OXFORD UNIVERSITY PRESS | Journal of Medical Entomology

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

Journal:	*Journal of Medical Entomology*
Manuscript ID	JME-2017-0335.R3
Manuscript Type:	Research
Date Submitted by the Author:	15-Mar-2018
Complete List of Authors:	Muttis, Evangelina; Centro de Estudios Parasitologicos y de Vectores, Insects Vectors Balsalobre, Agustin; Centro de Estudios Parasitologicos y de Vectores, Insects Vectors Chuchuy, Ailen; Centro de Estudios Parasitologicos y de Vectores, Insects Vectors Mangudo, Carolina; Instituto de Investigaciones en Energía No Convencional (INENCO, UNSa - CONICET), Insects Vectors Ciota, Alexander ; Wadsworth Center Center for Bioinformatics, Division of Infectious Disease Kramer, Laura; Wadsworth Center Center for Bioinformatics, Division of Infectious Disease Micieli, Maria; Centro de Estudios Parasitologicos y de Vectores, Insects Vectors
Please choose a section from the list:	Development, Life History
Field Keywords:	Vector Ecology, Development, Life History, Medical Entomology
Organism Keywords:	Aedes, Aedes aegypti, Culicidae, Diptera

María Victoria Micieli
Boulevard 120 s/n entre Av.60 y calle 64
054-221-4232327
Email: victoria@cepave.edu.ar

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

Evangelina Muttis[1], Agustin Balsalobre[1], Ailen Chuchuy[1], Carolina Mangudo[2], Alexander T. Ciota[3,4] , Laura D. Kramer [3,4], Micieli, María Victoria[1].

[1]Centro de Estudios Parasitológicos y de Vectores (CEPAVE-CCT-La Plata-CONICET – UNLP), Boulevard 120 e/61 y 62, 1900 La Plata, Argentina.

[2]Instituto de Investigaciones en Energía No Convencional (INENCO, UNSa - CONICET), Universidad Nacional de Salta, Av. Bolivia 5150, A4400FVY, Salta, Argentina.

[3]The Arbovirus Laboratory, Wadsworth Center, New York State Department of Health, Slingerlands, NY, United States of America

[4]Department of Biomedical Sciences, State University of New York at Albany School of Public Health, Albany, NY, United States of America

Abstract

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

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

Keywords: Mosquito, fitness, Argentina

Introduction

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

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

Here we present a comparative study about *Ae. aegypti* populations from Argentina in order to identify the life traits that respond to local adaptation and the traits that could be mostly influenced by temperature. In this sense, we selected four mosquito populations from three provinces: Salta from the Northwest, Buenos Aires from the South and Misiones from the Northeast area of this mosquito species distribution. Cohorts from each site were held at temperature values simulating a daily cycle, with an average of 25 ºC in order to determine their life-history traits and to make comparisons between populations. Additionally, we performed the study of the same populations by holding them at a daily temperature cycle which was approximately the same as the one registered at the surveyed area. The knowledge about the behavior of *Ae. aegypti* in different regions of the country, as well as the study of the same populations held at mean cycle temperature, will allow us to make inferences about the response of *Ae. aegypti* under different climatic scenarios that could be useful to define areas with greater potential of disease transmission.

Material and Methods

Study sites

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

Mosquitoes and environmental data collection

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

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

Experimental Procedures

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

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

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

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

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

Table life construction and definitions

The date and the total number of individuals that entered a given stage, died in that stage, and molted to the next stage were used as input for life table calculations (Deevey, 1947). The proportion of hatched eggs at the first submersion in water produced by the females of the cohort was used to estimate the number of initial eggs of each cohort. Daily mortality records were used to calculate survival as a function of age (lx). Survival (lx) was expressed as the percentage of individuals that reached the next instar/stage; the number of eggs laid daily was used to calculate the age-specific fecundity (mx), by dividing the total number of eggs laid each day(x) by the number of individuals alive at the end of that day. The (lx) and (mx) schedules allowed for the estimation of demographic parameters such as the intrinsic rate of natural increase (*r*), the net reproductive rate (*Ro*), and the mean generation time (*Tg*); complete definitions of these parameters and the formulas used for 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.

Demographic parameters. For each demographic parameter, we also estimated the confidence interval at a 95% significance level based on 1,000 bootstrap samples by random resampling with replacement from the initial individuals of each group. These calculations were carried out using a computer program developed in Delphi Language, cordially provided by Dr. Rabinovich. The statistical comparison of demographic parameters was carried out with the Student T-test for independent samples.

All statistical methods were performed using R software (version 3.3.2).

