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Comparison of overwintering survival and fertility of *Zaprionus indianus* (Diptera: Drosophilidae) flies from native and invaded ranges

N.J. Lavagnino, J.J. Fanara, J. Mensch

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4 Lavagnino NJ<sup>§</sup>, JJ Fanara & J Mensch<sup>§</sup>.

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6 Laboratorio de Evolución, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias  
7 Exactas y Naturales, Universidad de Buenos Aires. Argentina. IEGEBA-CONICET, Facultad de  
8 Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina.

9

10 <sup>§</sup>Corresponding author:

11

12 Departamento de Ecología, Genética y Evolución.

13 Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires.

14 Instituto de Ecología, Genética y Evolución de Buenos Aires, Consejo Nacional de Investigaciones  
15 Científicas y Técnicas.

16 Ciudad Universitaria, Pabellón II.

17 Buenos Aires 1428, Argentina.

18 Tel. 54-11-45763300.

19 Fax.54-11-4563384.

20 e-mail: nlavagnino@ege.fcen.uba.ar, jmensch@ege.fcen.uba.ar

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22 Running title: Overwintering traits in *Zaprionus indianus*.

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27 *Zaprionus indianus* is a fly species native to the Afrotropical biogeographic region that invaded the  
28 South American continent 20 years ago. Its southernmost record is 34°S in areas with temperate  
29 climates with cold winters. To better understand its invasion biology, we investigated physiological  
30 responses to winter-like abiotic conditions that may be relevant in *Z. indianus* geographic  
31 expansion. We characterized *Z. indianus* females reproductive traits (ovarian maturation and  
32 fertility) and survival in response to cold treatments with summer-like and winter-like photoperiods.  
33 We also compared these traits between native (Yokadouma, Africa) and invasive (Yuto, South  
34 America) range wild-derived flies. We showed that *Z. indianus* females have the ability to arrest  
35 ovarian maturation and maintain fertility following recovery from cold stress. The critical  
36 temperature for ovarian maturation of this species was estimated at c. 13°C, an intermediate value  
37 between those of tropical and temperate drosophilid species. Wild-derived females from Yuto  
38 responded to winter-like photoperiod by slowing down ovarian maturation at low but permissive  
39 temperatures of 14°C and 16°C and also delayed the start of oviposition after cold treatment. Yuto  
40 flies also survived better and recovered 20 % faster from chill coma than flies from Yokadouma.  
41 These results are consistent with a scenario of local adaptations or phenotypic plasticity in the  
42 invaded range, and suggest that photoperiod could act as modulator of ovarian arrest. Conversely,  
43 the fact that native range flies showed higher fertility after cold recovery than females from invaded  
44 range is not indicative of local adaptation. All in all, our findings report a set of physiological  
45 responses that would enable *Z. indianus* expansion to temperate and cold areas, but also results that  
46 are compatible with a limitation to the invasion process.

47

48 Key words: *Zaprionus indianus*; Overwintering; Low Temperature Physiology; Invaded Range;

49 Native Range.

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52 *Zaprionus indianus* Gupta 1970 (Diptera: Drosophilidae) is an invasive fly species native to the  
53 Afrotropical region that invaded the American continent 20 years ago (Commar *et al.*, 2012). In  
54 South America it was reported for the first time in 1999 in São Paulo city area near the Atlantic  
55 coast of Brazil (Vilela, 1999). Since this initial report, *Z. indianus* has been found in five of the 12  
56 countries of South America, with the southernmost record near 34°S (Castro and Valente, 2001;  
57 Goñi *et al.*, 2001; De Toni *et al.*, 2001; Vilela *et al.*, 2001; Goñi *et al.*, 2002; Tidon *et al.*, 2003;  
58 Santos *et al.*, 2003; Kato *et al.*, 2004; Oliveira *et al.*, 2009; Leao and Tidon, 2004; Soto *et al.*, 2006;  
59 Chaves and Tidon, 2008; Lavagnino *et al.*, 2008; Furtado *et al.*, 2009; Fernandes Rodrigues and  
60 Araujo, 2011; Pasini and Link, 2012; Ribeiro Barbosa *et al.*, 2012; Poppe *et al.*, 2014; Benítez Díaz,  
61 2015; Ferreira Mendes *et al.*, 2017; Vasconcelos *et al.*, 2017). In North America *Z. indianus* has  
62 been found at latitudes of 45 to 50°N (Renkema *et al.*, 2013; Holle *et al.*, 2019). Like many  
63 drosophilids, this fly feeds primarily on yeasts and bacteria on decomposing fruits (i.e., is a  
64 secondary pest), and in a specific event it has been reported as a primary pest of fig plants in Brazil  
65 (Vilela *et al.*, 2001). Understanding the factors that promote invasiveness of *Z. indianus* is therefore  
66 of biological and also economic interest. During its invasion into temperate regions of the  
67 Americas, *Z. indianus* has likely faced cooler temperatures and shorter day lengths compared to its  
68 native range in tropical Africa (see Commar *et al.*, 2012 for a review of the invasion process).  
69 Range expansion into high latitudes can impose selective pressures (e.g., due to cooler temperatures  
70 and shorter days in winter) that promote evolution by means of local adaptations in key  
71 physiological traits. For example, *Z. indianus* populations from cold environments in India have  
72 enhanced cold stress tolerance in eggs and pupae compared to populations from warmer areas  
73 (Ramniwas *et al.*, 2012). Invasion processes can also favor environmentally triggered phenotypic  
74 changes (i.e., phenotypic plasticity) in physiological traits. For example, *Z. indianus* acclimated to

75 low temperature and low humidity increase their desiccation resistance and levels of cuticular lipids  
76 (Kalra and Parkash, 2016; Girish et al., 2018). Both, local adaptation and phenotypic plasticity may  
77 facilitate *Z. indianus* range expansion by promoting survival at low temperatures and short  
78 photoperiods during winter at high latitudes.

79 Several studies have shown that fly species respond to winter cold thermal regimes by  
80 increasing cold tolerance (Bergland et al., 2014; Shearer et al., 2016; Mensch et al., 2017; Everman  
81 et al., 2018; Moghadam et al., 2019) or by slowing down or stopping reproductive maturation  
82 (Kimura, 1988; Saunders et al., 1989; Rossi-Stacconi et al., 2016; Mensch et al., 2017; Everman et  
83 al., 2018). Historical geographic origin and distribution influences the extent of cold tolerance and  
84 reproductive responses to winter abiotic conditions in many drosophilid species. For example,  
85 temperate *Drosophila buzzatii* females have a lower critical temperature (c. 11°C) for ovarian  
86 maturation than their tropical counterparts (c. 15°C), i e., tropical species interrupt ovarian  
87 development at higher temperatures (Mensch et al., 2017). Moreover, long-term cold exposure  
88 reduces reproductive capacity of tropical species of the *Drosophila buzzatii* complex, while females  
89 of temperate species enhance their fertility after cold-induced reproductive arrest (Mensch et al.,  
90 2017). Likewise, species originating from high latitudes and altitudes show cold tolerance capacities  
91 that allows them to survive at low temperatures for long periods, including *Drosophila*  
92 *pseudoobscura* (Crumpacker and Marinkovic, 1967), *Drosophila montana* (Vesala and Hoikkala,  
93 2011), *Drosophila suzukii* (Toxopeus et al., 2016), *Drosophila lutescens* and *Drosophila takahashii*  
94 (Fukatami, 1984). *Zaprionus indianus* is not the only drosophilid species that became invasive and  
95 expanded its geographical range, others fly species like *Drosophila melanogaster*, *Drosophila*  
96 *simulans* (Lachaise and Silvain, 2004) and *D. suzukii* (Rossi-Stacconi et al., 2016; Toxopeus et al.,  
97 2016; Stockton et al., 2018) have colonized different areas in the world. Given the characteristics of  
98 low temperature physiology of drosophilids, when range expansion for these species goes from  
99 tropical to temperate areas, key traits such as overwintering survival and fertility after long-term

100 cold exposure are particularly important. Therefore, for a species as *Z. indianus* that extended its  
101 range from tropical to novel temperate environments with cold winters, adaptation and plasticity in  
102 such traits could be crucial to cope with adverse abiotic conditions and in the determination of its  
103 geographic expansion.

