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

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

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

Key words: Zaprionus indianus; Overwintering; Low Temperature Physiology; Invaded Range; Native Range.
1. Introduction

*Zaprionus indianus* Gupta 1970 (Diptera: Drosophilidae) is an invasive fly species native to the Afrotropical region that invaded the American continent 20 years ago (Commar *et al.*, 2012). In South America it was reported for the first time in 1999 in São Paulo city area near the Atlantic coast of Brazil (Vilela, 1999). Since this initial report, *Z. indianus* has been found in five of the 12 countries of South America, with the southernmost record near 34°S (Castro and Valente, 2001; Goñi *et al.*, 2001; De Toni *et al.*, 2001; Vilela *et al.*, 2001; Goñi *et al.*, 2002; Tidon *et al.*, 2003; Santos *et al.*, 2003; Kato *et al.*, 2004; Oliveira *et al.*, 2009; Leao and Tidon, 2004; Soto *et al.*, 2006; Chaves and Tidon, 2008; Lavagnino *et al.*, 2008; Furtado *et al.*, 2009; Fernandes Rodrigues and Araujo, 2011; Pasini and Link, 2012; Ribeiro Barbosa *et al.*, 2012; Poppe *et al.*, 2014; Benítez Díaz, 2015; Ferreira Mendes *et al.*, 2017; Vasconcelos *et al.*, 2017). In North America *Z. indianus* has been found at latitudes of 45 to 50°N (Renkema *et al.*, 2013; Holle *et al.*, 2019). Like many drosophilids, this fly feeds primarily on yeasts and bacteria on decomposing fruits (i.e., is a secondary pest), and in a specific event it has been reported as a primary pest of fig plants in Brazil (Vilela *et al.*, 2001). Understanding the factors that promote invasiveness of *Z. indianus* is therefore of biological and also economic interest. During its invasion into temperate regions of the Americas, *Z. indianus* has likely faced cooler temperatures and shorter day lengths compared to its native range in tropical Africa (see Commar *et al.*, 2012 for a review of the invasion process). Range expansion into high latitudes can impose selective pressures (e.g., due to cooler temperatures and shorter days in winter) that promote evolution by means of local adaptations in key physiological traits. For example, *Z. indianus* populations from cold environments in India have enhanced cold stress tolerance in eggs and pupae compared to populations from warmer areas (Ramniwas *et al.*, 2012). Invasion processes can also favor environmentally triggered phenotypic changes (i.e., phenotypic plasticity) in physiological traits. For example, *Z. indianus* acclimated to
low temperature and low humidity increase their desiccation resistance and levels of cuticular lipids (Kalra and Parkash, 2016; Girish et al., 2018). Both, local adaptation and phenotypic plasticity may facilitate *Z. indianus* range expansion by promoting survival at low temperatures and short photoperiods during winter at high latitudes.

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

Testing hypotheses about the underlying causes of biological invasions of a given species requires comparing organisms that are in different stages of the invasion process (van Kleunen et al., 2010). Comparison of native range and invasive *Z. indianus* flies can inform our understanding of patterns, processes and mechanisms relevant to this invasion. To test physiological responses of *Z. indianus* to winter-like conditions (low temperatures and short days) that could limit its distribution (da Mata et al., 2010), we studied cold tolerance, ovarian maturation and survival after long-term exposure to cold and different photoperiods of native (Africa) and invasive range (South America) wild-derived flies. At the species level, we hypothesized that *Z. indianus* females would show immature ovaries after long-term exposure to winter-like conditions, but would recover fecundity following a shift to high temperature, indicating that induced reproductive arrest is possible and that females will be fecund and fertile (i.e., no fitness reduction as a consequence of exposure to prolonged winter-like conditions) for this species (similar to other drosophilids). At the intraspecific level, we hypothesized that females derived from the native range (Africa) would be more susceptible to low temperatures and winter-like photoperiod than those derived from invaded areas (South America). Specifically, we predicted that flies from the invasive range would show larger fertility recovery after prolonged cold exposure than wild-derived females from native range. In addition, as cold tolerance (measured in different metrics like chill coma temperature (CTmin), lethal temperature (LTe50), chill coma recovery time (CCRT)) strongly correlates with latitudinal distribution (Kellermann et al., 2012; Ramnivas et al., 2012, Andersen et al., 2015) we predicted that flies from the invaded range would show larger survival to chill coma and also display a faster recovery time than flies from the native range, indicating higher thermal tolerance to low
temperatures. Altogether these results would imply that survival and reproductive output maintenance after long-time exposure to challenging abiotic conditions correlate with the geographic expansion of *Z. indianus* to high latitudes.

