore, J,
39 U and O **chromosomes are almost fixed for** ‘warm’ adapted inversions. Thus, **if there is**
40 **little genetic variability remaining and temperatures continue increasing**, island
41 populations of *D. subobscura* might be **on** the threshold of **endangerment**. However,
42 apart from selection, genetic drift and inbreeding, **other processes**, such as phenotypic
43 plasticity or thermoregulatory behavior could be involved in the survival of the species’
44 populations. Finally, *D. subobscura* is a generalist species **that lives in** humanized
45 environments, and **this fact could favor its persistence on the island of Madeira.**

46 **Key words:** chromosomal inversions; climate change; temperature; selection;
47 thermoregulatory behavior

48 INTRODUCTION

49 Obviously, global warming has a profound impact on living organisms, but it can
50 provide an excellent opportunity to study the genetic components of adaptation
51 (Hoffmann & Sgrò 2011; Franks & Hoffmann 2012). For instance, in a given species a
52 possible experimental approach is to collect samples either over time or in distinct
53 localities of the distribution area with different climatic conditions and to study
54 differences in genetic composition (Solé *et al.* 2002; Hoffmann *et al.* 2004;
55 Heerwaarden & Hoffmann 2007). However, a large number of species could adapt to
56 global warming by migrating to other places. In this sense, it could be interesting to
57 study the effects of climatic changes on species located in small oceanic islands.
58 Compared with continental populations, they usually have reduced gene flow, whilst
59 genetic drift and inbreeding are important evolutionary factors (for recent examples see
60 Hoeck *et al.* 2010; Rogell *et al.* 2010; Mattila *et al.* 2012; Furlan *et al.* 2012; Wang *et*
61 *al.* 2014; Fountain *et al.* 2016; Funk *et al.* 2016).

62 Chromosomal inversion polymorphism is considered one of the most useful
63 genetic markers in studies of adaptation to global warming. Inversions were discovered
64 by Sturtevant (1921) and their adaptive value was widely recognized by Dobzhansky
65 (see Lewontin *et al.* 1981). These kind of chromosomal mutations are considered
66 cornerstones in the adaptation or speciation of a large number of organisms (see, for
67 instance, Feuk *et al.* 2005; Kirkpatrick & Barton 2006; Hoffmann & Rieseberg 2008;
68 McAllister *et al.* 2008; Kirkpatrick 2010; Nie *et al.* 2012; Ayala *et al.* 2014; Fuller *et al.*
69 2018). In particular, the role of inversions with regard to thermal adaptation was studied
70 in depth using different species of the *Drosophila* genus (for example, Dahlgard *et al.*
71 2001; Hoffmann *et al.* 2004; Anderson *et al.* 2003, 2005; Levitan 2003; Umina *et al.*

72 2005; Levitan & Etges 2005; Rane *et al.* 2015; Kapun *et al.* 2016). In this context, *D.*
73 *subobscura* can be considered a global warming model species. It presents a rich
74 chromosomal polymorphism, that is, a large number of different inversions and
75 combinations of inversions (overlapped or not) called arrangements (Krimbas 1992,
76 1993, Balanyà *et al.* 2009). This polymorphism is adaptive to many environmental
77 conditions and many inversions (and arrangements) have changed in frequency
78 according to global warming expectations (Orengo & Prevosti 1996; Rodríguez-Trelles
79 & Rodríguez 1998; Solé *et al.* 2002; Balanyà *et al.* 2004, 2006, 2009; Rezende *et al.*
80 2010; Zivanovic & Mestres 2011; Zivanovic *et al.* 2012, 2015; Orengo *et al.* 2016). All
81 these studies have been carried out either in populations located on the continent or on
82 islands close to the mainland. That is why we considered it would be interesting to
83 study the impact of global warming on an oceanic island population.

