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Dear Dr. Muttis,

It is a pleasure to accept your revised manuscript entitled "Factors related to *Aedes aegypti* (Diptera: Culicidae) populations and temperature determine differences on life-history traits with regional implications in disease transmission." in its current form for publication in the Journal of Medical Entomology.

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Thank you for your fine contribution. On behalf of the Editors of the Journal of Medical Entomology, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Phillip Kaufman
Editor, Journal of Medical Entomology
pkaufman@ufl.edu

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Factors related to *Aedes aegypti* (Diptera: Culicidae) populations and temperature determine differences on life-history traits with regional implications in disease transmission.

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6 **Factors related to *Aedes aegypti* (Diptera: Culicidae) populations and temperature**
7 **determine differences on life-history traits with regional implications in disease**
8 **transmission.**

9

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21

22

23 **Abstract**

24 *Aedes aegypti* (L.) (Diptera: Culicidae) is a vector of many medically significant viruses in
25 the Americas, including dengue virus, chikungunya virus and Zika virus. Traits such as
26 longevity, fecundity and feeding behavior contribute to the ability of *Ae. aegypti* to serve as

27 a vector of these pathogens. Both local environmental factors and population genetics could
28 contribute to variability in these traits. We performed a comparative study of *Ae. aegypti*
29 populations from four geographically and environmentally distinct collection sites in
30 Argentina in which the cohorts from each population were held at temperature values
31 simulating a daily cycle, with an average of 25 °C in order to identify the influence of
32 population on life-history traits. In addition, we performed the study of the same
33 populations held at a daily temperature cycle similar to that of the surveyed areas.
34 According to the results, Aguaray is the most outstanding population, showing features that
35 are important to achieve high fitness. Whereas La Plata gathers features consistent with low
36 fitness. Iguazu was outstanding in blood feeding rate while Posadas's population showed
37 intermediate values. Our results also demonstrate that climate change could differentially
38 affect unique populations, and that these differences have implications for the capacity for
39 *Ae. aegypti* to act as vectors for medically important arboviruses.

40

41 Keywords: Mosquito, fitness, Argentina

42

43

44 **Introduction**

45 *Aedes aegypti* is a highly successful invasive species that has become one of the most
46 common mosquito species biting humans in many tropical and subtropical cities. It is also a
47 vector of viruses causing several major tropical diseases including dengue, chikungunya,
48 yellow fever and Zika (Gubler, 2004; Rodriguez-Morales, 2015). *Aedes aegypti* has a wide
49 distribution in Argentina, from the northern border to the province of Neuquén in the south
50 (Grech et al., 2012), from subtropical to temperate climates. Like other insects, *Ae. aegypti*

51 development rates are a function of temperature (Christophers, 1960). However, several
52 studies performed in Argentina have shown that some life-history traits of *Ae. aegypti*
53 (immature and adults) varied between populations collected in different regions of the
54 country when they were reared at the same temperature (Dominguez et al., 2000; Tejerina
55 et al., 2009; Grech et al., 2010). This evidence of adaptation to local conditions is
56 supported by the fact that Argentinean *Ae. aegypti* populations showed high levels of
57 genetic polymorphism which suggest different origins from genetically distinct populations
58 (de Sousa et al., 2000; Rondán-Dueñas et al., 2009; Llinas and Gardenal, 2011).

59 Here we present a comparative study about *Ae. aegypti* populations from Argentina in
60 order to identify the life traits that respond to local adaptation and the traits that could be
61 mostly influenced by temperature. In this sense, we selected four mosquito populations
62 from three provinces: Salta from the Northwest, Buenos Aires from the South and Misiones
63 from the Northeast area of this mosquito species distribution. Cohorts from each site were
64 held at temperature values simulating a daily cycle, with an average of 25 °C in order to
65 determine their life-history traits and to make comparisons between populations.

66 Additionally, we performed the study of the same populations by holding them at a daily
67 temperature cycle which was approximately the same as the one registered at the surveyed
68 area. The knowledge about the behavior of *Ae. aegypti* in different regions of the country,
69 as well as the study of the same populations held at mean cycle temperature, will allow us
70 to make inferences about the response of *Ae. aegypti* under different climatic scenarios that
71 could be useful to define areas with greater potential of disease transmission.

72

73 **Material and Methods**

74 **Study sites**

75 We have selected four locations from three provinces of Argentina: Salta (Aguaray),
76 Buenos Aires (La Plata), and Misiones (Posadas and the Iguazu National Park) (Fig.1).
77 Aguaray (22° 14' 30" S 63° 44' 00" W) is located in an area characterized as a subtropical
78 montane moist forest with an annual mean temperature of 20 °C and a mean annual rainfall
79 of 950 mm. La Plata (34° 55' 07" S 57° 57' 15" W), as capital of the province of Buenos
80 Aires, is a highly populated area located in a region called Pampa, which has predominance
81 of plains and grasslands. The annual mean temperature is 16.5 °C and the mean annual
82 rainfall is 900 mm. Posadas (27° 21' 42" S - 55° 54' 15" W) and the Iguazu National Park
83 (25° 35' 49" S - 54° 34' 42" W) are located in a region called Paranaense Forest with an
84 annual mean temperature of 20 °C and a mean annual rainfall of 1800 mm. Although they
85 belong to the same province, these sites are different because Posadas is the capital of the
86 province with high anthropic disturbances, while Iguazu is mostly a forest area with little
87 human population, bordering Paraguay and Brazil (Burkart et al., 1999).