104         Testing hypotheses about the underlying causes of biological invasions of a given species  
105 requires comparing organisms that are in different stages of the invasion process (van Kleunen et  
106 al., 2010). Comparison of native range and invasive *Z. indianus* flies can inform our understanding  
107 of patterns, processes and mechanisms relevant to this invasion. To test physiological responses of  
108 *Z. indianus* to winter-like conditions (low temperatures and short days) that could limit its  
109 distribution (da Mata et al., 2010), we studied cold tolerance, ovarian maturation and survival after  
110 long-term exposure to cold and different photoperiods of native (Africa) and invasive range (South  
111 America) wild-derived flies. At the species level, we hypothesized that *Z. indianus* females would  
112 show immature ovaries after long-term exposure to winter-like conditions, but would recover  
113 fecundity following a shift to high temperature, indicating that induced reproductive arrest is  
114 possible and that females will be fecund and fertile (i. e., no fitness reduction as a consequence of  
115 exposure to prolonged winter-like conditions) for this species (similar to other drosophilids). At the  
116 intraspecific level, we hypothesized that females derived from the native range (Africa) would be  
117 more susceptible to low temperatures and winter-like photoperiod than those derived from invaded  
118 areas (South America). Specifically, we predicted that flies from the invasive range would show  
119 larger fertility recovery after prolonged cold exposure than wild-derived females from native range.  
120 In addition, as cold tolerance (measured in different metrics like chill coma temperature (CT<sub>min</sub>),  
121 lethal temperature (LT<sub>50</sub>), chill coma recovery time (CCRT)) strongly correlates with latitudinal  
122 distribution (Kellermann et al., 2012; Ramniwas et al., 2012, Andersen et al., 2015) we predicted  
123 that flies from the invaded range would show larger survival to chill coma and also display a faster  
124 recovery time than flies from the native range, indicating higher thermal tolerance to low

125 temperatures. Altogether these results would imply that survival and reproductive output  
126 maintenance after long-time exposure to challenging abiotic conditions correlate with the  
127 geographic expansion of *Z. indianus* to high latitudes.

128

## 129 **2. Materials and Methods**

130

### 131 *2.1. Insect collection and rearing*

132

133 *Zaprionus indianus* flies were collected from the invaded range in South America and in the native  
134 range in Africa. South American continent flies were collected by authors in Yuto (Province of  
135 Jujuy, Argentina, coordinates: 23° 35' 2.1" S, 61° 51' 1.6" W). African stock was obtained from the  
136 National *Drosophila* Species Stock Center (blogs.cornell.edu/drosophila; DSSC stock number:  
137 50001-1031.02) and were originally collected in Yokadouma (Cameroon, coordinates: 3° 31' 47.9"  
138 N, 15° 3' 14.3" E). Both stocks were set up by massive breeding using the offspring of several *Z.*  
139 *indianus* single gravid female collected in the wild. Thus, these stocks of wild-derived *Z. indianus*  
140 flies represent samples of similar genetic variation. All stocks were maintained by full-sib mating  
141 for more than 10 generations in 300-ml bottles, 4 bottles per stock, with standard fly laboratory  
142 medium of cornmeal-sugar-agar. Density was controlled by maintaining stocks with ~50 adults per  
143 bottle to avoid negative effects of high population density on *Z. indianus* developing larvae (David  
144 *et al.*, 2006). For each locality we obtained the minimum temperature of coldest month (BIO6) from  
145 WorldClim (www.worldclim.org,14/11/2018), data is the average for the years 1970-2000. The  
146 minimum temperature of the coldest month is 17.4°C in Yokadouma (Africa) and 8.3°C in Yuto  
147 (South America). This climatic data shows that wild-derived flies from Yuto (South America)  
148 population in the invaded range went through cold winters while flies from Yokadouma (Africa) in  
149 the native range are derived from a population where no low temperatures were experienced.

150

151 2.2. *Quantification of survival and ovarian maturation in long-term cold exposed females*

152

153 The experimental procedure used was a simplified version of the one described in detail in Mensch  
154 *et al.* (2017). Briefly, groups of ~100 newly emerged (< 6 h post-emergence) *Z. indianus* females  
155 from both stocks were exposed to four different cold treatments (10, 12, 14 and 16°C) for 20 days,  
156 combined with two alternative photoperiods simulating short winter days (10 h:14 h light:dark) and  
157 long summer days (14 h:10 h light:dark). This design resulted in 8 experimental treatments. Two  
158 replicates were performed for each combination of populations and treatments. After 20 days of  
159 cold exposure, all females were removed and survival was scored as the percentage of live females  
160 from the total number of females at the beginning of the cold treatment. For each replicate 15  
161 surviving females were dissected to evaluate the degree of ovarian development. Based on ovarian  
162 condition, we defined two types of females: reproductively arrested (RA) and mature females. RA  
163 females were defined by the presence of only pre-vitellogenic ovarioles in both ovaries (stages 1–7  
164 according to King, 1970), while mature females had at least one stage 8 (vitellogenic) oocyte in  
165 either ovary. Statistical analysis of survival and ovarian arrest under different environmental  
166 conditions were performed applying a logistic regression model with a binomial distribution to the  
167 entire data-set using the *glm* function of *lme4* package (Bates *et al.*, 2015) in R software, version  
168 3.3.1 (R Core Team, 2016). The full model included environmental temperature as a continuous  
169 predictor, origin (Yokadouma from native range in Africa and Yuto from invaded range in South  
170 America) and photoperiod (winter and summer) as fixed factors. Model fit was evaluated using  
171 *DHARMA* package (Hartig, 2019). For ovarian arrest analysis, since the triple interaction was  
172 significant (see supplementary table 1), separate analysis by photoperiod were performed. Multiple  
173 testing was corrected using a conservative Bonferroni correction.

174



## 175 2.3. Fertility measurements

176

177 In order to quantify reproductive recovery in females that went through cold treatment, we compared  
178 fertility scored as the number of adults (and larvae) per female of RA females and control females  
179 maintained at 25°C and 12 h:12 h light:dark photoperiod. Fertility of RA females was scored in flies  
180 exposed to 20 days at 12°C and winter-like photoperiod (10 h:14 h light:dark). For these assays,  
181 females were transferred to new vials at 25°C for three days; after that, all females were  
182 individually crossed with a pair of mature males that were reared at 25°C and 12 h:12 h (light:dark)  
183 photoperiod. Once mating had taken place, individual females were allowed to lay eggs for 5 days  
184 in 2 cm Petri dishes containing agar medium with yeast paste placed at the end of vials.  
185 Oviposition was performed each day on a different vial. Mating vials were kept at 25°C and 12  
186 h:12 h (light:dark) photoperiod. The number of larvae and adult progeny was recorded by visual  
187 inspection of vials daily. This procedure allowed to score fertility as the total number of larvae or  
188 adult progeny per female and also to quantify this parameter daily after cold treatment termination.  
189 The treatment of 12°C and winter-like photoperiod was chosen because such temperature is below  
190 the thermal limit that induce ovarian maturation in *Z. indianus* (see Figure 1) and also had a high  
191 survival (0.8 for Yokadouma and 0.84 for Yuto, Figure 2), which allowed to have a suitable number  
192 of RA females after cold treatment to score fertility. Statistical analysis of the comparison of  
193 fertility of RA females and control females was performed applying a General Linear Model using  
194 the *glm* function of *lme4* package in R software (Bates et al., 2015). The model included origin  
195 (Yokadouma from native range and Yuto from invaded range) and treatment (RA females vs  
196 control females) as fixed factors. Statistical analysis of mean number of larvae and adult progeny  
197 per female over time was performed applying a Generalized Linear Mixed Model with origin and  
198 time (day of quantification after cold treatment termination) as fixed factors and individual female  
199 (replicate) as a random factor. This statistical analysis was performed using the *lmer* function of

200 *lme4* package in R software was used (Bates et al., 2015). A binomial distribution was used in both  
201 analyses. Model fit was evaluated using *DHARMA* package (Hartig, 2019). Pairwise Tukey's post-  
202 hoc comparisons were performed using *emmeans* package (Lenth, 2018).