84 The chosen island was Madeira, located in the Atlantic Ocean at 978 km south
85 of Portugal, 700 km west of Africa and 450 km north of the Canary Is. Its total area is
86 796 km². It enjoys a mild climate with temperature variations through the year and
87 differences between the regions facing north and those facing south. These conditions
88 favor agricultural activity based on Mediterranean and tropical species. The island is
89 considered a hotspot of biodiversity with a large number of endemic species (Borges *et*
90 *al.* 2008). *D. subobscura* was reported for the first time on Madeira by Prevosti (1972),
91 although previous studies of Drosophilid fauna could have mistakenly classified this
92 species as *D. obscura* (Monclús 1984; Bächli & Báez 2002). Furthermore, within the
93 laurisilva environment, *D. subobscura* coexists with its closely related species of the
94 *obscura* group, *D. madeirensis*, which is endemic to the island (Monclús 1984). *D.*
95 *subobscura* from Madeira has been used to study distinct evolutionary problems by
96 means of diverse genetic markers (Pinto *et al.* 1997; Khadem *et al.* 1998, 2001; Brehm

97 *et al.* 2004; Herrig *et al.* 2014), but only two **previous** analyses of inversion
98 chromosomal polymorphism have been carried out (Prevosti 1972; Larruga *et al.* 1983).

99 Our main aim was to compare the current composition and frequencies of *D.*
100 *subobscura* inversion chromosomal polymorphism **on** Madeira with that reported
101 earlier. Also, we wanted to ascertain **whether predicted climatic effects of** global
102 warming could be **observed on the island analyzing rainfall and minimum, maximum**
103 **and mean temperatures, over years.** Finally, if variation both in inversions and climatic
104 change were observed, the final objective was to assess a possible relation between the
105 adaptive inversions and global warming.

106

107 **MATERIALS AND METHODS**

108 **Fly samples**

109 *D. subobscura* individuals were collected at Camacha (32°40'N 16°50'W), Curral das
110 Freiras (32°43'N 16°58'W) and Prazeres (32°45'N 17°12'W), during November and
111 December 2016 (Fig. 1). Flies were collected using open banana baits, sampled by
112 netting in the morning and late afternoon. The captured flies were put in bottles together
113 with some local vegetation and kept in darkness. The samples were transferred to the
114 laboratory, at the end of the day, where they were separated and classified. Males were
115 put together (20 to 30 individuals) in large plastic vials with Caroline Instant
116 *Drosophila* Medium (17-3200), whereas females were placed in individual vials and
117 allowed to lay eggs. All vials were sent to Barcelona for the chromosomal analysis.
118 Individual wild males (or sons of isofemale lines) were crossed with two virgin females
119 of the *chcu* homokaryotypic strain that carries the recessive mutations *ch* (cherry eyes)
120 and *cu* (curled wings), both located in the O chromosome. With regard to chromosomal

121 inversions, the strain is homokaryotypic A_{st}, J_{st}, U_{st}, E_{st} and O₃₊₄. Third instar F₁ larvae
122 were dissected to obtain the polytene chromosomes of the salivary glands, which were
123 stained and squashed in aceto-orcein solution. For the study of inversions we have
124 followed the classification and nomenclature of Kunze-Mühl & Müller (1958) and
125 Krimbas (1993). The inversion chromosomal polymorphisms from our Madeira
126 collections were compared with those obtained previously in Terreiro da Luta (32°41'N
127 16°53'W) and Curral das Freiras (32°43'N 16°58'W) in 1970 by Prevosti (1972) and in
128 Poiso (32°43'N 16°58'W) and Ribeiro (32°46'N 16°51'W) in 1978 by Larruga *et al.*
129 (1983).

130 **Statistical analyses and meteorological data**

131 All computations were carried out using R language (R Development Core Team,
132 2014). To compare the inversion chromosomal polymorphism between different
133 locations and/or years, a Fisher exact test was used and the corresponding *P*-values were
134 obtained using the bootstrap procedure (100,000 runs; statistically significant *P* < 0.05).
135 All analyses were carried out using the *fisher.test* function of the package *stats*. As
136 multiple testing was computed, results were corrected by means of the false discovery
137 rate (FDR) method (Benjamini & Hochberg 1995) using the function *p.adjust* (package
138 *stats*).

139 **To estimate the probability of appearance or disappearance of several inversions**
140 **in our Madeira collection (2016), the following permutation procedure was used. Using**
141 **Prevosti's collection as a base, we generated B=50,000 samples with replacement with**
142 **our Madeira sample size and we estimated the probability that infrequent inversions**
143 **were included in the 2016 collection. Also, we fixed the 2016 collection as a base and**
144 **Prevosti's plus Larruga's sample size, and then we generated B=50,000 samples with**

145 replacement to estimate the probability that the new inversions were included in the
146 previous samples.