88

89 **Mosquitoes and environmental data collection**

90 During February and March of 2014, peak population period of *Ae. aegypti* in Argentina
91 (Micieli and Campos, 2003; De Majo et al., 2013), mosquito eggs were obtained from
92 approximately 25 ovitraps from each location (Aguaray, La Plata and Posadas) while in the
93 Iguazu National Park it was possible to collect mosquito larvae only from seven artificial
94 containers due to the low availability of these mosquito habitats. The eggs were transported
95 to Centro de Estudios Parasitológicos y de Vectores (CEPAVE -CONICET-UNLP) in
96 plastic bags and identified as *Ae. aegypti* after larvae reached the fourth instar. These larvae
97 were used to build the colony from which F1 eggs were used in assays. For Iguazu
98 locations, field collected larvae were transported in plastic containers to a local laboratory.

99 Larvae identified as *Ae. aegypti* were used to rear adults from which F1 eggs were obtained
100 for transport to CEPAVE facilities to be used for assays. In each city, the daily temperature
101 and relative humidity were recorded between February 20 and March 20, 2014 using
102 HOBO data loggers (Onset, Cape Cod, MA) located at the collection sites, which were
103 protected from direct sunlight and rain. We determined the temperature range and the mean
104 value for each site: La Plata, 18-23 °C, average: 20 °C; Aguaray, 21-31 °C, average: 25 °C;
105 Posadas, 18-34 °C, average: 26 °C and Iguazu, 21-35 °C, average: 28 °C. These data were
106 used to build a curve of fluctuating daily temperatures that were used to program the
107 incubators for the experimental procedures (Fig. 2). A mean temperature range was
108 established from values generated at each of the four sites. This calculation provided a
109 mean range cycle of 20-30°C (Fig. 2). The mean relative humidity ($X \pm SD$) varied among
110 Iguazu ($75.45 \pm 11.63\%$), Aguaray ($77.61 \pm 6.22\%$), Posadas ($81.69 \pm 18.31\%$), and La
111 Plata ($86.99 \pm 4.04\%$).

112

113 **Experimental Procedures**

114 The colonies were maintained in the insectaries at CEPAVE following the protocol of
115 Gerberg et al. (1994) until sufficient numbers of eggs of the F1 generation were acquired to
116 carry out the experiments. The eggs were held at room temperature (20-27 °C) until the
117 beginning of the experiments, but for no longer than two months. When needed, eggs of
118 the first generation (F1) from each location were submerged overnight in 400 ml of
119 dechlorinated water in plastic bowls (170 mm diameter) for hatching in order to obtain 1st
120 instar larvae for the experiments.

121 The first set of trials was performed using the same cyclic temperature for all populations.
122 The daily temperatures recorded by hour in each location were averaged to build a mean
123 cyclic temperature curve that resulted in a daily minimum temperature of 20 °C and a
124 maximum of 30 °C, with a daily average of 25 °C (Fig. 2). The incubator temperature
125 parameters were set according to this cycle.

126 For each experiment, 100 1st instar larvae from each population were placed in groups of
127 25 larvae into one of four plastic flat trays (30 cm x 18 cm x 6 cm) filled with 750 ml of
128 dechlorinated water. Finely ground rabbit food (0.5 g) was added to the water to feed the
129 immature stages during the first two days of the experiment and 0.25 g of food were added
130 each subsequent day until pupation. Water was added as needed to maintain a 750 ml
131 volume. Larval instar and the number of dead larvae were recorded daily, as well as the day
132 of pupation. The pupae were transferred to plastic containers (8 cm x 3.5 cm diameter)
133 supplied with water and two to three raisins per container. After emergence, the adults were
134 sexed and transferred to a cardboard cage (25 cm x 22 cm diameter) for 3 to 5 days to allow
135 mating. Adults were offered a blood meal (restrained hamster (100 g) into each cage for 60
136 min), and fed with a 10% sugar solution from a cotton wick in 50-ml plastic flasks. After
137 feeding, the cages were held for 3 min at ≈ -20 °C in order to anesthetize the adults. Each
138 engorged female was moved to an individual plastic container (8 cm x 3.5 cm diameter)
139 containing a filter paper positioned over wet cotton to facilitate oviposition. A second blood
140 meal was offered 15 days after the first blood feeding after which the females were released
141 into a cardboard cage to commence the second gonotrophic cycle. Adults were checked
142 every day to record the number of deaths. The eggs laid during each oviposition were
143 counted daily and kept on their filter paper over cotton in a Petri dish and sealed using
144 parafilm to maintain humidity for 7-10 days to ensure embryogenesis. Thereafter,

145 individual filter papers were transferred into a plastic container with 250 ml of
146 dechlorinated water and 10 mg of yeast for hatching. The number of larvae was counted
147 after 48 hours.