203

#### 204 2.4. Cold-tolerance assay

205

206 Flies of 10 days of age from each population reared at 25°C and 12 h:12 h (light:dark) photoperiod  
207 were transferred without anesthesia in groups of 10-20 flies to vials set in boxes containing water at  
208 0°C. After 12 h at 0°C flies were allowed to recover at 25°C. Survival to chill coma was scored as  
209 the proportion of live flies from the total number of flies at the beginning of the chill coma  
210 treatment. Recovery from chill coma was individually measured as the time (in minutes) elapsed  
211 until flies could stand up on their legs (David et al., 1998). Statistical analysis of chill coma  
212 recovery time was performed applying a GLM using the *glm* function of *lme4* package in R  
213 software (Bates et al., 2015). The model included origin (Yokadouma from native range in Africa  
214 and Yuto from invaded range in South America) and sex (male and female) as fixed factors. A  
215 sigma distribution was used. Model fit was evaluated using *DHARMA* package (Hartig, 2019).

216

217

### 217 3. Results

218

219 3.1. Critical temperature for ovarian maturation for *Z. indianus* females from native and invaded  
220 ranges

221

222 Ovarian maturation as a function of temperature was fitted to a logistic curve (Figure 1), and from  
223 this equation, we could infer that 50% of all *Z. indianus* females exposed to cold treatments attained  
224 ovarian maturation at a critical temperature of c. 13.3°C. Critical temperature for ovarian

225 maturation is similar between different origins and photoperiod (Figure 1). Nevertheless, statistical  
226 analysis showed that there was a significant photoperiod  $\times$  temperature  $\times$  origin interaction  
227 affecting ovarian maturation (Supplementary Table 1). Thus the combination of photoperiod and  
228 temperature had impacts on ovarian maturation, that were also dependent on the biogeographic  
229 origin of females (Supplementary Table 1). In addition, females from both origins showed ovarian  
230 maturation above the critical temperature, except for a small proportion of females from Yuto  
231 (South America) that still arrested their ovaries at 14°C and 16°C under winter-like photoperiod  
232 (Figure 1, Table 1).

233

### 234 3.2. *Survival following cold treatments for Z. indianus females from native and invaded ranges*

235

236 Temperature, photoperiod and the origin  $\times$  photoperiod interaction affected *Z. indianus* female  
237 survival following 20 days at low temperatures (Table 2). Females exposed to long-term cold  
238 treatments showed high survival (>75%) in all treatments except at 10°C (Table 2, Figure 2). The  
239 lowest survival was found in Yokadouma (Africa) derived females when exposed to 10°C and  
240 winter-like photoperiod (Figure 2). Females from both origins that were exposed to 12°C and  
241 winter-like photoperiod showed high survival (>75%) (Figure 2) and all arrested ovarian  
242 development (Figure 1). These RA females were used to evaluate fertility recovery after cold  
243 treatment.

244

### 245 3.3. *Fertility recovery of reproductively arrested females from native and invaded ranges*

246

247 Reproductively arrested females from both origins maintained fertility following cold recovery,  
248 although females from different origins showed different patterns (Figure 3A). Reproductively  
249 arrested females from Yokadouma (Africa) showed higher fertility than control females not exposed

250 to cold treatments (GLM,  $F_{1,12} = 8.54$ ,  $P = 0.013$ ) while fertility of females from Yuto (South  
251 America) did not differ significantly from control flies (GLM,  $F_{1,9} = 3.1$ ,  $p = 0.077$ ) (Figure 3A).  
252 The mean number of offspring from RA females was 40.83 larvae and 23.00 adults per female from  
253 Yokadouma (Africa) and 32.00 larvae and 16.17 adults per female from Yuto (South America). The  
254 number of offspring (estimated as larvae or adults) for these RA females varied over time following  
255 mating and there also was an interaction with the origin of RA females (Figure 3B, Table 3).  
256 Females from Yokadouma (Africa) had significant larger number of larvae (Tukey contrast,  
257  $estimate_{30,95} = 20.54$ ,  $P = 0.017$ ) and adult progeny (Tukey contrast,  $estimate_{27,96} = 9.74$ ,  $P =$   
258  $0.0082$ ) than Yuto (South America) females 24 h after mating, but this difference disappeared at 48  
259 h (Tukey contrasts, Larvae:  $estimate_{30,95} = -6.67$ ,  $P = 0.863$ ; Adult:  $estimate_{27,96} = 1.64$ ,  $P = 0.986$ )  
260 and 72 h (Tukey contrast, Larvae:  $estimate_{30,95} = -4.75$ ,  $P = 0.964$ ; Adult:  $estimate_{27,96} = -2.45$ ,  $P =$   
261  $0.926$ ) after mating (Figure 3B).

262

### 263 3.4. Chill coma recovery time of *Z. indianus* flies from native and invaded ranges

264

265 The proportion of flies that survived following chill coma (12 h at 0°C) was 0.31 for Yokadouma  
266 (Africa) flies and 0.66 for flies from Yuto (South America). Chill coma recovery time of *Z.*  
267 *indianus* flies was affected by the geographical origin and by the sex of flies (Table 4, Figure 4).  
268 Wild-derived flies from Yuto (South America) recovered faster from chill coma than flies from  
269 Yokadouma (Africa), with median recovery times of 101 minutes and 126 minutes, respectively.  
270 For both origins, females (median = 103 minutes) recovered faster than males (median = 129.5).  
271 These results suggest that flies from the invaded range had higher cold tolerance than flies from the  
272 native range.

273

274

## 4. Discussion

275

276 In the present study we report that *Z. indianus* females are able to arrest ovarian development when  
277 exposed to long-term cold exposure, indicating that cold-induced reproductive arrest is possible for  
278 this species, similar to other drosophilids (Vesala and Hoikkala, 2011; Kubrak et al., 2014;  
279 Toxopeus et al., 2016; Mensch et al., 2017). We also determined that the critical temperature to  
280 induce ovarian maturation for this species is c. 13.3°C, a value for this physiological parameter that  
281 is larger than the values for temperate drosophilids (11°C) and slightly lower than the thermal limit  
282 of tropical drosophilids (14-15°C) (Mensch et al., 2017). We demonstrated that *Z. indianus*  
283 reproductively arrested females maintained fertility following recovery from challenging abiotic  
284 conditions, and therefore does not experience a drastic fitness reduction as a consequence of low  
285 temperature exposure. Thus, although *Z. indianus* originated in tropical areas, it can display  
286 reproductive overwintering characteristics similar to temperate species. These characteristics may  
287 facilitate *Z. indianus* invasion to high latitudes with temperate and cold climates, since the ability to  
288 tolerate challenging abiotic conditions may determine the spread potential of any given species  
289 (Ramniwas et al., 2012; Kalra and Parkash, 2016; Girish et al., 2018).

290 In addition to species-level responses to winter-like conditions, we expected to find  
291 intraspecific differences between *Z. indianus* from native and invaded range for overwintering  
292 traits. Intraspecific differences in these traits could be indicative of adaptations or phenotypic  
293 plasticity in invaded range populations. In effect, we found that wild-derived females from Yuto  
294 (South America) responded to a winter (short day) photoperiod by slowing down ovarian  
295 maturation at low but permissive temperatures of 14°C and 16°C; and also delayed the start of  
296 oviposition after cold treatment (oviposition peak at 48 hs). These results could be signs of a deeper  
297 dormancy-like response only for Yuto females. Also, a greater proportion of Yuto flies survived  
298 chill coma than flies from Yokaouma and the former recovered more quickly from chill coma as  
299 well, indicating that flies from colder climates presented larger survival to chill coma treatment and

300 also faster chill coma recovery times, i. e. higher cold tolerance in the invasive population.

301 Enhanced cold tolerance in the South American flies could be interpreted as local adaptation to

302 colder climate, in any case this results show that cold tolerance may be part of a physiological

303 responses that facilitate expansion of *Z. indianus* into cold areas. However, we also found opposing

304 results regarding local adaptations in the invaded population in terms of fertility recovery after long-

305 term cold exposure, since native range flies from Yokadouma (Africa) showed higher fertility

306 recovery than females from Yuto (South America). Also, females from native and invaded range

307 show very similar critical temperatures for ovarian maturation, which means that invaded range

308 females are not being less affected in ovarian maturation by challenging abiotic conditions of low

309 temperatures and winter-like photoperiod. Ovarian maturation shows latitudinal cline variation

310 among natural populations of other drosophilid species, indicating that this trait could be related to

311 adaptation to temperate environments (Schmidt et al., 2005). However, this has not been the case

312 for the pattern found in *Z. indianus*. We speculate that while enhanced cold tolerance may facilitate

313 invasion of *Z. indianus* to southern areas of South America, limited adaptation or plasticity of the

314 reproductive arrest response may limit this expansion. It should be noted that differences in the

315 phenotypic values of traits among flies from different origins that have different environments are

316 indicative of the action of natural selection that produces local adaptation; but as Reznick & Travis

317 (1996) points out, this evidence can only be taken as a first step for proving adaptation in the wild.

