Campos R, Zanotti G, Di Battista CM, Gimenez JO, Fischer S. 2020: Differential inhibition of egg hatching in *Aedes aegypti* populations from localities with different winter conditions

This is a preprint (pre-review)-copyedited, author-produced version of an article accepted for publication in the ***Bulletin of Entomological Research*** *following peer review. The version of record is available at:*
https://www.cambridge.org/core/journals/bulletin-of-entomological-research/article/abs/differential-inhibition-of-egg-hatching-in-aedes-aegypti-populations-from-localities-with-different-winter-conditions/4B126F236C3824F08265E214CC3C7363
DOI: https://doi.org/10.1017/S0007485320000681

……

Differential inhibition of egg hatching in *Aedes aegypti* populations from localities with different winter conditions

Raúl E. Campos[1], Gabriela Zanotti[2], Cristian M. Di Battista[1], Javier O. Gimenez[3], Sylvia Fischer[2]

1 Instituto de Limnología “Dr. Raúl A. Ringuelet”, Universidad Nacional de La Plata-CONICET, CCT La Plata, Boulevard 120 y 62 Nº 1437, La Plata (B 1900), Buenos Aires, Argentina.

2 Departamento de Ecología, Genética y Evolución, and Instituto IEGEBA (UBA-CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires. Ciudad Universitaria, Pabellón 2, 4to piso. Laboratorio 54. C1428EHA, Buenos Aires, Argentina.

3 Universidad Nacional del Nordeste (UNNE), Instituto de Medicina Regional, Área de Entomología, Avda. Las Heras, 727, 3500, Resistencia, Chaco, Argentina.

Corresponding author: Sylvia Fischer

Departamento de Ecología, Genética y Evolución, and Instituto de Ecología, Genética y Evolución de Buenos Aires (UBA-CONICET), Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria, Pabellón 2, 4to piso, Laboratorio 54. C1428EHA, Buenos Aires, Argentina.

sylvia@ege.fcen.uba.ar

Running head: Egg hatching of different *Aedes aegypti* populations

Abstract

In Argentina, the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae) is distributed from subtropical to temperate climates. Here, we hypothesized that the expansion of *Ae. aegypti* into colder regions is favoured by high phenotypic plasticity and an adaptive inhibition of egg hatching at low temperatures. Thus, we investigated the hatching response of eggs of three populations: one from a subtropical region (Resistencia: Re) and two from temperate regions (Buenos Aires City: BA, and San Bernardo: SB) of Argentina. Eggs collected in the field were raised in three experimental colonies. F1 eggs were acclimated for seven days prior to immersion at 7.6°C or 22ºC (control eggs). Five immersion temperatures were tested: 7.6, 10.3, 11.8, 14.1 and 16°C (range of mean winter temperatures of the three localities). A second immersion at 22°C was performed two weeks later to assess the inhibition to hatch under favourable conditions. After the first immersion, we compared the proportions of hatched eggs and dead larvae between treatments, whereas after the second immersion we compared the hatching response between the three populations. The factors that most influenced the egg hatching response were the geographical origin of the populations and the immersion temperature, but not the acclimation temperature. The proportions of hatching and larval mortality at low temperatures were higher for Re than for BA and SB, whereas the hatching response at ambient temperature was lower for SB than for BA and Re. The results support the hypothesis that populations from colder regions show an adaptive inhibition of egg hatching.

Keywords: *Aedes aegypti* – Hatching response - Low temperature – Acclimation - Mortality - Argentina

Introduction

Invasive species are of great concern because of their effects on native species and ecosystems and/or human activities and health (Lounibos & Kramer, 2016). The expansion of invasive species to novel environments is conditioned by the ability of invaders to persist under the new conditions, especially if these are stressful. Over short time scales, the most important mechanism that enhances survival is phenotypic plasticity (Chown & Terblanche, 2007; Whitman & Agrawal, 2009), whereas, over longer time scales, another important factor that may also aid in the naturalization process (Huey et al., 2005; Lee et al., 2007) is rapid evolutionary adaptation (i.e. natural selection).

A major factor limiting the range expansion of a species is the tolerance to extreme temperatures (Chen & Kang, 2005; Chown & Terblanche, 2007). In regions that experience seasonal fluctuations in temperature, the ability of a species to tolerate low winter temperatures may be especially important, and several examples of rapid changes in cold tolerance after invasion or range expansion have been documented (Sinclair et al., 2012). Organisms expanding their distribution range towards colder climate may survive the lower temperature season in a dormant stage (reviewed in Tauber et al., 1986). Such strategy is common in many mosquito species of the genus *Aedes*, which overwinter under unfavourable conditions as dormant eggs (Vinogradova, 2007; Denlinger & Armbruster, 2014).