148 The general procedure for a second set of experimental assays was similar to the above
149 mentioned, but it was performed using the range of temperatures measured at the sample
150 site of each population. Three replicates of 100 1st instar larvae from each population were
151 used for these experimental assays . All these studies were conducted at CEPAVE insectary
152 facilities. We used an approximately photoperiod 14:10 (L:D) according to summer season
153 across all experiments in the incubator and the relative humidity level was maintained
154 between 70% and 80%.

155

156 **Table life construction and definitions**

157 The date and the total number of individuals that entered a given stage, died in that stage,
158 and molted to the next stage were used as input for life table calculations (Deevey, 1947).
159 The proportion of hatched eggs at the first submersion in water produced by the females of
160 the cohort was used to estimate the number of initial eggs of each cohort. Daily mortality
161 records were used to calculate survival as a function of age (l_x). Survival (l_x) was
162 expressed as the percentage of individuals that reached the next instar/stage; the number of
163 eggs laid daily was used to calculate the age-specific fecundity (m_x), by dividing the total
164 number of eggs laid each day(x) by the number of individuals alive at the end of that day.
165 The (l_x) and (m_x) schedules allowed for the estimation of demographic parameters such as
166 the intrinsic rate of natural increase (r), the net reproductive rate (R_0), and the mean
167 generation time (T_g); complete definitions of these parameters and the formulas used for
168 their calculation are given in Rabinovich and Nieves (2011). The length of the gonotrophic

169 cycle (GC) is equivalent to the number of days between the blood meal and the first batch
170 of eggs (mean time between the first and last day for each female's batch of eggs). The
171 length of the second GC was regarded as the number of days between the second blood
172 meal (approximately 14 days after the first blood meal) and the second batch of eggs. Life
173 fecundity is understood as the mean number of laid eggs per female calculated from
174 individual female oviposition during all its life; and the egg hatch rate is equivalent to the
175 number of larvae/eggs. The blood-feeding rate is the number of blood-fed females over the
176 total number of females exposed to feeding.

177

178 **Statistical analyses**

179 Three sets of analyses were performed in order to compare the life table traits including
180 demographic parameters. The first analysis was conducted among populations held at
181 common mean cycle temperature (25 °C, range: 20-30 °C), the second analysis was
182 performed among populations held at the temperature cycle recorded from each site, while
183 the third analysis was a comparison of the demographic parameters and some life table
184 traits (fecundity, blood feeding rate) under the two temperatures regimes (specific-site and
185 mean) by each population.

186 ***Life table traits***

187 *Immature stages.* Hatching rate and mortality were analyzed by Chi-squared test. Larval
188 and pupal development times were analyzed by Mann-Whitney Test.

189 *Adults.* Adult female survival was analyzed by Log-rank (Mantel-Cox) Test. The sex ratio
190 and blood-feeding rate were analyzed by Chi-squared test. The length of the GC and life
191 fecundity was analyzed by Kruskal Wallis Test.

192 *Demographic parameters.* For each demographic parameter, we also estimated the
193 confidence interval at a 95% significance level based on 1,000 bootstrap samples by
194 random resampling with replacement from the initial individuals of each group. These
195 calculations were carried out using a computer program developed in Delphi Language,
196 cordially provided by Dr. Rabinovich. The statistical comparison of demographic
197 parameters was carried out with the Student T-test for independent samples.
198 All statistical methods were performed using R software (version 3.3.2).

199

200 **Results**

201 *Aedes aegypti* populations response at common mean cycle temperature

202 *Immature stages*

203 The lowest rate of hatching was observed on Iguazu's population (78%), whereas the
204 percentage obtained in cohorts from other sites was higher than 80%. However, a
205 significant difference was detected only between Iguazu and La Plata ($p < 0.05$, chi-square
206 test).

207 The specific mortality of the 1st and 3rd larval instar was different among populations
208 ($p < 0.05$, chi-square test) while in the 2nd and 4th larval instar there were no significant
209 differences. However, the immature mortality from 1st instar to pupa was not significantly
210 different between the locations (Table 1).

211 The mean development times from 1st instar larvae to the pupal stage were statistically
212 different among populations ($p < 0.01$, Mann–Whitney test), with a range of 8.9 days
213 (Iguazu) to 10.5 days (La Plata). The development time of Iguazu's population was
214 significantly shorter ($p < 0.01$, Mann–Whitney test) compared to the other populations,

215 which was primarily a result of decreased larval development time from the 2nd to the 4th
216 instar larvae (Table 1).

217 *Adult traits*

218 *Adult female's survival*

219 The median female survival was 27 days for Iguazu, 35 days for Posadas, 37 days for La
220 Plata and 38 days for Aguaray. No significant differences were found among populations.