318 Also, this interpretation is based only on two populations collected in the wild, therefore the

319 generality of the indications of adaptation must be further confirmed by expanding the number of

320 samples of both origins, or by conducting reciprocal transplant experiments (e.g., Pelini et al.,

321 2009). Another possible caveat that should be mentioned is the possibility that laboratory adaptation

322 or inbreeding could have impacted our traits estimates. However, given that the populations

323 analyzed were maintained at large population sizes and controlled environmental laboratory

324 conditions (standard laboratory medium, temperature, photoperiod, humidity) the possibility that

325 these laboratory processes have differentially affected the stocks is low. Also, several studies have  
326 shown that for drosophilids these factors affect intraspecific variation less than interspecific  
327 variation (Andersen et al., 2015; Maclean et al., 2018).

328 It has been widely shown that day length is an anticipatory and reliable cue of seasonal  
329 change, even more than environmental temperature. Thus, physiological response to changes in day  
330 length could be relevant in the biology of organisms facing seasonal changes. In flies, the fact that  
331 photoperiod has a role in the modulation of trait expression could be indicative of flies adaptive  
332 response to life at high latitudes (Bradshaw & Holzapfel, 2010). We found that photoperiod  
333 modulated *Z. indianus* ovarian arrest, since invaded range females slowed down ovarian maturation  
334 and delayed the start of oviposition after cold treatment only under the winter (short day)  
335 photoperiod. Thus, *Z. indianus* is similar to other drosophilid species, whose thermal resistance and  
336 life history traits are affected by photoperiod (Lanciani et al., 1990; Lanciani et al., 1992; Hori and  
337 Kimura, 1998; Sørensen and Loeschcke, 2002; Hoffmann et al., 2005; Vesala and Hoikkala,  
338 2011; Vesala et al., 2012; Bauerfeind et al., 2014; Moghadam et al., 2019). Photoperiod is also a  
339 relevant abiotic factor that could have an impact on *Z. indianus* overwintering survival and fertility,  
340 especially considering the invasion process of this species towards high latitudes to the south of the  
341 South American continent where there are marked seasonal fluctuations of day length.

342

343

## 5. Conclusions

344

345 We identified a set of overwintering traits that could facilitate geographical expansion of *Z.*  
346 *indianus* to southern latitudes of South America. *Z. indianus* likely does not experience a drastic  
347 reduction in fitness in temperate climates with cold winters, given that females show ovarian arrest  
348 followed by a recovery in fertility when exposed to challenging abiotic conditions. We found mixed  
349 evidence both for and against scenarios of local adaptations or phenotypic plasticity in the invaded

350 range; and also showed that photoperiod acted as modulator of ovarian arrest. With respect to  
351 overwintering mechanisms, *Z. indianus* seems to have characteristics of both tropical and temperate  
352 species. Overall, variation in reproductive and cold tolerance physiology may enable (and limit)  
353 geographical expansion of *Z. indianus* to high latitudes of the American continent.

354

355

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356

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### References

365

- 366 Andersen, J.L., Manenti, T., Sørensen, J.G., MacMillan, H.A., Loeschcke, V., Overgaard, J., 2015.  
367 How to assess *Drosophila* cold tolerance: chill coma temperature and lower lethal temperature are  
368 the best predictors of cold distribution limits. *Funct. Ecol.* 29, 55-65.
- 369 Bauerfeind, S.S., Kellermann, V., Moghadam, N.N., Loeschcke, V., Fischer, K., 2014. Temperature  
370 and photoperiod affect stress resistance traits in *Drosophila melanogaster*. *Physiol. Entomol.* 39,  
371 237-246.
- 372 Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using  
373 *lme4*. *J. Stat. Sof.* 67, 1-48.



- 374 Benítez Díaz, E.A., 2015. Primer registro de la Mosca Africana del Higo, *Zaprionus indianus*  
375 (Diptera: Drosophilidae) en Paraguay. B. Mus. Nac. Hist. Nat. Par. 19, 100-110.
- 376 Bergland, A.O., Behrman, E.L., O'Brien, K.R., Schmidt, P.S., Petrov, D.A., 2014. Genomic  
377 evidence of rapid and stable adaptive oscillations over seasonal time scales in *Drosophila*. PLoS  
378 Genet. 10, e1004775.
- 379 Bitner-Mathé, B.C., David, J.R., 2015. Genetic variability and phenotypic plasticity of metric  
380 thoracic traits in an invasive drosophilid in America. Genetica. 143, 441-451.
- 381 Bradshaw, W.E., Holzapfel, C.M., 2010. Insects at not so low temperature: climate change in the  
382 temperate zone and its biotic consequences, in: Denlinger, D.L., Lee, R.E. (Eds.), Low Temperature  
383 Biology of Insects. Cambridge University Press, Cambridge, pp. 242-275.
- 384 Castro, F.L., Valente, V.L.S., 2001. *Zaprionus indianus* invading communities in the southern  
385 Brazilian city of Porto Alegre. Dros. Inf. Serv. 84, 15-17.
- 386 Chaves, N.B., Tidon, R., 2008. Biogeographical aspects of drosophilids (Diptera, Drosophilidae) of  
387 the Brazilian savanna. Rev. Bras. Entomol. 52, 340-348.
- 388 Commar, L.S., Galego L.G., Ceron, C.R., Carareto, C.M.A., 2012. Taxonomic and evolutionary  
389 analysis of *Zaprionus indianus* and its colonization of Palearctic and Neotropical regions. Genet.  
390 Mol. Biol. 35, 395-406.
- 391 Crumpacker, D., Marinkovic, D., 1967. Preliminary evidence of cold temperature resistance in  
392 *Drosophila pseudoobscura*. Ame. Nat. 101, 505-514.
- 393 da Mata, R.A., Tidon, R., Côrtes, L.G., De Marco, P., Diniz-Filho, J.A.F., 2010. Invasive and  
394 flexible: niche shift in the drosophilid *Zaprionus indianus* (Insecta, Diptera). Biol. Invasions. 12,  
395 1231-1241.
- 396 David, J.R., Araripe, L.O., Bitner-Mathé, B.C., Capy, P., Goñi, B., Klaczko, L.B., Legout, H.,  
397 Martins, M.B., Vouidibio, J., Yassin, A., Moreteau, B., 2006. Sexual dimorphism of body size and

398 sternopleural bristle number: a comparison of geographic populations of an invasive cosmopolitan  
399 drosophilid. *Genetica*. 128, 109-122.

400 David, R.J., Gibert, P., Pla, E., Petavy, G., Karan, D., Moreteau, B., 1998. Cold stress tolerance in  
401 *Drosophila*: analysis of chill coma recovery in *D. melanogaster*. *J. Therm. Biol.* 23, 291-299.

402 De Toni, D.C., Hofmann, P.R.P., Valente, V.L.S., 2001. First record of *Zaprionus indianus*  
403 (Diptera, Drosophilidae) in the State of Santa Catarina, Brazil. *Biotemas*. 14, 71-85.

404 Everman, E.R., Freda, P.J., Brown, M., Schieferecke, A.J., Ragland, G.J., Morgan, T.J., 2018.  
405 Ovary development and cold tolerance of the invasive pest *Drosophila suzukii* (Matsumura) in the  
406 central plains of Kansas, United States. *Environ. Entomol.* 47, 1013-1023.

407 Fernandes Rodrigues, D.R., Araújo, E.L., 2011. Ocorrência de *Zaprionus indianus* gupta (Diptera:  
408 Drosophilidae) em frutos de juazeiro *Ziziphus joazeiro* Mart. (rhamnaceae) no estado do Rio  
409 Grande do Norte. *Rev. Bras. Frutic.* 33, 1356-1358.