147 Finally, to place the Madeira polymorphism with regard to other Palearctic and
148 American *D. subobscura* populations, comparisons between O chromosome inversion
149 polymorphisms (the most studied chromosome in this species) were carried out (Table
150 S1). The Bhattacharyya distance (Bhattacharyya 1946) was used and a Principal
151 Coordinate Analysis was carried out with this set of populations (Balanyà *et al.* 2006;
152 Mestres *et al.* 2009). The computations were carried out using the *mds* function from
153 *vegan* package and graphically displayed with the *eqsplot* function of *MASS* package.
154 Finally, GEVA-Ward was chosen as cluster method, because it is considered excellent
155 for chromosomal inversion data (Irigoién *et al.* 2010; Zivanovic *et al.* 2016). The cluster
156 was obtained using the *hclust* function of *stats* package of R. To measure how faithfully
157 the cluster preserved the pairwise distances between the original data, the Pearson
158 cophenetic correlation was computed (*cophenetic* function of *stats* package).

159 Values of minimum (Tmin.), maximum (Tmax.) and mean (Tmean)
160 temperatures and rainfall were obtained from Funchal Meteorological Station. Although
161 this is not one of our trapping sites, it allows for the tracking of meteorological variables
162 on the island. To analyze the possible effect of global warming in Madeira, we used
163 data from each October (the month before trapping the flies) between 1961 and 2016.
164 For each of the three temperatures (Tmin., Tmax and Tmean), a temporal series was
165 computed using Statgraphics software (Statgraphics Technologies, Inc. USA). As
166 rainfall was appreciably erratic, a temporal series was not computed. Finally, the CTI
167 (Chromosomal Thermal Index, Arenas *et al.* 2018) was computed to measure the
168 thermal adaptation of the whole karyotype. The index varies from 0 to 1, where 1 means
169 that only 'warm' adapted inversions are present. Chromosomes were classified as

170 'warm' or 'cold' using the well-established criterion of Menozzi & Krimbas (1992) and
171 Rego *et al.* (2010). Thus, in Madeira the following inversions (or arrangements) were
172 considered 'warm': A_2 , J_1 , U_{1+2} , U_{1+8+2} , E_{1+2+9} , $E_{1+2+9+12}$ and O_{3+4} , whereas A_{st} , J_{st} , U_{st} ,
173 E_{st} and O_{st} were classified as 'cold'. The remaining inversions were considered
174 **unrelated to** thermal adaptation.

175

176 **RESULTS**

177 **Inversion chromosomal polymorphism**

178 It is interesting to compare the chromosomal polymorphism from this study (Table 1)
179 with data from previous studies (Prevosti 1972; Larruga *et al.* 1983). From a qualitative
180 point of view, the chromosomal composition is rather similar. However, the inversions
181 A_{2+6} , $E_{1+2+9+3}$ and O_{st} , previously detected **by** Prevosti (1972), are absent **in both** Larruga
182 *et al.* (1983) and the present research. **The probabilities that these inversions were**
183 **included in our 2016 sample were estimated as 0.6501, 0.8558 and 0.9997, respectively.**
184 **Therefore, the most likely scenario is that they disappeared from the Madeira**
185 **populations. Also, in** the present study, inversions U_{st} and U_1 were detected for the first
186 time **and** a new inversion, never **before** reported, **was observed** in the species
187 ($E_{1+2+9+new}$). **The probabilities that these inversions were present in the previous Madeira**
188 **collections were estimated as 0.9536, 0.9538 and 0.9576, respectively. Thus our**
189 **hypothesis is that they appeared recently.** The detection, for the **first time, of common**
190 **inversions in the species distribution area (albeit with low frequencies)** seems to
191 indicate a certain level of gene flow from the continent. The observation of new
192 inversions in *D. subobscura* is a recurrent phenomenon previously reported (Orengo &
193 Prevosti 1996; Zivanovic & Sperlich 2000; Solé *et al.* 2002; Balanyà *et al.* 2003, 2004),

194 but their fate is usually extinction (Sperlich & Pfriem 1986; Powell 1997). Finally, it is
195 worth **pointing** out that, although the O chromosome is highly polymorphic for
196 inversions (Krimbas 1992, 1993), our Madeira collections are almost monomorphic for
197 O_{3+4} (Table 1).