221 *Sex ratio*

222 Sex ratios were as follows: 0.60 for Iguazu, 0.86 for Aguaray, 0.95 for Posadas, and 1.17
223 for La Plata. However, no significant differences were detected.

224 *Adult reproductive features*

225 The *Ae. aegypti* populations from Posadas and La Plata had a significantly higher blood
226 feeding rate than those from Iguazu and Aguaray at the first GC ($p < 0.00001$, chi-squared
227 test), but no differences were detected at the second GC. The life fecundity and the length
228 of the first and second GC were not significantly different between populations (Table 2).

229 Iguazu females laid the fewest total eggs ($n=847$), due to a low oviposition rate (0.60
230 laying/fed female) in relation to the other populations (0.84 for Aguaray and 0.95 for La
231 Plata and Posadas).

232 Oviposition patterns for each GC varied among populations. For the first GC, Iguazu
233 females laid eggs over two days, while in other populations oviposition was distributed
234 over more than four days, the most extensive being the population from La Plata (6 days)
235 (Fig. 3). On the first day of oviposition, females from Iguazu and Posadas laid 80% of their
236 eggs (752 and 2,509 eggs, respectively), while females from Aguaray and La Plata laid
237 approximately 60% of their total, equating to 1,056 and 1,895 eggs, respectively. The
238 second GC showed the same pattern of oviposition, with the females of Iguazu's population

239 laying all their eggs during the first day of oviposition, and the females from Aguaray and
240 La Plata laying eggs over 2 or 3 days, respectively (Fig 3). Oviposition time by Iguazu's
241 population was significantly shorter (days) compared to Aguaray and La Plata ($p < 0.05$,
242 Kruskal-Wallis test).

243 ***Demographic parameters***

244 The mean generation time (T_g) was significantly different among the four populations
245 studied ($p < 0.001$, t-test). The highest T_g was measured with Aguaray's population (28.2
246 days) and the lowest (23.0 days) with Posadas's population (Table 3). The net reproductive
247 rate (R_o) also was significantly different among populations ($p < 0.001$, t-test). The highest
248 value for R_o was measured for La Plata, which was 4-fold higher than the one for Iguazu
249 ($p < 0.05$, t-student test). The intrinsic rate of natural increase (r) was statistically different
250 among the populations ($p < 0.05$, t-student test) with the exception of La Plata and Posadas
251 (Table 3).

252

253 ***Aedes aegypti* populations response at specific-site temperature cycles**

254 ***Immature traits***

255 The lowest rate of hatching of 41.96% ($p < 0.05$, chi-square test) was measured at 18-23 °C
256 in La Plata's population, while Aguaray (21-31°C) presented the highest percentage of
257 hatching, 81.08% ($p < 0.05$, chi-square test). The significantly lowest immature mortality,
258 3.33 %, ($p < 0.05$, chi-square test) and the lowest mean development time, 8.3 days (larvae-
259 pupa) ($p < 0.01$, Mann-Whitney test) also were found at 21-31 °C in Aguaray's population,
260 while the longest mean development time was found in La Plata at 18-23 °C ($p < 0.01$,
261 Mann-Whitney test) (Table 1).

262 ***Adult traits***

263 The lowest female survival was found in Iguazu at 21-35 °C (27 days, $p < 0.03$, Log-rank
264 Test) while the greatest (41 days, $p < 0.01$, Log-rank Test) was found in La Plata (18-23 °C).
265 The lowest blood feeding rate (34 %) was found in La Plata ($p < 0.05$, chi-squared test) and
266 the highest blood feeding rate at both, first (96 %) and second (75 %) gonotrophic cycles
267 ($p < 0.001$, chi-squared test) was found in Iguazu at 21-35 °C (Table 2). The highest life
268 fecundity (110 eggs/female) was measured in Aguaray at 21-31 °C ($p < 0.05$, Kruskal Wallis
269 Test) (Table 2).

270 ***Demographic parameter***

271 The shortest mean generation time (27 days) was found in Aguaray at 21-31 °C and the
272 longest (44 days) was found in La Plata at 18-23 °C ($p < 0.05$, t-test) (Table 3). The lowest
273 net reproductive rate, 3.14, was found in La Plata while the highest, 22.1, was found in
274 Aguaray ($p < 0.05$, t-test) (Table 3). Likewise, the lowest intrinsic rate of natural increase,
275 0.027, was found at 18-23 °C, La Plata population while the highest value, 0.126, was
276 recorded at 21-31 °C in Aguaray ($p < 0.05$, t-test) (Table 3).