The yellow fever mosquito *Aedes aegypti* is the main vector of arboviruses like the dengue, Zika and chikungunya viruses (Lounibos & Kramer, 2016), which affect the health of millions of people around the world (Mayer et al., 2017). This mosquito of tropical origin is abundant in tropical and subtropical regions (Kraemer et al., 2015). It is considered an invasive species (Lounibos & Kramer, 2016), whose distribution range has been predicted to expand in the future mostly in tropical and subtropical areas (Kraemer et al., 2019). This is because the main limitations assumed to limit expansion towards colder regions are low winter temperatures (Brady et al., 2014). However, during recent decades, *Ae. aegypti* has increased its geographic range, colonizing also temperate climate regions (Eisen et al., 2014), where the limit for successful establishment is assumed to be the ability to overwinter successfully (Eisen et al., 2014; Medlock et al., 2015; Lima et al., 2016). The dormant eggs of *Ae. aegypti* are much more tolerant to low temperatures than the larval, pupal or adult stages (Davis, 1932). Thus, the successful establishment in temperate or cold regions should be aided by an adequate strategy to remain in the egg stage during the cold season (e.g. by inhibiting the hatching of eggs during periods when temperatures are not favourable to complete development).

In Argentina, after the continent-wide programme implemented between 1930 and 1960 to control *Ae. aegypti*, the species was considered eradicated in 1965 (Soper, 1967). However, it was detected again in the provinces of Misiones and Formosa in 1986 (Curto et al., 2002). In 1991, it was first recorded more than 1000 km southwards, in the metropolitan area of Buenos Aires (Campos, 1993), where abundances increased steadily during the following two decades (Fischer et al., 2017). In addition, *Ae. aegypti* has expanded towards colder climate areas, including the provinces of Buenos Aires (Zanotti et al., 2015), La Pampa (Rossi et al., 2006; Diez et al., 2014), Mendoza (Domínguez & Lagos, 2001), Neuquén (Grech et al., 2013), San Luis (Visintin et al., 2009), San Juan (Carrizo Páez et al., 2016), and Río Negro (Rubio et al., 2020).

Within Buenos Aires province, the distribution has expanded to the south, currently covering localities such as Tandil (Rubio et al., 2020), Dolores, and various cities on the Atlantic coast, including San Bernardo and Villa Gesell (Zanotti et al., 2015). In several of the recently colonized localities, average winter temperatures (June-August) are lower than 9.5ºC, and the period of monthly average temperatures below 12ºC extends for 4-5 months. These conditions are considered unfavourable for immature development of *Ae. aegypti* (Eisen et al., 2014). However, in most temperate regions (including those in Argentina), during the winter season, there are also short periods of higher temperatures (Rusticucci et al., 2003), which could trigger the hatching of the dormant eggs. When colder conditions return, the unfavourable temperatures may prevent the newly hatched larvae from completing their development and/or increase their mortality (De Majo et al., 2017). Such mortality is expected to be larger in the coldest regions, such as near the edge of the distribution, and thus an adaptive response to inhibit egg hatching and preserve the highest number of eggs as possible for the next reproductive season should be observed.

In this study, we hypothesized that the range expansion of *Ae. aegypti* towards colder regions in Argentina is aided by an adaptive inhibition of the egg hatching response at low temperatures or by a high phenotypic plasticity to environmental conditions, without a specific adaptation in populations from colder areas. Thus, the aim of the present study was to investigate the hatching response of eggs of three populations: one from a subtropical region (Resistencia: Re) and two from temperate regions (Buenos Aires City: BA, and San Bernardo: SB) of Argentina.

Materials and methods

Populations studied

Three populations of *Ae. aegypti*, collected from Re, BA and SB, which are localities with contrasting winter temperatures and durations, were studied.

Re (Resistencia) (27° 27' 0.78" S - 58° 59' 33.98" W) is a city located towards the north east of Argentina, in the province of Chaco (Figure 1). The city covers an area of 562 km^2 and has near 290,700 inhabitants (INDEC, 2010). The climate is subtropical without a dry season, with annual mean temperature of 21.3ºC and cumulative rainfall of 1324 mm. Mean monthly temperatures are above 15ºC during the whole year (Figure 2). Thus, the temperature can be considered favourable for the development of *Ae. aegypti* throughout the year. This locality has been invaded by *Ae. aegypti* since 1997 (Stein & Oria, 2002).