198 We compared the chromosomal polymorphism composition in Curral das Freiras
199 between our sample and **Prevosti's** (1972) and no significant differences were obtained
200 for any chromosome (A, $P = 0.725$; J, $P = 1$; U, $P = 0.358$; E, $P = 0.801$ and O, $P = 1$).
201 **In our collections**, as no significant differences were observed between Camacha, Curral
202 das Freiras and Prazeres, it is possible to consider all the *D. subobscura* samples from
203 Madeira as a unique population (Table S2). **We observed significant differences in**
204 chromosomal compositions **between** the three studies: Prevosti (1972), Larruga *et al.*
205 (1983) and the present research, when all chromosomes were considered together (Table
206 2). Moreover, **we detected** significant differences **in** E and O chromosomes between the
207 **two former samples**. This could be a product of an increase in frequency of E_{st} , E_{1+2} and
208 E_{1+2+9} and a decrease of $E_{1+2+9+12}$ ($E_{1+2+9+3}$ disappeared in the second study).
209 **Furthermore**, in the second study, O_{st} was absent and O_{3+4} increased. The loss of O_{st} and
210 the increase of E_{1+2+9} are in **accordance** with global warming expectations **although this**
211 **loss could equally be due to genetic drift**. The increase of E_{st} (considered a 'cold'
212 inversion) **is** most probably **attributable** to genetic drift. In the comparison between **the**
213 **second study and the present research** only the A chromosome was significant, due to an
214 increase of A_{st} and a decrease of A_2 . This result is **at odds with** the global warming
215 hypothesis, because A_{st} and A_2 are considered 'cold' and 'warm', respectively. Finally,
216 when comparing both extreme samples (Prevosti (1972) and the present study), A and O
217 chromosomes showed significant variations: A_{st} **increased** and A_2 **decreased, we saw an**

218 **increase in** O_{3+4} whereas O_{st} was not detected in the present analysis. Therefore, these
219 results could be interpreted in the same way as those previously explained.

220 The multivariate analysis using the O chromosome frequencies in natural
221 populations showed that collections from **the island of Madeira were clearly**
222 **differentiated from other populations** (Fig. 2). In the principal coordinate analysis, first,
223 second and third axes explained the 50.77%, 19.72% and 15.89%, respectively (Fig. 2
224 (a)). Four groups are manifestly differentiated: from left to right it is possible to observe
225 the collections from Madeira, the group of Balkan populations, the remaining European
226 collections and the American populations. European populations are distributed along
227 the second axis and **follow** a clinal distribution, with Mediterranean samples at the
228 bottom, **central and north European at the top**. American populations are separated, due
229 to the strong founder event, and **are** sorted along latitude. However, although they
230 belong to different hemispheres, North and South American populations are mixed in
231 the graphic, following a climatic pattern. According to weather, Atlantic climate
232 populations are **at** the top of the group, **regardless** of their American origin and the same
233 happens with Mediterranean climate populations, which appear at the bottom of the
234 group. Equivalent results were obtained from the cluster analysis (Fig. 2 (b)). The
235 cophenetic correlation coefficient was 0.813, indicating that the tree properly describes
236 the genetic distance between populations. According to the O chromosome inversion
237 polymorphism, the first partition separated **off the** Madeira collection. The second
238 divergence was between American and European populations. Inside the first group,
239 samples with **a** Mediterranean climate were clustered together (including samples from
240 North and South America), whereas the second group contained those belonging to **an**
241 Atlantic climate (also with North and South American populations). Analyzing the
242 European cluster, two groups are clearly differentiated: one containing the populations

243 with a Mediterranean climate, the other Atlantic. In the Mediterranean group it is
244 possible to detect the populations from Majorca (Calvià), Catalonia and other Iberian
245 Peninsula samples. In the other large European group, two clusters can be observed: one
246 with Balkan populations and the other with the remaining European collections. In this
247 last group, the first cluster contained the French populations and one Catalan sample
248 and the second, the central and north European samples are grouped.

249

250 Climatic change and chromosomal inversions

251 Temperatures showed a significant increase, according with global warming
252 expectations. For Tmin, Tmax and Tmean, the *P* values were lower than 0.0001 in all
253 cases. Graphical displays and lineal trends for these temperatures are presented in Fig.
254 S1. The rainfall pattern was irregular, in accordance with global warming expectations.
255 These results are in agreement with those reported in Santos *et al.* (2004), Cropper &
256 Hanna (2014) and Tomé *et al.* (2014).