Results

Aedes aegypti populations response at common mean cycle temperature

Immature stages

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

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

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

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

Demographic parameters

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

Aedes aegypti populations response at specific-site temperature cycles

Immature traits

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

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

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

Moreover, we identified populations with unique traits. Iguazu female were shown to have the lowest survival rate and, concordantly, the shortest oviposition periods. Iguazu females completed oviposition in one or two days, which represents at least half the time of the other populations. La Plata's population had the lowest blood feeding rate. However, the females had the longest survival, which could permit time for a third GC. When this population was held at an average temperature of 25 ºC, the blood feeding rate increased to very high values, while the survival remained high. In addition, the demographic parameters improved substantially. The combination of these effects could have implications for virus transmission in a climatic change scenario with a warmer environment. These populations could feed more frequently and for a longer period of time. This site is also distinctive with its long-term oviposition pattern, which could be related to high female survival, and this behavior could give them greater dispersion capacity and more possibilities of immature survival.

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

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).

Acknowledgments

The authors would like to thank Dr. Fabricio Tejerina and Neris Gauto for providing us with the populations of mosquitoes used for establishing the mosquito colonies from Misiones. We also want to thank Graciela Minardi for her assistance in statistical analysis. We appreciate the financial support of the *Fulbright Visiting Scholar Program* and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

References

Burkart, R., N. Barbaro, O. Sanchez and D. A. Gomez.-1999. Eco-Regiones de la Argentina. Administracion de Parques Nacionales, Buenos Aires. 43 pp

Carrington, L. B., S. N. Seifert, N. H. Willits, L. Lambrechts, and T. W. Scott. 2013. Large diurnal temperature fluctuations negatively influence *Aedes aegypti* (Diptera: Culicidae) life history traits. J. Med. Entomol. 50: 43-51.

Christophers, S. 1960. *Aedes aegypti* (L.) the yellow fever mosquito: its life history, bionomics and structure. Cambridge University Press, London.

Deevey, E. S. J. 1947. Life tables for natural populations of animals. Q. Rev. Biol. 22: 283-314.

De Majo, M. S, S. Fischer, M. Otero, and N. Schweigmann. 2013. Effects of thermal heterogeneity and egg mortality on differences in the population dynamics of *Aedes aegypti* (Diptera: Culicidae) over short distances in temperate Argentina. J. Med. Entomol. 50: 543-555.

De Sousa, G., G. Avilles, and C. N. Gardenal. 2000. Allozymic polymorphism in *Aedes aegypti* populations from Argentina. J. Am. Mosq. Control. Assoc. 16: 206-209.

Domínguez, M. C., F. F. Ludueña-Almeida, and W. R. Almirón. 2000. Dinámica poblacional de *Aedes aegypti* (Diptera : Culicidae) en Córdoba Capital. Revista de la Sociedad Entomológica Argentina. 59: 41-50.

Gerberg, E. J, D. R. Barnard, and R. A. Ward. 1994. Manual for mosquito rearing and experimental techniques. J. Am. Mosq. Control. Assoc, Inc. Lake Charles.

Grech, M. G., F. Ludueña-almeida, and W. R. Almirón. 2010. Bionomics of *Aedes aegypti* subpopulations (Diptera : Culicidae) from Argentina. J. Vector. Ecol. 35: 277-285.

Grech, M., A. Visintin, M. Laurito, E. Estallo, P. Lorenzo, I. Roccia, M. Korin, F. Goya, F. Ludueña Almeida, and W. R. Almirón. 2012. New records of mosquito species (Diptera: Culicidae) from Neuquén and La Rioja provinces, Argentina. Revista de Saúde Pública. 46: 387-389.

406 **Gubler, D. J. 2004.** The changing epidemiology of yellow fever and dengue, 1900 to 2003:
407 full circle?. Comp. Immunol. Microbiol. Infect. Dis. 27:319-330.
408 **Llinás, G. A., C. N. Gardenal. 2011.** Introduction of different lineages of *Aedes aegypti* in
409 Argentina. J. Am. Mosq. Control. Assoc. 27: 429-432.
410 **Micieli, M. V., and R. E. Campos. 2003**. Oviposition activity and seasonal pattern of a
411 population of *Aedes (Stegomyia) aegypti* (L.) (Diptera: Culicidae) in subtropical Argentina
412 Mem. Inst. Oswaldo Cruz. 98: 659-663.
413 **Mohammed, A. and D. D. Chadee. 2011.** Effects of different temperature regimens on the
414 development of *Aedes aegypti* (L.)(Diptera: Culicidae) mosquitoes. Acta Tropica. 119: 38-
415 43.
416 **Rabinovich, J. E., and E. L. Nieves. 2011.** Vital Statistics of Triatominae (Hemiptera:
417 Reduviidae) under laboratory conditions. III. *Rhodnius neglectus*. J. Med. Entomol. 48:775-
418 787.
419 **Rodriguez-Morales, A. J. 2015**. Dengue and chikungunya were not enough : now also
420 Zika arrived. Archivos de Medicina. 11: 1-5.
421 **Rondan Dueñas, J., G. Albrieu Llinás, M. Panzatta-Dutari, and C. Gardenal. 2009.**
422 Two different routes of colonization of *Aedes aegypti* in Argentina from neighboring
423 countries. J. Med. Entomol. 46: 1344-1354.
424 **Tejerina, E. F., F. F. L. Almeida, and W. R. Almirón**. **2009.** Bionomics of *Aedes*
425 *aegypti* subpopulations (Diptera: Culicidae) from Misiones Province, northeastern
426 Argentina. Acta Tropica. 109: 45-49.