410 Ferreira Mendes, M., Berti Valer, F., Aleixo Vieira, J.G., Laner Blauth, M., Gottschalk, M.S., 2017.  
411 Diversity of Drosophilidae (Insecta, Diptera) in the Restinga forest of southern Brazil. *Rev. Bras.*  
412 *Entomol.* 61, 248-256.

413 Fukatami, A., 1984. Cold temperature resistance in *Drosophila lutescens* and *D. takahashii*. *Jpn. J.*  
414 *Genet.* 59, 61-70.

415 Furtado, I.S., Martins, M.B., Costa, J.E., 2009. First record of *Zaprionus indianus* (Diptera:  
416 Drosophilidae) in the Urucu Petroleum Province in Amazonas, Brazil. *Dros. Inf. Serv.* 92, 17-18.

417 Girish, T.N., Pradeep, B.E., Parkash, R., 2018. Heat- and humidity-induced plastic changes in body  
418 lipids and starvation resistance in the tropical fly *Zaprionus indianus* during wet and dry seasons. *J.*  
419 *Exp. Biol.* 221, jeb174482.

420 Goñi, B., Fresia, P., Calviño, M., Ferreiro, M.J., Valente, V.L.S., 2001. First record of *Zaprionus*  
421 *indianus* Gupta, 1970 (Diptera, Drosophilidae) in southern localities of Uruguay. *Dros. Inf. Serv.*  
422 84, 61-65.

- 423 Goñi, B., Martinez, M.E., Techera, G., Fresia, P., 2002. Increased frequencies of *Zaprionus*  
424 *indianus* Gupta, 1970 (Diptera, Drosophilidae) in Uruguay. Dros. Inf. Serv. 85, 75-80.
- 425 Gupta, J.P., 1970. Description of a new species of *Phorticella* and *Zaprionus* (Drosophilidae) from  
426 India. P. Indian Nat. Sci. Acad. B. 36, 62-70.
- 427 Hartig, F., 2019. DHARMA: Residual Diagnostics for Hierarchical (Multi-Level / Mixed).  
428 Regression Models. R package version 0.2.1. <https://CRAN.R-project.org/package=DHARMA>
- 429 Hoffmann, A.A., Scott, M., Partridge, L., Hallas, R., 2003. Overwintering in *Drosophila*  
430 *melanogaster*: outdoor field cage experiments on clinal and laboratory selected populations help to  
431 elucidate traits under selection. J. Evolution. Biol. 16, 614-623.
- 432 Hoffmann, A. A., Shirriffs, J., Scott, M., 2005. Relative importance of plastic vs genetic factors in  
433 adaptive differentiation: Geographical variation for stress resistance in *Drosophila melanogaster*  
434 from eastern Australia. Funct. Ecol. 19, 222-227.
- 435 Holle, S. G., Tran, A. K., Burkness, E. C., Ebbenga, D. N., Hutchison, W. D., 2019. First detections  
436 of *Zaprionus indianus* (Diptera: Drosophilidae) in Minnesota. J. Entomol. Sci. 54, 99-102.
- 437 Hori, Y., Kimura, M.T., 1998. Relationship between cold stupor and cold tolerance in *Drosophila*  
438 (Diptera: Drosophilidae). Environ. Entomol. 27, 1297-1302.
- 439 Izquierdo, J.I., 1991. How does *Drosophila melanogaster* overwinter?. Entomol. Exp. Appl. 59, 51-  
440 58.
- 441 Karan, D., Moreteau, B., David, J.R., 1999. Growth temperature and reaction norms of  
442 morphometrical traits in a tropical drosophilid: *Zaprionus indianus*. Heredity. 83, 398-407.
- 443 Kalra, B., Parkash, R., 2016. Effects of saturation deficit on desiccation resistance and water  
444 balance in seasonal populations of the tropical drosophilid *Zaprionus indianus*. J. Evolution. Biol.  
445 219, 3237-3245.

- 446 Kalra, B., Tamang, A. M., Parkash, R., 2017. Cross-tolerance effects due to adult heat hardening,  
447 desiccation and starvation acclimation of tropical drosophilid-*Zaprionus indianus*. Comp. Biochem.  
448 Phys. A. 209, 65-73.
- 449 Kato, C.M., Foureaux, L.V., César, R.A., Torres, M.P., 2004. Occurrence of *Zaprionus indianus*  
450 Gupta, 1970 (Diptera: Drosophilidae) in Minas Gerais State. Ciênc Agrotec. 28, 454-455.
- 451 Keller, A., 2007. *Drosophila melanogaster's* history as a human commensal. Curr. Biol. 17, R77-  
452 R81.
- 453 Kellermann, V., Overgaard, J., Hoffmann, A. A., Fløjgaard, C., Svenning, J. C., Loeschcke, V.,  
454 2012. Upper thermal limits of *Drosophila* are linked to species distributions and strongly  
455 constrained phylogenetically. Proc. Natl. Acad. Sci. USA, 109, 16228-16233.
- 456 Kimura, M.T., 1988. Adaptations to temperate climates and evolution of overwintering strategies in  
457 the *Drosophila melanogaster* species group. Evolution. 42, 1288-1297.
- 458 King, R.C., 1970. Ovarian development in *Drosophila melanogaster*. Academic Press, New York.
- 459 Lachaise, D., Silvain, J.F., 2004. How two afrotropical endemics made two cosmopolitan human  
460 commensals: the *Drosophila Melanogaster*-*D. Simulans* palaeogeographic riddle. Genetica. 120,  
461 17-39.
- 462 Kubrak, O.I., Kucerova, L., Theopold, U., Nassel, D.R., 2014. The sleeping beauty: how  
463 reproductive diapause affects hormone signaling, metabolism, immune response and somatic  
464 maintenance in *Drosophila melanogaster*. PLoS ONE. 9, e113051.
- 465 Lanciani, C.A., Giesel, J.T., Anderson, J.F., Emerson, S.S., 1990. Photoperiod-induced changes in  
466 metabolic response to temperature in *Drosophila melanogaster* Meigen. Funct. Ecol. 4, 41-45.
- 467 Lanciani, C.A., Lipp, K.E., Giesel, J. T., 1992. The effect of photoperiod on cold tolerance in  
468 *Drosophila melanogaster*. J. Therm. Biol. 17, 147-148.

- 469 Lavagnino, N.J., Carreira, V.P., Mensch, J., Hasson, E., Fanara, J.J., 2008. Geographic distribution  
470 and hosts of *Zaprionus indianus* (Diptera: Drosophilidae) in North-Eastern Argentina. Rev. Soc.  
471 Entomolo. Arg. 67, 189-192.
- 472 Leao, B.F.D., Tidon, R., 2004. Newly invading species exploiting native host-plants: the case of the  
473 African *Zaprionus indianus* (Gupta) in the Brazilian Cerrado (Diptera, Drosophilidae). Ann. Soc.  
474 Entomol. Fr. 40, 285-290.
- 475 Lenth, R., 2018. emmeans: Estimated Marginal Means, aka Least-Squares Means. R package  
476 version 1.3.1. <https://CRAN.R-project.org/package=emmeans>
- 477 Loh, R., Bitner-Mathé, B.C., 2005. Variability of wing size and shape in three populations of a  
478 recent Brazilian invader, *Zaprionus indianus* (Diptera: Drosophilidae), from different habitats.  
479 Genetica. 125, 271-281.
- 480 Loh, R., David, J.R., Debat, V., Bitner-Mathé, B.C., 2008. Adaptation to different climates results  
481 in divergent phenotypic plasticity of wing size and shape in an invasive drosophilid. J. Genet. 87,  
482 209-217.
- 483 Maclean, H.J., Kristensen, T.N., Sørensen, J.G., Overgaard, J., 2018. Laboratory maintenance does  
484 not alter ecological and physiological patterns among species: a *Drosophila* case study. J. Evol.  
485 Biol. 31, 530-542.
- 486 Mensch, J., Hurtado, J., Zermoglio, P.F., de la Vega, G., Rolandi, C., Schilman, P.E., Markow,  
487 T.A., Hasson, E., 2017. Enhanced fertility and chill tolerance after cold-induced reproductive arrest  
488 in females of temperate species of the *Drosophila buzzatii* complex. J. Exp. Biol. 220, 713-721.
- 489 Mitrovski, P., Hoffmann, A.A., 2001. Postponed reproduction as an adaptation to winter conditions  
490 in *Drosophila melanogaster*: evidence for clinal variation under semi-natural conditions. P. Roy.  
491 Soc. B- Biol. Sci. 268, 2163-2168.