BA (Buenos Aires City) (34° 36' 13.26" S - 58° 22' 53.61" W) is located in the north east of Buenos Aires province, on the coast of the Río de la Plata river (Figure 1). The city covers an area of 203 km^2, and has a population of approximately three million inhabitants (INDEC, 2010). Buenos Aires City is part of an urban agglomeration with 13 million inhabitants in an area of 2680 km^2, called the Metropolitan Area of Buenos Aires. The climate is temperate humid, with an annual mean temperature of 16.8ºC and cumulative rainfall of 1040 mm. Mean monthly temperatures are below 12ºC from June to August (Figure 2). Thus, the period unfavourable for *Ae. aegypti* development lasts approximately three months, and, during this period, the overall average temperature is 11ºC.

Finally, SB (San Bernardo) (36° 41' 10.92" S -56° 40' 45.11" W) is located in the south east of Buenos Aires province, on the coast of the Atlantic Ocean (Figure 1). This is a small city of 4.3 km^2, with 8133 permanent inhabitants (INDEC, 2010), whose population increases during the summer because it is a touristic location. The climate is temperate oceanic, with an annual mean temperature of 15ºC and a cumulative rainfall of 953 mm. Mean monthly temperatures are below 12ºC from May to September (Figure 2). Thus, in this case, the period considered unfavourable for *Ae. aegypti* development lasts approximately five months, and, during this period, the overall average temperature is 10.4ºC.

Figure 1: Location of the localities from which the *Aedes aegypti* populations studied were collected. Re: Resistencia, BA: Buenos Aires City, SB: San Bernardo.

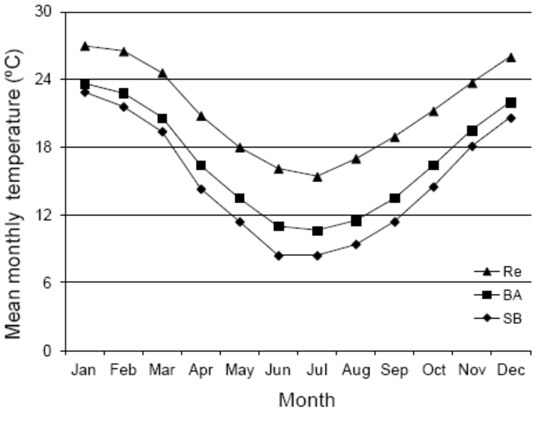

Figure 2: Mean monthly temperatures for Resistencia (Re), Buenos Aires City (BA) and San Bernardo (SB) obtained from climate-data.org.

Source of eggs for the experiment

The eggs used in the experiment were obtained from three experimental colonies (one for each locality) maintained at 24ºC under a photoperiod of 12:12 (Light:Dark). The colonies were initiated simultaneously, starting from field-collected eggs. The eggs were collected with ovitraps in late summer and early fall (February-March) of the same year. After hatching, the larvae were fed *ad libitum* with a solution of powdered baker´s yeast. A few days after the emergence of the last adults, they were provided access to a guinea pig to obtain a blood meal. Blood-fed females were separated and transferred to individual cages (6 cm in height x 3 cm in diameter), which contained a wooden paddle (a tongue depressor used for medical purposes) for egg laying and a raisin as a source of sugar. The individual cages were inspected daily, the paddles with eggs were maintained under the same conditions of photoperiod and temperature for at least one week to ensure the complete development of the embryos, and the females were separated and killed by freezing. All the eggs were collected within two weeks. A total of 250 replicates (where each replica was a paddle with eggs from the same female) were obtained: 77 from Re, 81 from BA, and 88 from SB.

Experimental design

The experiment consisted in the immersion at low temperatures of eggs from each population analyzed, previous acclimated to low temperatures. For acclimation, paddles with eggs were stored for seven days prior to immersion in a cold chamber (commercial fridge) at 7.6ºC. Control eggs were stored for the same period of time at room temperature of 22ºC. After this, five immersion temperatures were tested: 7.6, 10.3, 11.8, 14.1 and 16ºC, which aimed to represent approximately the range of the mean winter temperatures of the three localities from where the mosquito eggs were collected. For each population, treatments consisted in the combination of acclimation and immersion temperatures, and between 6 and 10 replicates (substrate with 7 to 135 eggs from the same female) from each population were assigned to each treatment.