257 We analyzed the chromosomal polymorphism for all available Madeiran
258 collections but considered only those inversions classified as ‘warm’ or ‘cold’, using the
259 CTI index (Table 3). These values ranged between 0.714 and 0.781 and can be
260 considered high, being surpassed only by the sample from Etna on Sicily (0.958),
261 analyzed by Prevosti *et al.* (1984). No significant differences were detected for the
262 comparisons between the CTI values of our whole Madeira sample and the two earlier
263 samples (Table S3), which could indicate that thermal adapted chromosomal inversions
264 have not changed in frequency over time.

265

266 DISCUSSION

267 There is **widespread** concern **about the** different phenomena that can damage our planet,
268 and one of **the most urgent** is climate change (Ripple *et al.* 2017). The way in which
269 living organisms are able to adapt **to** survive this environmental change is an essential
270 issue in evolutionary biology. **Studies of *Drosophila* genus have proved it** to be an
271 excellent biological model (Levitan 2003; Levitan & Etges 2005; Umina *et al.* 2005;
272 Overgaard *et al.* 2014; Tobler *et al.* 2015). **The *D. subobscura* species has been**
273 **especially interesting** due to its rich chromosomal inversion polymorphism and the
274 accumulation of a large number of studies carried out in different biogeographic areas,
275 over time (Orengo & Prevosti 1996; Rodríguez-Trelles & Rodríguez 1998; Solé *et al.*
276 2002; Balanyà *et al.* 2004, 2006, 2009; Rezende *et al.* 2010; Zivanovic & Mestres 2011;
277 Zivanovic *et al.* 2012, 2015; Orengo *et al.* 2016). However, it was scientifically
278 valuable to study the inversion chromosomal polymorphism of this species in an
279 isolated place with low gene flow, **such as the island of Madeira**. As this is a small
280 island and its *D. subobscura* populations have suffered many bottlenecks (**due**, for
281 instance, to forest fires or floods), their effective population sizes (N_e) are expected to
282 be small. In these conditions, genetic drift and inbreeding could be evolutionary
283 mechanisms with **relatively dramatic** effects. In natural *D. subobscura* populations, the
284 number of generations per year has been estimated **at** 4-6 (Begon 1976; Mestres *et al.*
285 2001). Therefore, selection and/or genetic drift plus inbreeding could have been **active**
286 **through 32-48 and 152-228 generations (representing the time lapses between first and**
287 **second studies and second and third, respectively).**

288 From our study, it seems that all populations, at least in the **southern** half of the
289 island were **fairly** uniform. For this reason, we analyzed the inversion polymorphism,
290 considering Madeira as a single population. Although this island is **distant** from the
291 continent, **the** tourist industry **means** large amounts of food are imported, mainly from

292 continental Portugal. Fruits and other vegetables could act as carriers for *D. subobscura*
293 individuals, most likely in the larvae or pupa stages. However, the composition of the
294 chromosomal inversions was stable over time and, in the present study, only two
295 Palearctic inversions were detected for the first time on Madeira. There are two
296 competing hypotheses for their presence: either they were already present on the island
297 but remained undetected or they are newly arrived from the continent. The first is
298 improbable due to the large sample sizes analyzed by Prevosti (1972) and Larruga *et al.*
299 (1983). We think that continental inversions are able to reach Madeira via unintentional
300 human transport, but would soon disappear due to genetic drift or selection, because
301 they are non-adaptive to the particular island environment. Although significant
302 differences were observed for several particular chromosome comparison between the
303 three collections from Madeira (Prevosti 1972; Larruga *et al.* 1983; present study), the
304 chromosomal composition over time is fairly constant in the type of inversions and their
305 relative abundance, even though eight (32-48 generations) and thirty-eight years (152-
306 228 generations) have elapsed between these three collections. Different reasons could
307 explain some of the particular differences detected. For instance, Larruga *et al.* (1983)
308 like us, collected the samples in the autumn, but Prevosti (1972) trapped flies in the
309 summer. Furthermore, we sampled *D. subobscura* individuals just after extensive forest
310 fires in August and floods in October had ravaged the island, and these events could
311 have produced population bottlenecks altering the inversion frequencies.