277

278 ***Aedes aegypti* populations response at two different temperature cycles**

279 Iguazu showed the highest blood feeding rate at its site-specific temperature cycle of 21-35
280 °C (0.96 for GC1 and 0.75 for GC2), in comparison to a mean temperature cycle of 20-30
281 °C (0.45 for GC1 and 0.28 for GC2) for both GCs ($p < 0.05$, chi-square test). Instead, La
282 Plata had the highest blood feeding rate at a mean temperature cycle of 20-30 °C, in
283 comparison to its site-specific temperature cycle of 18-23 °C (only for the first GC, 0.89 vs.
284 0.34) ($p < 0.05$, chi-square test). Aguaray did not show significant differences in any GC
285 between both temperature cycles (20-30 °C vs. 21-31 °C). The blood feeding rate for
286 Posadas's population presented a different behavior for each GC. For the first GC, the

287 highest value was found at the mean temperature cycle, 0.95, in comparison to the site-
288 specific temperature cycle (18-34 °C), 0.58. On the other hand, for the second GC, the
289 highest value was found at the site-specific temperature cycle, 0.25, in comparison to the
290 mean temperature cycle, 0.03.

291 Life fecundity was significantly different ($p < 0.05$, Kruskal Wallis Test) between both
292 temperature cycles for Iguazu, La Plata, and Posadas, with the highest number of eggs
293 recorded at a mean temperature cycle. For Aguaray's population, no significant difference
294 was detected between cycles. The analysis of the demographic parameters (T_g , R_0 and r)
295 between two temperature cycles (mean temperature cycle vs. site-specific temperature
296 cycle) for each population showed significant differences ($p < 0.001$, t-test). Iguazu (33.69
297 vs. 24.26), La Plata (43.96 vs. 25.21), and Posadas (28.50 vs. 23.02) presented a higher
298 mean generation time at their site-specific temperature cycles, in comparison to a mean
299 temperature cycle, with the exception of Aguaray (27 vs. 28.15). Iguazu (10.98 vs. 7) and
300 Aguaray (22.1 vs. 16.65) presented a higher net reproductive rate at their site-specific
301 temperature cycles, in comparison to a mean temperature cycle, whereas in La Plata (3.14
302 vs. 29.96) and Posadas (8.7 vs. 27.12) the opposite behavior was shown. Iguazu (0.08 vs.
303 0.07), La Plata (0.14 vs. 0.02), and Posadas (0.14 vs. 0.07) presented a higher intrinsic rate
304 of natural increase at a mean temperature cycle in comparison to their site-specific
305 temperature cycles, with the exception of Aguaray (0.10 vs. 0.12).

306

307 **Discussion**

308 The comparative study of *Ae. aegypti* populations allowed us to identify the life history
309 traits that respond to local adaptation and the traits that most likely could be influenced by
310 temperature. Some characteristics were significantly different between populations held at

311 the same temperature cycle, such as rate of hatching, mean development time and blood
312 feeding rate in the first GC. Even more relevant are the differences among populations in
313 the demographic parameters showing specific-population responses. These differences
314 cannot be explained on the basis of temperature; therefore, part of this variation is due to
315 population-related factors. On the other hand, some traits did not vary among populations
316 held at mean cyclic temperature: immature mortality, sex ratio, blood feeding rate in the
317 second GC, length of GC, life fecundity and female survival. These results suggest that
318 these traits are more dependent on temperature. Moreover when we compare some traits
319 such as blood feeding rate, lifetime fecundity and population-level traits at two different
320 temperature cycles; we were able to demonstrate significant differences when the variation
321 of the average temperature was at least one degree. More studies are needed in order to
322 confirm these effects.

323 Previous studies have demonstrated that fluctuating temperatures impact the bionomics of
324 *Ae. aegypti* (Mohammed and Chadee, 2011; Carrington et al., 2013) but studies comparing
325 different *Ae. aegypti* populations from Argentina also showed differences in life cycle traits
326 due to local adaptations (Tejerina et al., 2009; Grech et al., 2010). Grech et al. (2010)
327 studied three populations from Argentina (San Javier, Misiones; Oran, Salta; and Cordoba
328 City, Cordoba) at the same temperature range (18.5-28 °C) and found similarities in some
329 traits (sex ratio, immature survival and mean development time larva-pupa). Moreover,
330 differences among population traits were registered: fecundity, net reproductive rate and
331 intrinsic rate of natural increase. Our results corroborate these data with the exception of
332 the mean development time and fecundity.

333 We additionally studied the populations held at daily cycling temperatures based on one
334 month of temperature recordings in the populations source area. Because data from a single

335 survey was used, this does not include variation during this peak population period of *Ae.*
336 *aegypti*, nor does include variation across a year or over the years. We identified Aguaray
337 (mean: 25 °C) as the population with the highest fitness, La Plata (mean: 20 °C) with the
338 lowest fitness, and Posadas (mean: 26 °C) and Iguazu (mean: 28 °C) with intermediate
339 fitness levels.

