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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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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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20

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 ºC 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 ºC 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 ºC 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 ºC 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 ºC 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 ℃, average: 20 ℃; Aguaray, 21-31 ℃, average: 25 ℃;

105   Posadas, 18-34 ℃, average: 26 ℃ and Iguazu, 21-35 ℃, average: 28 ℃. 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℃ (Fig. 2). The mean relative humidity (X±SD) varied among

110   Iguazu (75.45 ± 11.63%), Aguaray (77.61 ± 6.22%), Posadas (81.69 ± 18.31%), and La

111   Plata (86.99 ± 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 ℃) 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 ºC and a

124   maximum of 30 ºC, with a daily average of 25 ºC (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 ºC 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 (lx). Survival (lx) 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 (mx), 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 (lx) and (mx) schedules allowed for the estimation of demographic parameters such as

166   the intrinsic rate of natural increase (*r*), the net reproductive rate (*Ro*), and the mean

167   generation time (*Tg*); 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 ºC, range: 20-30 ºC), 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 (*Tg*) was significantly different among the four populations

245   studied (p<0.001, t-test). The highest *Tg* 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 (*Ro*) also was significantly different among populations (p<0.001, t-test). The highest

248   value for *Ro* 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 ℃

256   in La Plata's population, while Aguaray (21-31℃) 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 ℃ in Aguaray's population,

260   while the longest mean development time was found in La Plata at 18-23 ℃ (p<0.01,

261   Mann–Whitney test) (Table 1).

262   ***Adult traits***

263   The lowest female survival was found in Iguazu at 21-35 ºC (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 ºC).

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 ºC (Table 2). The highest life

268   fecundity (110 eggs/female) was measured in Aguaray at 21-31 ºC (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 ºC and the

272   longest (44 days) was found in La Plata at 18-23 ºC (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 ºC, La Plata population while the highest value, 0.126, was

276   recorded at 21-31 ºC 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   ºC (0.96 for GC1 and 0.75 for GC2), in comparison to a mean temperature cycle of 20-30

281   ºC (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 ºC, in

283   comparison to its site-specific temperature cycle of 18-23 ºC (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 ºC vs. 21-31 ºC). 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 ºC), 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 (Tg, 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 ºC) 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   *aegypty*, nor does include variation across a year or over the years. We identified Aguaray

337   (mean: 25 ºC) as the population with the highest fitness, La Plata (mean: 20 ºC) with the

338   lowest fitness, and Posadas (mean: 26 ºC) and Iguazu (mean: 28 ºC) 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 ºC, 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.

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Ethics Statement

This research was conducted according to Argentine laws following the procedures and

protocols approved by Ethics Committee for Research on Laboratory Animals, Farm and

Obtained from Nature of National Council of Scientific and Technical Research

(CONICET) (Resolution 1047, section 2, annex II) and subsequently by National Agency

for the Promotion of Science and Technology of Argentina (ANPCYT) (PICT 2015-0665).

Sample collection was carried out under official permits granted by Ministerio de Asuntos

Agrarios de la Provincia de Buenos Aires (File 22500-23675/13). For collection at the

Iguazu National Park, Permit NEA 326 was issued by the Administración de Parques

Nacionales, Argentina (Guía Tránsito 002978).

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429   **Legends**

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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).
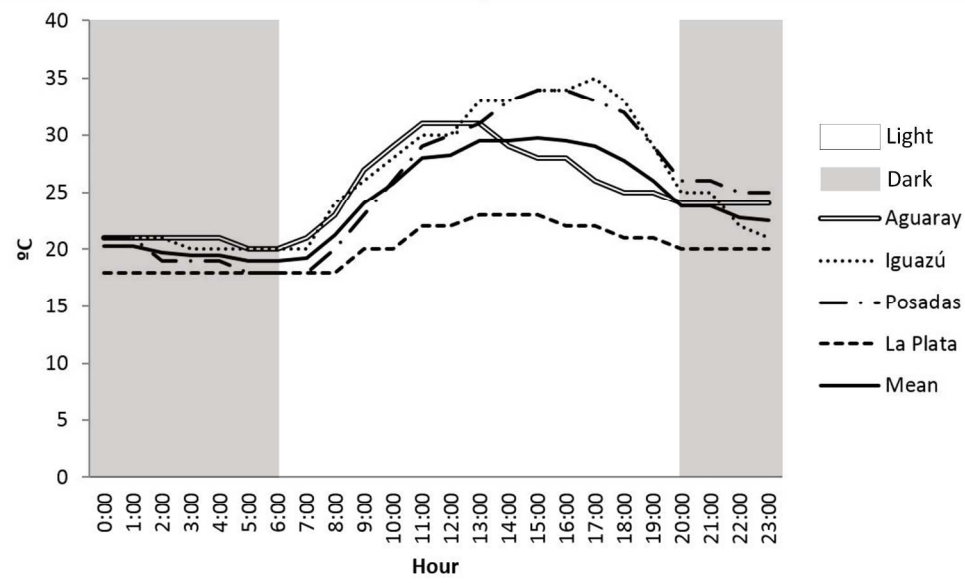
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437   **Figure 3**. Oviposition by *Aedes aegypti* from four populations in Argentina. The total

438   number of laid eggs/day is shown in the same bar, for the first (black) and the second (gray)

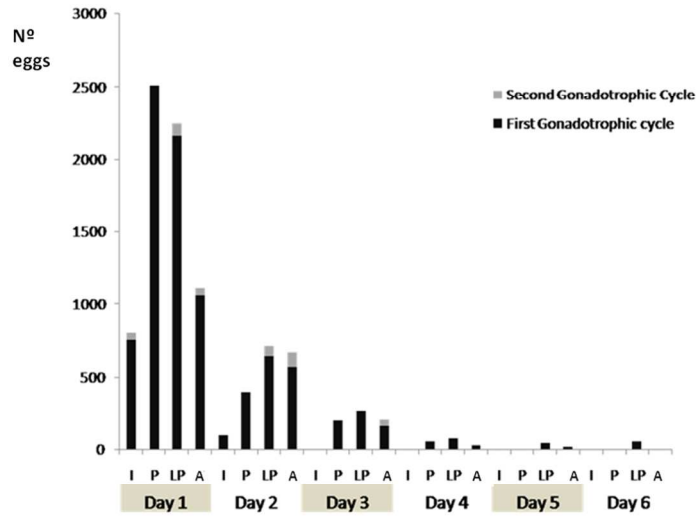439   gonotrophic cycles. I= Iguazu, LP=La Plata, P=Posadas, A= Aguaray.

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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

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

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

[3]DT: 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).~~

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

[5]M: 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.

[6]Note: 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	
		1st GC[2]	2nd GC	1st GC	2nd GC	1st GC	2nd GC	1st GC	2nd 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)

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

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

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

[4]Life 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 parameterRange [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 (*Tg*) (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 (*Ro*)	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 (*r*)	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 (*Tg*) (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 (*Ro*)	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 (*r*)	0.075c	0.057-0.0929	0.027d	0.015-0.040	0.079b	0.063-0.096	0.126a	0.110-0.143

[1]Temperature parameterrange at which the populations were held. Mean cycle: 20-30ºC. Site cycle: Iguazu (21-35ºC), La Plata (18-23ºC), Posadas (18-34ºC), Aguaray (21-31ºC) (Fig. 2).

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Avg[2]: average; values within row followed by a different letter were significantly different between populations (p<0.05, t-student test) within temperature parameter.

L-U[3]: Lower–Upper 95% limits for a confidence levelintervals.

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