312 If we focus only on the chromosomal inversions considered thermally adapted
313 (Krimbas & Menozzi 1992; Rego *et al.* 2010; Arenas *et al.* 2018), although small
314 fluctuations have been observed, in general their composition has not changed over
315 time. The consistency of the CTI values is very similar in the three collections available
316 and no significant differences were detected. The similarity of the CTI values over time

317 contrasted **greatly** with the dramatic global warming observed when analyzing T_{min} ,
318 T_{max} and T_{mean} **over a fifty-five-year period (an increase of around 2°C)**. Also, the
319 erratic pattern of rainfall during these years was **in accordance with the expectations of**
320 **climate change**. The main evolutionary question is: why **has** thermally adapted
321 chromosomal polymorphism **on Madeira not responded** to global warming? It was
322 demonstrated that the chromosomal polymorphism of the island is poor and particular
323 when compared to closer mainland populations: **a product of a historic founder event**
324 and adaptations to **the** special island environment. This situation was **demonstrated**
325 **beyond** doubt when comparing the O chromosome inversion composition **on Madeira**
326 **with** other *D. subobscura* populations from the whole distribution area, using
327 multivariate analyses (PCA and cluster). Moreover, *D. subobscura* **on** the island seems
328 to be close to the thermal adaptation limit, because ‘warm’ inversions appear **in**
329 dramatically high frequencies for three of the chromosomes (0.903, 0.994 and 0.987, for
330 J, U and O, respectively). However, ‘cold’ inversions are present in non-negligible
331 frequencies for the A (0.228) and E (0.257) chromosomes. Two considerations must be
332 taken into account: the inversions considered ‘warm’ or ‘cold’ probably do not **imply** a
333 direct adaptive effect of temperature (Santos *et al.* 2005) and other karyotypic regions
334 not covered by inversions could contain thermal adaptation genes (Arenas *et al.* 2018).
335 For instance, the A_{st} frequency (considered as a ‘cold’ inversion) that we detected **on**
336 Madeira (0.228, collected in autumn/winter) was significantly higher than that found by
337 Prevosti (1972) (0.110, but trapped in summer) and Larruga *et al.* (1983) (0.115, also
338 collected in autumn), **which** could indicate that this inversion **might response to**
339 **something other than** temperature, because it has changed over the years in the opposite
340 direction **to that** expected. However, the different trapping seasons and/or genetic drift
341 could explain these differences. On the other hand, although thermal adapted genes in

342 *D. subobscura* could be distributed throughout the whole karyotype, a larger than
343 expected number of these genes were located inside inverted regions (Laayouni *et al.*
344 2007).

345 The particular chromosomal polymorphism composition seems to be properly
346 adapted to the environmental and biotic conditions of the island, and gene flow appears
347 irrelevant. In this situation, other possibilities must be considered in front of **the increase**
348 **of temperature**. In continental populations, in addition to selection of the available
349 genetic variability, other explanations are possible. Thus, *D. subobscura* individuals can
350 migrate to areas of their thermal preference, because it is a species with a high capacity
351 **for** dispersion (Greuter 1963; Loukas & Krimbas 1979; Serra *et al.* 1987) and reacts
352 according to its thermal preference (Rego *et al.* 2010; Dolgova *et al.* 2010).

353 Consequently, variations in the continental latitudinal clines according to global
354 warming expectations could **also be** attributed to a greater or lesser extent to migration
355 (Santos 2017). As this explanation is not applicable to an isolated island, other
356 hypotheses have to be explored, **such as**, for example, thermal plasticity (Kelly 2019;
357 Bonamour *et al.* 2019). In this case, the direct influence of environmental factors
358 (including temperature) on the development of individual phenotypes is considered a
359 key element in the phenotypic change of populations and their persistence (Chevin *et al.*
360 2010; Fragata *et al.* 2016). However, phenotypic plasticity could involve many fitness
361 costs **for** the individuals, regardless of the phenotype expressed (Dewitt *et al.* 1998).
362 Furthermore, there is a limit to the effectiveness of plastic response (Mitchell *et al.*
363 2011). Another possibility is that selection on thermal related traits is compensated by
364 fly behavior (Huey & Pascual 2009). In this sense, **the** Bogert effect is defined as the
365 ectoderm thermoregulatory behavior which compensates for environmental temperature
366 variation (Huey *et al.* 2003; Castañeda *et al.* 2013). It was reported that behavior is

367 important in the *Drosophila* genus to maintain temperature within a physiological
368 permissive range (Dillon *et al.* 2009). For instance, these thermoregulatory behaviors
369 could manifest as variations in seasonal and daily activity patterns or displacements to
370 beneficial microclimates (Stevenson 1985).