340 Moreover, we identified populations with unique traits. Iguazu female were shown to have
341 the lowest survival rate and, concordantly, the shortest oviposition periods. Iguazu females
342 completed oviposition in one or two days, which represents at least half the time of the
343 other populations. La Plata's population had the lowest blood feeding rate. However, the
344 females had the longest survival, which could permit time for a third GC. When this
345 population was held at an average temperature of 25 °C, the blood feeding rate increased to
346 very high values, while the survival remained high. In addition, the demographic
347 parameters improved substantially. The combination of these effects could have
348 implications for virus transmission in a climatic change scenario with a warmer
349 environment. These populations could feed more frequently and for a longer period of time.

350 This site is also distinctive with its long-term oviposition pattern, which could be related to
351 high female survival, and this behavior could give them greater dispersion capacity and
352 more possibilities of immature survival.

353 Taken together, these studies of different mosquito populations at site-based temperature
354 and mean temperature demonstrate that these populations could respond differently at
355 specific climatic change scenarios and that the capacity for local adaptation may be
356 differential. These results provide insight into the relative role of the environment and
357 mosquito genetics in the variability of life cycle traits and into how such variability might
358 contribute to regional differences in disease transmission.

359

360 **Ethics Statement**

361 This research was conducted according to Argentine laws following the procedures and
362 protocols approved by Ethics Committee for Research on Laboratory Animals, Farm and
363 Obtained from Nature of National Council of Scientific and Technical Research
364 (CONICET) (Resolution 1047, section 2, annex II) and subsequently by National Agency
365 for the Promotion of Science and Technology of Argentina (ANPCYT) (PICT 2015-0665).
366 Sample collection was carried out under official permits granted by Ministerio de Asuntos
367 Agrarios de la Provincia de Buenos Aires (File 22500-23675/13). For collection at the
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369 Nacionales, Argentina (Guía Tránsito 002978).

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371

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378

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429 **Legends**

430

431 **Figure 1.** Four sampling locations of *Aedes aegypti* populations in Argentina.

432

433 **Figure 2.** Temperature cycle used during experiments measuring *Ae. aegypti* life-history
434 parameters for four Argentinean populations: Specific-site cyclic temperature (Aguaray,
435 Iguazu, Posadas, La Plata) and mean cyclic temperature (Mean).

436

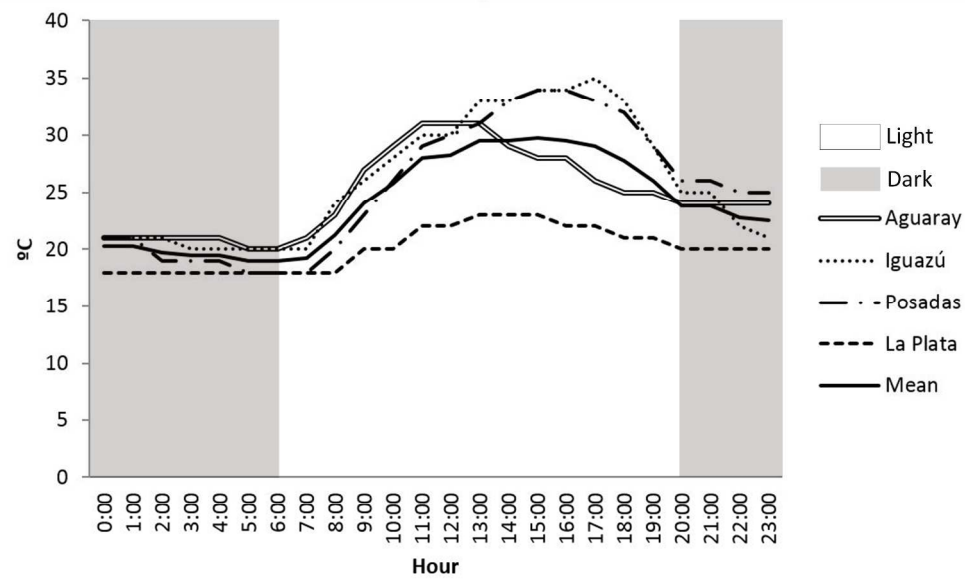
437 **Figure 3.** Oviposition by *Aedes aegypti* from four populations in Argentina. The total
438 number of laid eggs/day is shown in the same bar, for the first (black) and the second (gray)
439 gonotrophic cycles. I= Iguazu, LP=La Plata, P=Posadas, A= Aguaray.

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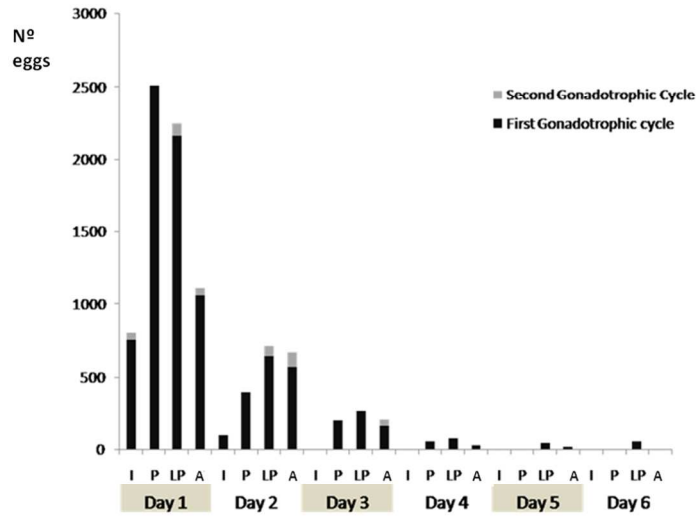
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53x53mm (300 x 300 DPI)