2. Materials and Methods

2.1. Insect collection and rearing

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

The experimental procedure used was a simplified version of the one described in detail in Mensch et al. (2017). Briefly, groups of ~100 newly emerged (< 6 h post-emergence) Z. indianus females from both stocks were exposed to four different cold treatments (10, 12, 14 and 16°C) for 20 days, combined with two alternative photoperiods simulating short winter days (10 h:14 h light:dark) and long summer days (14 h:10 h light:dark). This design resulted in 8 experimental treatments. Two replicates were performed for each combination of populations and treatments. After 20 days of cold exposure, all females were removed and survival was scored as the percentage of live females from the total number of females at the beginning of the cold treatment. For each replicate 15 surviving females were dissected to evaluate the degree of ovarian development. Based on ovarian condition, we defined two types of females: reproductively arrested (RA) and mature females. RA females were defined by the presence of only pre-vitellogenic ovarioles in both ovaries (stages 1–7 according to King, 1970), while mature females had at least one stage 8 (vitellogenic) oocyte in either ovary. Statistical analysis of survival and ovarian arrest under different environmental conditions were performed applying a logistic regression model with a binomial distribution to the entire data-set using the glm function of lme4 package (Bates et al., 2015) in R software, version 3.3.1 (R Core Team, 2016). The full model included environmental temperature as a continuous predictor, origin (Yokadouma from native range in Africa and Yuto from invaded range in South America) and photoperiod (winter and summer) as fixed factors. Model fit was evaluated using DHARMa package (Hartig, 2019). For ovarian arrest analysis, since the triple interaction was significant (see supplementary table 1), separate analysis by photoperiod were performed. Multiple testing was corrected using a conservative Bonferroni correction.
In order to quantify reproductive recovery in females that went through cold treatment, we compared fertility scored as the number of adults (and larvae) per female of RA females and control females maintained at 25°C and 12 h:12 h light:dark photoperiod. Fertility of RA females was scored in flies exposed to 20 days at 12°C and winter-like photoperiod (10 h:14 h light:dark). For these assays, females were transferred to new vials at 25°C for three days; after that, all females were individually crossed with a pair of mature males that were reared at 25°C and 12 h:12 h (light:dark) photoperiod. Once mating had taken place, individual females were allowed to lay eggs for 5 days in 2 cm Petri dishes containing agar medium with yeast paste placed at the end of vials. Oviposition was performed each day on a different vial. Mating vials were kept at 25°C and 12 h:12 h (light:dark) photoperiod. The number of larvae and adult progeny was recorded by visual inspection of vials daily. This procedure allowed to score fertility as the total number of larvae or adult progeny per female and also to quantify this parameter daily after cold treatment termination. The treatment of 12°C and winter-like photoperiod was chosen because such temperature is below the thermal limit that induce ovarian maturation in *Z. indianus* (see Figure 1) and also had a high survival (0.8 for Yokadouma and 0.84 for Yuto, Figure 2), which allowed to have a suitable number of RA females after cold treatment to score fertility. Statistical analysis of the comparison of fertility of RA females and control females was performed applying a General Linear Model using the *glm* function of *lme4* package in R software (Bates et al., 2015). The model included origin (Yokadouma from native range and Yuto from invaded range) and treatment (RA females vs control females) as fixed factors. Statistical analysis of mean number of larvae and adult progeny per female over time was performed applying a Generalized Linear Mixed Model with origin and time (day of quantification after cold treatment termination) as fixed factors and individual female (replicate) as a random factor. This statistical analysis was performed using the *lmer* function of...
The `lme4` package in R software was used (Bates et al., 2015). A binomial distribution was used in both analyses. Model fit was evaluated using `DHARMa` package (Hartig, 2019). Pairwise Tukey’s post-hoc comparisons were performed using `emmeans` package (Lenth, 2018).

2.4. Cold-tolerance assay

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

3. Results

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

Ovarian maturation as a function of temperature was fitted to a logistic curve (Figure 1), and from this equation, we could infer that 50% of all Z. indianus females exposed to cold treatments attained ovarian maturation at a critical temperature of c. 13.3°C. Critical temperature for ovarian
maturation is similar between different origins and photoperiod (Figure 1). Nevertheless, statistical analysis showed that there was a significant photoperiod × temperature × origin interaction affecting ovarian maturation (Supplementary Table 1). Thus the combination of photoperiod and temperature had impacts on ovarian maturation, that were also dependent on the biogeographic origin of females (Supplementary Table 1). In addition, females from both origins showed ovarian maturation above the critical temperature, except for a small proportion of females from Yuto (South America) that still arrested their ovaries at 14°C and 16°C under winter-like photoperiod (Figure 1, Table 1).