427

428

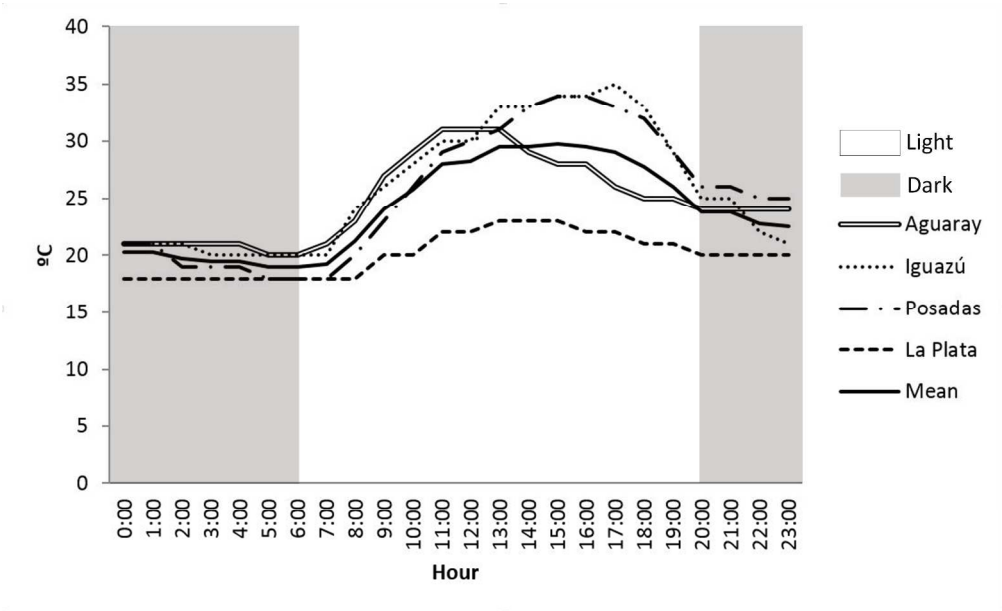
Legends

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

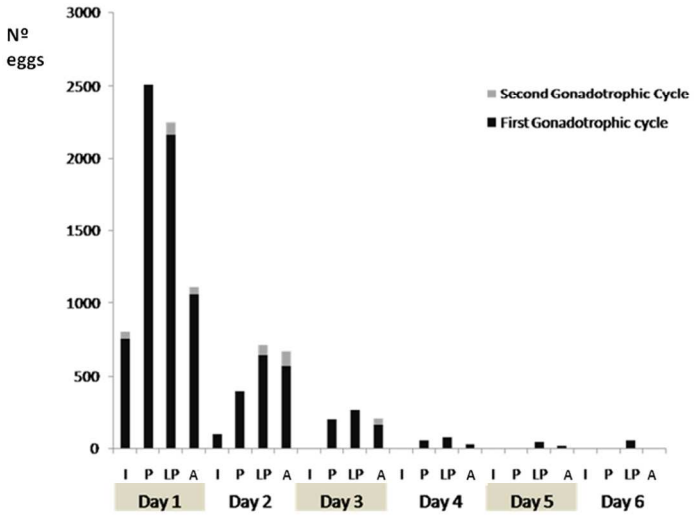
Figure 2. Temperature cycle used during experiments measuring *Ae. aegypti* life-history parameters for four Argentinean populations: Specific-site cyclic temperature (Aguaray, Iguazu, Posadas, La Plata) and mean cyclic temperature (Mean).

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

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.

		Iguazú				La Plata				Posadas				Aguaray			
T[1]	Instar/Stage~~/Instar~~	*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.

		Iguazu		La Plata		Posadas		Aguaray	
T[1]	Reproductive feature	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 parameter~~Range~~ [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 parameter~~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).

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 ~~level~~intervals.

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

Formatted: Line spacing: Double