290x177mm (300 x 300 DPI)



104x60mm (300 x 300 DPI)

Table 1. Life-history traits of *Aedes aegypti* immature stages from four populations in Argentina. The four populations were held at a mean temperature cycle and, secondarily, at a temperature cycle based on data recorded in each source site. The daily high and low temperatures recorded in each source area were averaged to construct a mean temperature cycle.

T ¹	Instar/Stage/Instar	Iguazú					La Plata				Posadas				Aguaray		
		N ²	DT ³	L-U ⁴	M ⁵	N	DT	L-U	M	N	DT	L-U	M	N	DT	L-U	M
Mean cycle	Egg ⁶	128	1.00	---	21.88a	113	1.00	---	11.50b	116	1.00	---	13.79ab	122	1.00	---	18.03ab
	Larvae I	98	1.52b	1.42-1.62	2.00ab	100	1.50b	1.39-1.61	0.00b	100	1.89a	1.76-2.03	5.00a	100	1.49b	1.39-1.59	0.00ab
	Larvae II	97	1.12b	1.04-1.21	1.02a	99	1.66a	1.55-1.77	1.00a	95	1.15b	1.08-1.22	2.11a	96	1.77a	1.56-1.98	4.00a
	Larvae III	96	1.29b	1.20-1.38	1.03ab	94	1.66a	1.56-1.76	5.05a	93	1.46b	1.32-1.60	0.00b	94	1.67a	1.54-1.80	2.08ab
	Larvae IV	95	2.79c	2.62-2.96	1.04a	92	3.55a	3.38-3.73	2.13a	93	3.24b	3.06-3.42	0.00a	94	3.12b	2.99-3.25	0.00a
	Pupal	88	2.25b	2.15-2.35	7.37a	89	2.18b	2.10-2.26	3.26ab	92	2.68a	2.54-2.83	1.08b	93	2.14b	2.05-2.23	1.06b
	LI-Pupal	88	8.94d	8.72-9.17	12.00a	89	10.45a	10.19-10.71	11.00a	92	10.38b	9.97-10.79	8.00a	93	10.05c	9.76-10.35	7.00a
Site cycle	Egg ¹	525	1.00	---	42.86b	715	1.00	---	58.04a	435	1.00	---	31.03c	370	1.00	---	18.92d
	Larvae I	291	1.95c	1.78-2.12	3.00b	297	2.09b	1.98-2.21	1.00b	267	2.84a	2.68-3.00	11.00a	297	2.03b	1.92-2.13	1.00b
	Larvae II	285	1.43b	1.34-1.52	2.06a	291	2.90a	2.79-3.02	2.02a	263	1.38b	1.30-1.46	1.50a	294	1.12c	1.08-1.16	1.01a
	Larvae III	276	1.47b	1.38-1.57	3.16a	287	2.87a	2.77-2.97	1.37ab	261	1.13c	1.06-1.19	0.76bc	294	1.05c	1.02-1.07	0.00c
	Larvae IV	270	3.53b	3.43-3.63	2.17a	274	5.30a	5.20-5.40	4.53a	255	2.89c	2.70-3.07	2.30a	293	1.94d	1.88-1.99	0.34b
	Pupal	264	1.76d	1.70-1.81	2.22ab	260	3.49a	3.43-3.55	5.11a	254	2.06c	2.01-2.11	0.39b	290	2.20b	2.15-2.25	1.02b
	LI-Pupal	264	10.14b	9.71-10.24	12.00a	260	15.84a	16.21-16.83	13.33a	254	10.30b	9.64-10.37	15.33a	290	8.34c	8.16-8.44	3.33b

¹T: temperature range at which the populations were held. Mean cycle: 20-30°C. Site cycle: Iguazu (21-35°C), La Plata (18-23°C), Posadas (18-34°C), Aguaray (21-31°C).(Fig. 2).

²N: number of individuals that completed each instar/stage.

³DT: average development time of instar/stage (Days); values within row followed by a different letter were significantly different between populations ($p < 0.05$, Mann-Whitney Test), within temperature parameter. ~~values followed by a different letter were significantly different between groups ($p < 0.05$, Mann-Whitney Test).~~

⁴L-U: Lower–Upper 95% ~~limits for a confidence level~~intervals.

⁵M: stage-specific mortality (%); values within row followed by a different letter were significantly different between populations ($p < 0.05$, Chi-squared Test) within temperature parameter.

⁶Note: the number of eggs for the life table analyses was estimated. In this trait, mortality M is equivalent to the percentage of unhatched eggs. The statistical tests presented here were performed separately for each temperature cycle.