3.2. Survival following cold treatments for Z. indi anus females from native and invaded ranges

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

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

Reproductively arrested females from both origins maintained fertility following cold recovery, although females from different origins showed different patterns (Figure 3A). Reproductively arrested females from Yokadouma (Africa) showed higher fertility than control females not exposed
to cold treatments (GLM, $F_{1,12} = 8.54$, $P = 0.013$) while fertility of females from Yuto (South America) did not differ significantly from control flies (GLM, $F_{1,9} = 3.1$, $p = 0.077$) (Figure 3A).

The mean number of offspring from RA females was 40.83 larvae and 23.00 adults per female from Yokadouma (Africa) and 32.00 larvae and 16.17 adults per female from Yuto (South America). The number of offspring (estimated as larvae or adults) for these RA females varied over time following mating and there also was an interaction with the origin of RA females (Figure 3B, Table 3).

Females from Yokadouma (Africa) had significant larger number of larvae (Tukey contrast, estimate$_{30, 95} = 20.54$, $P = 0.017$) and adult progeny (Tukey contrast, estimate$_{27, 96} = 9.74$, $P = 0.0082$) than Yuto (South America) females 24 h after mating, but this difference disappeared at 48 h (Tukey contrasts, Larvae: estimate$_{30, 95} = -6.67$, $P = 0.863$; Adult: estimate$_{27, 96} = 1.64$, $P = 0.986$) and 72 h (Tukey contrast, Larvae: estimate$_{30, 95} = -4.75$, $P = 0.964$; Adult: estimate$_{27, 96} = -2.45$, $P = 0.926$) after mating (Figure 3B).

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

The proportion of flies that survived following chill coma (12 h at 0°C) was 0.31 for Yokadouma (Africa) flies and 0.66 for flies from Yuto (South America). Chill coma recovery time of *Z. indianus* flies was affected by the geographical origin and by the sex of flies (Table 4, Figure 4).

Wild-derived flies from Yuto (South America) recovered faster from chill coma than flies from Yokadouma (Africa), with median recovery times of 101 minutes and 126 minutes, respectively. For both origins, females (median = 103 minutes) recovered faster than males (median = 129.5).

These results suggest that flies from the invaded range had higher cold tolerance than flies from the native range.

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

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

Enhanced cold tolerance in the South American flies could be interpreted as local adaptation to
colder climate, in any case this results show that cold tolerance may be part of a physiological
responses that facilitate expansion of *Z. indianus* into cold areas. However, we also found opposing
results regarding local adaptations in the invaded population in terms of fertility recovery after long-
term cold exposure, since native range flies from Yokadouma (Africa) showed higher fertility
recovery than females from Yuto (South America). Also, females from native and invaded range
show very similar critical temperatures for ovarian maturation, which means that invaded range
females are not being less affected in ovarian maturation by challenging abiotic conditions of low
temperatures and winter-like photoperiod. Ovarian maturation shows latitudinal cline variation
among natural populations of other drosophilid species, indicating that this trait could be related to
adaptation to temperate environments (Schmidt et al., 2005). However, this has not been the case
for the pattern found in *Z. indianus*. We speculate that while enhanced cold tolerance may facilitate
invasion of *Z. indianus* to southern areas of South America, limited adaptation or plasticity of the
reproductive arrest response may limit this expansion. It should be noted that differences in the
phenotypic values of traits among flies from different origins that have different environments are
indicative of the action of natural selection that produces local adaptation; but as Reznick & Travis
(1996) points out, this evidence can only be taken as a first step for proving adaptation in the wild.

Also, this interpretation is based only on two populations collected in the wild, therefore the
generality of the indications of adaptation must be further confirmed by expanding the number of
samples of both origins, or by conducting reciprocal transplant experiments (e.g., Pelini et al.,
2009). Another possible caveat that should be mentioned is the possibility that laboratory adaptation
or inbreeding could have impacted our traits estimates. However, given that the populations
analyzed were maintained at large population sizes and controlled environmental laboratory
conditions (standard laboratory medium, temperature, photoperiod, humidity) the possibility that
these laboratory processes have differentially affected the stocks is low. Also, several studies have shown that for drosophilids these factors affect intraspecific variation less than interspecific variation (Andersen et al., 2015; Maclean et al., 2018).

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

5. Conclusions

We identified a set of overwintering traits that could facilitate geographical expansion of *Z. indianus* to southern latitudes of South America. *Z. indianus* likely does not experience a drastic reduction in fitness in temperate climates with cold winters, given that females show ovarian arrest followed by a recovery in fertility when exposed to challenging abiotic conditions. We found mixed evidence both for and against scenarios of local adaptations or phenotypic plasticity in the invaded
range; and also showed that photoperiod acted as modulator of ovarian arrest. With respect to overwintering mechanisms, *Z. indianus* seems to have characteristics of both tropical and temperate species. Overall, variation in reproductive and cold tolerance physiology may enable (and limit) geographical expansion of *Z. indianus* to high latitudes of the American continent.