371 Global warming is altering both, the terrestrial and marine ecosystems of
372 Madeira (Cruz *et al.* 2009; Clemente *et al.* 2014). Moreover, some extinctions and
373 invasions have been reported (see, for instance, Gardiner 2003; Wirtz 2005; Ribeiro *et*
374 *al.* 2009). These changes could even be harmful for the human population on the island,
375 producing economic losses and health problems (Carvalho *et al.* 2013; Liu-Helmersson
376 *et al.* 2016). Given the present situation, we are unable to predict the future of *D.*
377 *subobscura* populations on Madeira. The particular ecosystem alterations inflicted by
378 global warming may not be a serious problem for the species, because it is a generalist
379 species closely associated with human or humanized environments (Krimbas 1992,
380 1993). However, the direct impact of temperature on *D. subobscura* could pose a
381 difficult problem to overcome, due to its limited genetic variability, plasticity and
382 mobility in such a small and isolated island as Madeira. For all these reasons, *D.*
383 *subobscura* could be an excellent model species for studying the effects global warming
384 on the evolution of organisms inhabiting isolated oceanic islands.

385

386

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395

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673

674 **SUPPORTING INFORMATION**

675 Additional Supporting Information may be found online in the Supporting Information
676 section at the end of the article.

677

678 **Table S1.** Populations from Madeira, Europe and America used in the comparisons for
679 the O chromosome inversion polymorphisms.

680 **Table S2.** *P* values of the Fisher exact test in all comparisons between the inversion
681 chromosomal polymorphisms of the three populations analyzed on Madeira.

682 **Table S3.** Results of the *CTI* index comparisons of the three collections analyzed on
683 Madeira Is.

684

685 **Figure S1.** Lineal trends and graphical displays for *T*_{min.}, *T*_{max.}, *T*_{mean} and Rainfall
686 (years 1961-2016).

687

688

689 **Figure 1** Map of Madeira **showing locations of** the populations studied (Prazeres,
690 Curral das Freiras and Camacha). The capital of the island, Funchal, is also shown, as a
691 reference.

692

693 **Figure 2** Multivariate analysis of the O chromosome inversion polymorphism in natural
694 populations of *D. subobscura*. (a) Principal Coordinate Analysis. Four groups of
695 populations were clearly determined, from left to right: Madeira samples (white
696 diamond, \diamond), Balkan populations (dark square, \blacksquare), remaining European samples (white
697 circle, \circ) and American populations (dark circle, \bullet). (b) GEVA-Ward cluster study. The
698 first partition separated Madeira collections from other *D. subobscura* collections. In
699 both analyses, the populations analyzed were: 1. Montpellier (France), 2. Lagrasse
700 (France), 3. Queralbs (Catalonia, Iberian Peninsula), 4. Riba-roja (Iberian Peninsula), 5.
701 Calvià (Majorca Is.), 6. Punta Umbría (Iberian Peninsula), 7. Málaga (Iberian
702 Peninsula), 8. Groningen (The Netherlands), 9. Louvaine-la-Neuve (Belgium), 10.
703 Villars (France), 11. Tübingen (Germany), 12. Vienna (Austria), 13. Leuk
704 (Switzerland), 14. Santiago de Chile (Chile), 15. Chillán (Chile), 16. Laja (Chile), 17.
705 Valdivia (Chile), 18. Puerto Montt (Chile), 19. Coyhaique (Chile), 20. Gilroy (USA),
706 21. Davis (USA), 22. Eureka (USA), 23. Medford (USA), 24. Salem (USA), 25.
707 Centralia (USA), 26. Bellingham (USA), 27. Port Hardy (Canada), 28. Kamariste
708 (Serbia), 29. Petnica (Serbia), 30. Zanjic (Montenegro), 31. Font Grogga 2004
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713 2014 (Catalonia, Iberian Peninsula), 39. Font Grogga 2015 (Catalonia, Iberian

714 Peninsula), 40. Camacha (Madeira), 41. Curral das Freiras 2016 (Madeira), 42. Prazeres
715 (Madeira), 43. **Terreiro** da Luta (Madeira), 44. Curral das Freiras 1970 (Madeira), 45.
716 Poiso (Madeira), 46. Ribeiro (Madeira) and 47. Adraga (continental Portugal). (**Prevosti**
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718 **Balanyà *et al.* 2003, 2004; Araúz *et al.* 2009; Fragata *et al.* 2010; Calabria 2012;**
719 **Galludo *et al.* 2018).**

720