- 492 Moghadam, N.N., Kurbalija Novicic, Z., Pertoldi, C., Kristensen, T.N., Bahrndorff, S. 2019. Effects  
493 of photoperiod on life history and thermal stress resistance traits across populations of *Drosophila*  
494 *subobscura*. Ecol. Evol. 9, 2743-2754.
- 495 Oliveira, G.F., de Melo, K.P.S., Garcia, A.C.L., Rohde, C., 2009. First record of *Zaprionus*  
496 *indianus* (Diptera, Drosophilidae) in Fernando de Noronha, an Oceanic Island of Pernambuco State,  
497 Brazil. Dros. Inf. Serv. 92, 18-20.
- 498 Pasini, M.P.B., Link, D., 2011. Efficiency of different traps to capture *Zaprionus indianus* (Diptera:  
499 Drosophilidae) in fig orchard in Santa Maria county, Rio Grande do Sul state, Brazil. Int. J. Agr.  
500 Soil Sci. 1, 349-354.
- 501 Pelini, S.L., Dzurisin, J.D.K., Prior, K.M., Williams, C.M., Marisco, T.D., Sinclair, B.J., Hellmann,  
502 J.J., 2009. Translocation experiments with butterflies reveal limits to enhancement of poleward  
503 populations under climate change. Proceedings of the National Academy of Sciences USA. 106,  
504 11160-11165.
- 505 Poppe, J.L., Schmitz, H.J., Grimaldi, D., Da Silva Valente V.L., 2014. High diversity of  
506 Drosophilidae (Insecta, Diptera) in the Pampas Biome of South America, with descriptions of new  
507 *Rhinoleucophenga* species. Zootaxa. 3779, 215-245.
- 508 Ramniwas, S., Kajla B., Parkash, R., 2012. Extreme physiological tolerance leads the wide  
509 distribution of *Zaprionus indianus* (Diptera: Drosophilidae) in temperate world. Acta Entomol.  
510 Sinica. 55, 1295-1305.
- 511 Renkema, J. M., Miller, M., Fraser, H., Légaré, J. P. H., Hallett, R. H., 2013. First records of  
512 *Zaprionus indianus* Gupta (Diptera: Drosophilidae) from commercial fruit fields in Ontario and  
513 Quebec, Canada. J. ent. Soc. Ont. 144, 125-130.
- 514 Reznick, D., Travis, J., (1996). The empirical study of adaptation in natural populations, in: Rose,  
515 M. R., Lauder, G. V. (Eds.), Adaptation. Academic Press, San Diego, pp. 243-289.

516 Ribeiro Barbosa, M.R., Gracioli, G., Paiva, F., 2012. Diptera, Drosophilidae, *Zaprionus indianus*  
517 Gupta, 1970: distribution extension for the state of Mato Grosso do Sul, Brazil. Check List. 8, 175-  
518 176.

519 R Core Team, 2016. A language and environment for statistical computing. [http://www.R-](http://www.R-project.org/)  
520 [project.org/](http://www.R-project.org/)

521 Rossi-Stacconi, M.V., Kaur, R., Mazzoni, V., Ometto, L., Grassi, A., Gottardello, A., Rota-Stabelli,  
522 O., Anfora, G., 2016. Multiple lines of evidence for reproductive winter diapause in the invasive  
523 pest *Drosophila sukuzii*: useful clues for control strategies. J. Pest Sci. 89, 689-700.

524 Santos, J., Rieger, T., Campos, S., Nascimento, A., Félix, P., Silva, S., Freitas, F.M.R., 2003.  
525 Colonization of northeast region of Brazil by the drosophilid flies *Drosophila malerkotliana* and  
526 *Zaprionus indianus*, a new potential insect pest for Brazilian fruticulture. Dros. Inf. Serv. 86, 83-  
527 125.

528 Saunders, D.S., Henrich, V.C., Gilbert, L.I., 1989. Induction of diapause in *Drosophila*  
529 *melanogaster*: photoperiodic regulation and the impact of arrhythmic clock mutations on time  
530 measurement. P. Natl. Acad. Sci. USA. 86, 3748-3752.

531 Schmidt, P.S., Matzkin, L., Ippolito, M., Eanes, W. F., 2005. Geographic variation in diapause  
532 incidence, life-history traits, and climatic adaptation in *Drosophila melanogaster*. Evolution. 59,  
533 1721-1732.

534 Schou, M.F., Loeschcke, V., Kristensen, T.N., 2015. Strong costs and benefits of winter  
535 acclimatization in *Drosophila melanogaster*. PLoS ONE. 10, e0130307.

536 Shearer, P.W., West, J.D., Walton, V.M., Brown, P.H., Svetec, N., Chiu, J.C., 2016 Seasonal cues  
537 induce phenotypic plasticity of *Drosophila sukuzii* to enhance winter survival. BMC Ecol. 16, 11.

538 Sørensen, J.G., Loeschcke, V., 2002. Natural adaptation to environmental stress via physiological  
539 clock-regulation of stress resistance in *Drosophila*. Ecol. Lett. 5, 16-19.

- 540 Soto, I., Corio, C., Fanara, J.J., Hasson, E., 2006. First record of *Zaprionus indianus* Gupta 1970  
541 (Diptera, Drosophilidae) in Argentina. *Dros. Inf. Serv.* 89, 13-14.
- 542 Stockton, D.G., Wallingford, A.K., Loeb, G.M., 2018. Phenotypic plasticity promotes  
543 overwintering survival in a globally invasive crop pest, *Drosophila suzukii*. *Insects.* 9, 105.
- 544 Tidon, R., Leite, D.F., Ferreira Dobbin Leao, B., 2003. Impact of the colonization of *Zaprionus*  
545 *indianus* (Diptera, Drosophilidae) in different ecosystems of the Neotropical Region: 2 years after  
546 the invasion. *Biol. Conserv.* 112, 299-305.
- 547 Toxopeus, J., Jakobs, R., Ferguson, L.V., Garipey, T.D., Sinclair, B.J., 2016. Reproductive arrest  
548 and stress resistance in winter-acclimated *Drosophila suzukii*. *J. Insect Physiol.* 89, 37-51.
- 549 van Kleunen, M., Dawson, W., Schlaepfer, D., Jeschke, J.M., Fischer, M., 2010. Are invaders  
550 different? A conceptual framework of comparative approaches for assessing determinants of  
551 invasiveness. *Ecol. Lett.* 13, 947-958.
- 552 Vasconcelos, A., Azevedo, T., Santos, R.S., 2017. Primeiro registro de *Zaprionus indianus* Gupta  
553 (Diptera: Drosophilidae) no Estado do Acre. *EntomoBrasilis.* 10, 0-63.
- 554 Vesala, L., Hoikkala, A., 2011. Effects of photoperiodically induced reproductive diapause and cold  
555 hardening on the cold tolerance of *Drosophila montana*. *J. Insect Physiol.* 57, 46-51.
- 556 Vesala, L., Salminen, T. S., Kankare, M., Hoikkala, A., 2012 Photoperiodic regulation of cold  
557 tolerance and expression levels of *regucalcin* gene in *Drosophila montana*. *J. Insect Physiol.* 58,  
558 704-709.
- 559 Vilela, C.R., 1999. Is *Zaprionus indianus* Gupta, 1970 (Diptera: Drosophilidae) currently  
560 colonizing the Neotropical region? *Dros. Inf. Serv.* 82, 37-39.
- 561 Vilela, C.R., Teixeira, E.P., Stein, C.P., 2001. Mosca africana do figo, *Zaprionus indianus* (Diptera:  
562 Drosophilidae), in Vilela, E.F., Zucchi, R.A. & Cantor, F. (Eds.), *Historico e impacto das pragas*  
563 *introduzidas no Brasil*. Holos, Ribeirao Preto, Brasil, pp. 48-52.



564 Yassin, A., Abou-Youssef, A.Y., Bitner-Mathé, B.C., Capy, P., David, J.R., 2007. Developmental  
565 stress in wild-living drosophilids inferred from biometry: Metric and meristic traits react differently  
566 to heterogeneous environmental conditions. *Ecol. Entomol.* 32, 698-706.