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Table 1. Logistic regression analysis of environmental factors (temperature and photoperiod) affecting reproductive arrest in *Z. indianus* flies from different biogeographic origin (native and invaded range). The analysis for winter-like photoperiod and summer-like photoperiod are shown separately since the full model presented significant interactions (see supplementary table 1).

Significance threshold factor using a Bonferroni correction was $P < 0.0015$.

<table>
<thead>
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<th>Effect</th>
<th>Winter Estimate ($X^2$)</th>
<th>Winter $P$</th>
<th>Summer Estimate ($X^2$)</th>
<th>Summer $P$</th>
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<td>197.58</td>
<td>&lt;0.0015</td>
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<td>Origin</td>
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<td>&lt;0.0015</td>
<td>1.53</td>
<td>0.216</td>
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<tr>
<td>Temperature × Origin</td>
<td>17.48</td>
<td>&lt;0.0015</td>
<td>2.92</td>
<td>0.087</td>
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</table>

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

<table>
<thead>
<tr>
<th>Effect</th>
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<th>$P$</th>
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<td>Photoperiod</td>
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<tr>
<td>Origin × Photoperiod</td>
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<td>0.01</td>
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Table 3. Generalized Linear Mixed Model analysis of the comparison of fertility of cold induced RA females of *Z. indianus* from Yokadouma (Africa) in the native range and Yuto (South America) in the invaded range (origin effect) at different times after mating (time effect). Fertility was estimated as the number of larvae and adults per female.

<table>
<thead>
<tr>
<th>Effect</th>
<th>Larvae per females</th>
<th>Adults per female</th>
</tr>
</thead>
</table>
Table 4. General Linear Model analysis of the comparison of chill coma recovery time of females and males (sex effect) flies from Yokadouma (Africa) in the native range (N=17) and Yuto (South America) in the invaded range (N=59) (origin effect).
Fig. 1. Ovarian maturation for *Z. indianus* females from Yokadouma (Africa) and Yuto (South America) in winter (left panel) and summer (right panel) photoperiods. Ovarian maturation was scored as the presence of at least one stage 8 (vitellogenic) oocyte in either ovary. Error bars are standard deviations. Vertical dashed lines indicate the thermal threshold for the induction of ovarian maturation. Yokadouma (Africa) flies showed 50% induction of ovarian maturation at 13.34°C in winter-like photoperiod and 13.01°C in summer-like photoperiod, whereas Yuto (South America) flies reached 50% ovarian maturation at 13.58°C in winter-like photoperiod and 13.34°C in summer-like photoperiod.

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

Fig. 3. Fertility recovery of reproductively arrested *Z. indianus* females from Yokadouma (Africa) and Yuto (South America). (A) Number of adult offspring obtained from reproductively-arrested (RA) females exposed to cold treatment (dark gray) and control mature females not exposed to cold treatment (light gray) for Yokadouma (Africa) (N=7) and Yuto (South America) (N=8) populations of *Z. indianus*. Data are the mean (±s.e.m.) number of adult progeny derived from single females. (B) Fertility recovery of reproductively arrested *Z. indianus* across days after mating. The mean (±s.e.m.) number of larvae and adults per female was determined from vials containing eggs laid 24, 48, and 72 h after mating. Left panel shows fertility measured as number of larvae per female and right panel shows fertility measured as number of adult offspring per female. **P<0.05.

Fig. 4. Chill coma recovery time (in minutes) of wild-derived *Z. indianus* flies from Yokadouma (Africa) and Yuto (South America). Sample sizes were N=17 for Yokadouma (Africa) and N=59.
for Yuto (South America). Boxplot lines represent the median and box boundaries are the upper and lower quartiles (e.g. 25th percentiles). Error bars represent the maximum and minimum values.

**P<0.05.**
### Supplementary Table I. Logistic regression analysis of environmental factors (temperature and photoperiod) affecting reproductive arrest in *Z. indianus* populations from different biogeographic origin (native and invaded range).

<table>
<thead>
<tr>
<th>Effect</th>
<th>Estimate ($X^2$)</th>
<th>P-value</th>
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<tr>
<td>Temperature</td>
<td>392.03</td>
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</tr>
<tr>
<td>Origin</td>
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<td>0.0244</td>
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<tr>
<td>Photoperiod</td>
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<tr>
<td>Temperature × Origin</td>
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<tr>
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<td>0.0037</td>
</tr>
<tr>
<td>Temperature × Origin × Photoperiod</td>
<td>16.83</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Vitae

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• 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.