Table 2. Reproductive features under first and second gonotrophic cycle of four populations of *Aedes aegypti* from Argentina held at a mean temperature and at a site-specific temperature cycle based on data recorded in each source area. The daily temperatures recorded in each location were averaged to build a mean temperature cycle.

T ¹	Reproductive feature	Iguazu		La Plata		Posadas		Aguaray	
		1 st GC ²	2 nd GC	1 st GC	2 nd GC	1 st GC	2 nd GC	1 st GC	2 nd GC
Mean cycle	Feeding female/total female	14/31	2/7	42/47	4/27	38/40	1/26	25/43	3/12
	Blood feeding rate	0.45b	0.28a	0.89a	0.14a	0.95a	0.03a	0.58b	0.25a
	Gravid females	9	1	40	3	36	0	21	2
	Fecundity ³	94.11	51.00	80.75	51.66	87.38	0	87.19	101.5
	Life fecundity ⁴	99.8 ±25.62a		84.7 ±30.42a		87.4 ±23.17a		94.1±39.09a	
	Length of GC (d)	4.45a	7a	7.69a	9.25a	6.01a	0	7.3a	7.2a
	Range of GC (d)	(4-6)	(7-7)	(4-27)	(4-15)	(4-26)	0	(4-22)	(4-14)
Site cycle	Feeding female/total female	82/85	36/48	32/94	5/28	58/99	11/44	77/106	21/63
	Blood feeding rate	0.96a	0.75a	0.34c	0.18b	0.58b	0.25b	0.72b	0.33b
	Gravid females	82	36	32	5	58	11	77	21
	Fecundity	62.81	51.19	55.12	46.8	65.29	46	93.76	62.14
	Life fecundity	69.2±49.03b		61.3±33.7b		73.7±42.64b		110.7±46.76a	
	Length of GC (d)	8a	6a	14a	9a	8a	4a	6a	9a
	Range of GC (d)	(1-15)	(1-14)	(5-53)	(6-13)	(3-16)	(1-8)	(2-15)	(2-23)

¹T: temperature range at which the populations were held. Mean cycle: 20-30°C. Site cycle: Iguazu (21-35°C), La Plata (18-23°C), Posadas (18-34°C), Aguaray (21-31°C) (Fig. 2).

²GC: number of days between the blood meal and the beginning of oviposition. After first feeding (GC1) and after second feeding (GC2).

³Fecundity: mean number of laid eggs per female and per GC.

⁴Life fecundity: mean number of laid eggs per female calculated from individual female oviposition during all its life.

Length of gonotrophic cycle and life fecundity were analyzed by Kruskal-Wallis test. The blood feeding rate was analyzed by Chi-square Test. Values within row followed by a different letter were significantly different between populations within temperature parameter and GC.

Note: The statistical tests presented here were performed separately for each temperature cycle.

Table 3. Demographic parameters of *Aedes aegypti* from four populations of Argentina held at a mean temperature cycle and at four site-specific temperature cycles. The daily temperatures recorded in each population source location were averaged to build a mean temperature cycle.

Temperature parameter ¹	Demographic parameter	Iguazu		La Plata		Posadas		Aguaray	
		Avg ²	L-U ³	Avg	L-U	Avg	L-U	Avg	L-U
Mean cycle	Mean generation time (<i>Tg</i>) (days)	24.26c	24.06-24.48	25.21b	25.02-25.41	23.02d	22.93-23.12	28.15a	27.90-28.41
	Net reproductive rate (<i>Ro</i>)	7.007d	6.607-7.407	29.96a	29.32-30.60	27.12b	26.402-27.839	16.65c	16.15-17.16
	Intrinsic rate of natural increase (<i>r</i>)	0.083c	0.051-0.114	0.142a	0.119-0.167	0.145a	0.116-0.174	0.108b	0.084-0.132
Site cycle	Mean generation time (<i>Tg</i>) (days)	33.693b	33.508-33.907	43.968a	43.735-44.239	28.506c	28.380-28.639	27.008d	26.843-27.178
	Net reproductive rate (<i>Ro</i>)	10.984b	10.648-11.321	3.149d	3.002-3.296	8.703c	8.446-8.959	22.100a	21.708-22.491
	Intrinsic rate of natural increase (<i>r</i>)	0.075c	0.057-0.0929	0.027d	0.015-0.040	0.079b	0.063-0.096	0.126a	0.110-0.143

¹Temperature ~~parameter~~ ~~range~~ at which the populations were held. Mean cycle: 20-30°C. Site cycle: Iguazu (21-35°C), La Plata (18-23°C), Posadas (18-34°C), Aguaray (21-31°C) (Fig. 2).

Avg²: average; values within row followed by a different letter were significantly different between populations (p<0.05, t-student test) within temperature parameter.

L-U³: Lower–Upper 95% ~~limits for a~~ confidence ~~level~~ intervals.

Note: The statistical tests presented here were performed separately for each temperature cycle.

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