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567 **Table 1.** Logistic regression analysis of environmental factors (temperature and photoperiod)  
 568 affecting reproductive arrest in *Z. indianus* flies from different biogeographic origin (native and  
 569 invaded range). The analysis for winter-like photoperiod and summer-like photoperiod are shown  
 570 separately since the full model presented significant interactions (see supplementary table 1).  
 571 Significance threshold factor using a Bonferroni correction was  $P < 0.0015$ .

Effect	Winter		Summer	
	Estimate ( $X^2$ )	P	Estimate ( $X^2$ )	P
Temperature	195.35	<0.0015	197.58	<0.0015
Origin	14.92	<0.0015	1.53	0.216
Temperature $\times$ Origin	17.48	<0.0015	2.92	0.087

572

573

574 **Table 2.** Logistic regression analysis of environmental factors (temperature and photoperiod)  
 575 affecting survival after cold treatments in *Z. indianus* populations from different biogeographic  
 576 origins (native and invaded range). Non-significant interactions are not shown.

Effect	Estimate ( $X^2$ )	P
Temperature	186.91	<0.001
Origin	0.06	0.8
Photoperiod	12.27	<0.001
Origin $\times$ Photoperiod	6.56	0.01

577

578

579 **Table 3.** Generalized Linear Mixed Model analysis of the comparison of fertility of cold induced  
 580 RA females of *Z. indianus* from Yokadouma (Africa) in the native range and Yuto (South America)  
 581 in the invaded range (origin effect) at different times after mating (time effect). Fertility was  
 582 estimated as the number of larvae and adults per female.

Larvae per females	Adults per female
--------------------	-------------------

Effect	Estimate ( $X^2$ )	P	Estimate ( $X^2$ )	P
Origin	0.81	0.3	1.64	0.2
Time	14.26	<0.001	26.99	<0.001
Origin $\times$ Time	13.5	0.0012	14.33	<0.001

583

584

585 **Table 4.** General Linear Model analysis of the comparison of chill coma recovery time of females  
 586 and males (sex effect) flies from Yokadouma (Africa) in the native range (N=17) and Yuto (South  
 587 America) in the invaded range (N=59) (origin effect).

588

Effect	Estimate ( $X^2$ )	P
Origin	11.62	<0.001
Sex	10.89	<0.001
Origin $\times$ Sex	0.003	0.95

589

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590 **Fig. 1.** Ovarian maturation for *Z. indianus* females from Yokadouma (Africa) and Yuto (South  
591 America) in winter (left panel) and summer (right panel) photoperiods. Ovarian maturation was  
592 scored as the presence of at least one stage 8 (vitellogenic) oocyte in either ovary. Error bars are  
593 standard deviations. Vertical dashed lines indicate the thermal threshold for the induction of ovarian  
594 maturation. Yokadouma (Africa) flies showed 50% induction of ovarian maturation at 13.34°C in  
595 winter-like photoperiod and 13.01°C in summer-like photoperiod, whereas Yuto (South America)  
596 flies reached 50% ovarian maturation at 13.58°C in winter-like photoperiod and 13.34°C in  
597 summer-like photoperiod.

598

599 **Fig. 2.** Survival following 20 day cold treatments for *Z. indianus* females from Yokadouma (Africa)  
600 and Yuto (South America) in winter (left panel) and summer (right panel) photoperiods. Survival  
601 was measured as the percentage of live females from the total number of females that started cold  
602 treatments. Error bars are standard deviations.

603

604 **Fig. 3.** Fertility recovery of reproductively arrested *Z. indianus* females from Yokadouma (Africa)  
605 and Yuto (South America). (A) Number of adult offspring obtained from reproductively-arrested  
606 (RA) females exposed to cold treatment (dark gray) and control mature females not exposed to cold  
607 treatment (light gray) for Yokadouma (Africa) (N=7) and Yuto (South America) (N=8) populations  
608 of *Z. indianus*. Data are the mean ( $\pm$ s.e.m.) number of adult progeny derived from single females.

609 (B) Fertility recovery of reproductively arrested *Z. indianus* across days after mating. The mean  
610 ( $\pm$ s.e.m.) number of larvae and adults per female was determined from vials containing eggs laid  
611 24, 48, and 72 h after mating. Left panel shows fertility measured as number of larvae per female  
612 and right panel shows fertility measured as number of adult offspring per female. \*\*P<0.05.

613

614 **Fig. 4.** Chill coma recovery time (in minutes) of wild-derived *Z. indianus* flies from Yokadouma  
615 (Africa) and Yuto (South America). Sample sizes were N=17 for Yokadouma (Africa) and N=59

616 for Yuto (South America). Boxplot lines represent the median and box boundaries are the upper and  
617 lower quartiles (e.g. 25th percentiles). Error bars represent the maximum and minimum values.  
618 \*\*P<0.05.

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619 **Appendix A**

620

621 **Supplementary Table 1.** Logistic regression analysis of environmental factors (temperature and  
622 photoperiod) affecting reproductive arrest in *Z. indianus* populations from different biogeographic  
623 origin (native and invaded range).

624

Effect	Estimate ( $X^2$ )	P-value
Temperature	392.03	<0.001
Origin	5.06	0.0244
Photoperiod	9.20	0.0024
Temperature × Origin	3.58	0.0584
Temperature × Photoperiod	1.21	0.271
Origin × Photoperiod	8.43	0.0037
Temperature × Origin × Photoperiod	16.83	<0.001

625

626 **Vitae**

627

628 **Nicolás José Lavagnino.** Research Associate at the Department of Ecology, Genetics and  
629 Evolution at University of Buenos Aires, Argentina and at the National Council of Scientific and  
630 Technical Research (CONICET), Argentina. B.S. in Biological Sciences and Ph.D. in Biology at  
631 the University of Buenos Aires, Argentina. Scientific interests on patterns, processes and  
632 mechanisms of biological invasions using different species of drosophilids as models organisms.  
633 Uses methodological approaches ranging from ecophysiology to ecological evolutionary ecology  
634 and molecular evolution.

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636 **Juan José Fanara.** Principal Investigator and Associate Professor at the Department of Ecology,  
637 Genetics and Evolution at University of Buenos Aires, Argentina and at the National Council of  
638 Scientific and Technical Research (CONICET, Argentina). B.S. in Biological Sciences and Ph.D. in  
639 Biology at the University of Buenos Aires, Argentina. Postdoctoral fellow at Mackay Lab in North  
640 Carolina State University, USA. Scientific interests on processes and mechanisms related to  
641 adaptation, mainly in natural populations, using different species of drosophilids as models  
642 organisms for the investigations.

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644 **Julián Mensch.** Research Associate at the Department of Ecology, Genetics and Evolution at  
645 University of Buenos Aires, Argentina and at the National Council of Scientific and Technical  
646 Research (CONICET), Argentina. B.S. in Biological Sciences and Ph.D. in Biology at the  
647 University of Buenos Aires, Argentina. Scientific interests on how the ectotherms deal with  
648 seasonality in terms of growth, survival and reproduction. Uses methodological approaches ranging  
649 from ecophysiology to evolutionary and molecular biology with aims of reaching an integrated  
650 understanding of climatic adaptations at different organization levels.



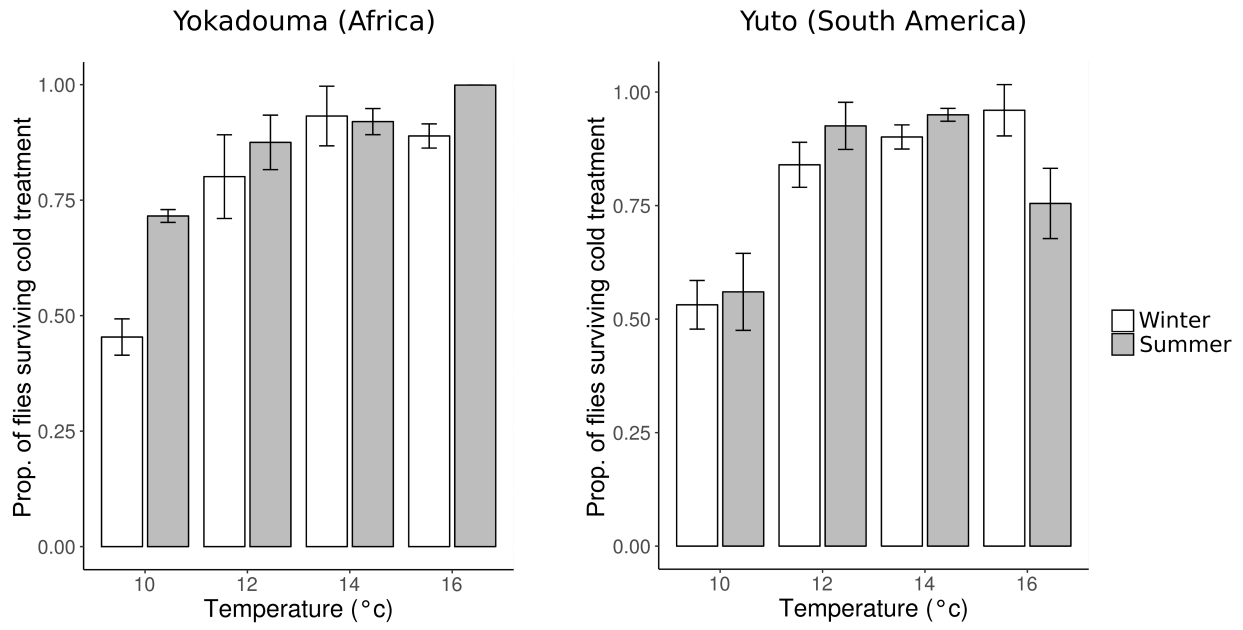
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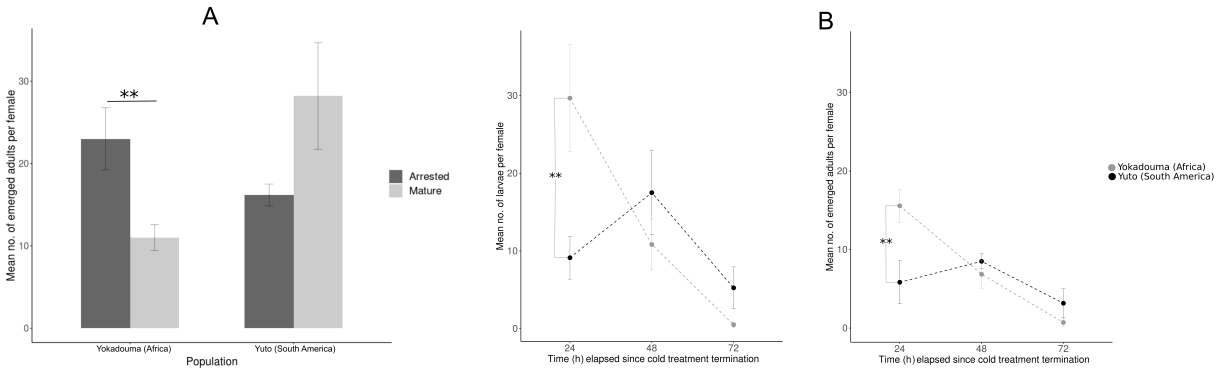




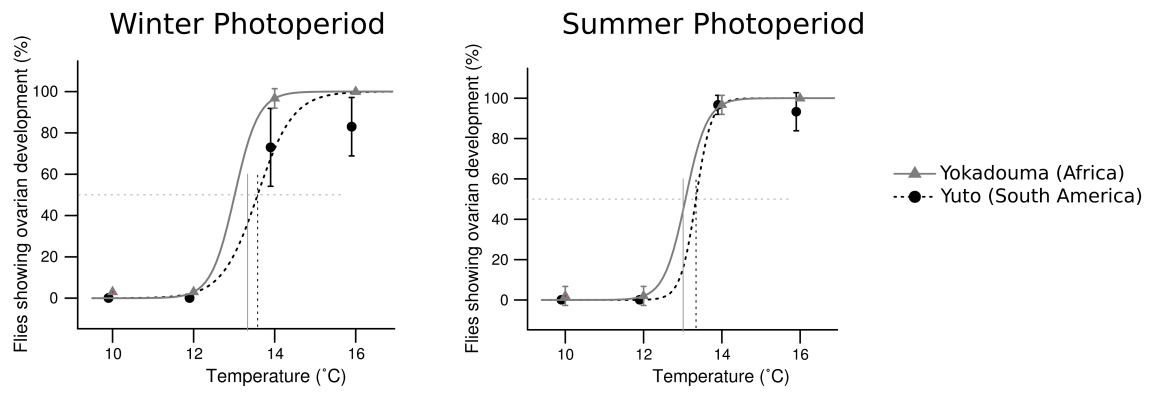


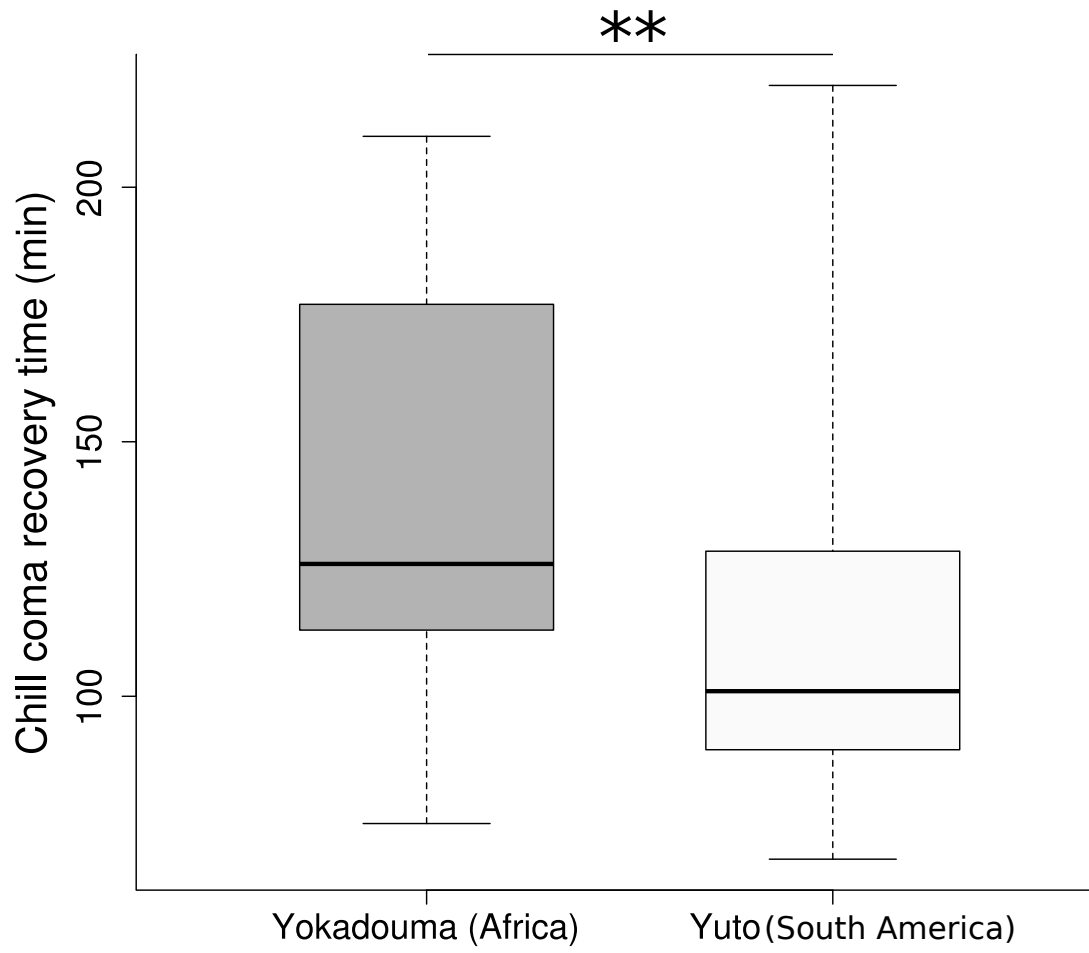
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Journal Pre-proof





- We characterized physiological traits responses to winter like conditions in *Z. indianus*.
- *Z. indianus* females arrested ovarian maturation in response to cold treatments.
- Flies recovered fertility after winter-like conditions.
- Photoperiod acted as modulator of ovarian arrest only in invaded range flies.
- Invaded range flies survived better and recovered faster from chill coma.