For immersion, paddles with eggs were individually placed in hatching tubes (50 ml Falcon® tubes), containing 40 ml of reverse osmosis filter water. The hatching tubes were located in thermal baths at the corresponding temperature prior to immersion to stabilize the experimental temperature. After 48 hours, the paddles were transferred to a dry tube placed at room temperature, and the live and dead larvae in each tube were counted.

A second immersion at 22ºC was performed two weeks later, to assess the viability of the remaining eggs. To this end, the eggs were immersed in a new hatching tube, containing a 40-ml solution of 47 mg powdered baker´s yeast/l of filtered water. The addition of yeast aimed to generate a stronger stimulus than water only, as previous experiments have shown that a larger number of eggs hatch under these conditions (Byttebier et al., 2014). After 48 hours, the number of live and dead larvae in each replicate was counted. Since this process took about a week, not all replicates were counted at the same time. Also, on each substrate, the number of intact eggs was counted, and the intact eggs were bleached with a solution of sodium hypochlorite to allow direct observation of the embryos. Creamy-white embryos with visible eyes, abdominal segmentation, and a hatching spine were considered viable, while those without these characteristics were considered nonviable (Farnesi et al., 2009).

Data analysis

For each population (all treatments pooled), we calculated the percentages of hatched eggs (100 * number of hatched eggs / total number of viable eggs), the percentage of replicates with some hatching (100 * number of replicates with at least one hatched egg / total number of replicates), and the percentage of dead larvae (100 * number of dead larvae / total number of larvae observed) after the first immersion.

For each replicate, the number of viable eggs was calculated as the sum of the total number of larvae observed during the two immersions and the number of viable embryos counted after the bleaching process. The proportion of eggs hatched during the first immersion was calculated as the number of larvae observed divided by the number of initial viable eggs. To analyze the predisposition to hatch under favourable conditions, the proportion of eggs hatched during the second immersion in yeast solution at ambient temperature was calculated as the number of larvae divided by the number of viable eggs, which was obtained as the number of remaining viable embryos plus the number of larvae counted during the immersion.

For the immersion at low temperatures, the effects of acclimation, immersion temperature, population, and all the possible two-way interactions on the hatching response were analyzed with a generalized linear model (GLM). The response variable was the number of larvae observed after the first immersion of the total number of viable eggs of each replicate. Immersion temperatures were considered as continuous variables, whereas the acclimation treatment and population were included as categorical variables. Non-significant terms were sequentially deleted from the full model. The quasi binomial family with the logit link function was used, because, in a first exploration of the data with the binomial distribution, an over dispersion in the resulting model was detected (Zuur et al., 2009). The same analysis was used to analyze the number of dead larvae of the total number of larvae observed during the first immersion.

For the second immersion at ambient temperature, the predispositions to hatch were analyzed graphically. To this end, the replicates were ordered by increasing value within each population, and plotted together to visualize the variation in behaviour of each population.

The GLM analyses were performed with the R package, Version 3.6.2, (R Core Team, 2019), accessed through a user friendly interface in Infostat Software (Di Rienzo et al., 2019). For post-hoc comparisons among populations, the Fisher LSD rank test was used (Conover, 1999).

Results

A total of 17348 viable eggs were used, 1442 (8.3%) of which hatched during the first immersion when analyzing all the treatments together. Approximately one third of the replicates showed some hatching response during the first immersion, and this proportion was similar among populations. The hatching response during the first immersion was low, although with some differences among populations: lowest in SB, intermediate in BA, and highest in Re (Table 1).

Table 1: Proportions of hatching responses and larval mortality for three populations of *Aedes aegypti* of Argentina. In parentheses: the number of hatched or dead larvae from the total analyzed.

	Resistencia	Buenos Aires City	San Bernardo
Proportion of replicates with some hatching after first immersion	0.351 (27/77)	0.346 (28/81)	0.307 (27/88)
Proportion of eggs hatched after first immersion	0.131 (708/5400)	0.076 (449/5929)	0.047 (285/6019)
Proportion of dead larvae after first immersion	0.316 (224/708)	0.200 (90/449)	0.056 (16/285)
Proportion of eggs hatched after second immersion	0.378 (1772/4692)	0.365 (1999/5480)	0.184 (1056/5734)

A large variability in the hatching response of different replicates was observed, with most replicates showing a low or null hatching response (Figure 3). Some replicates exhibited a high hatching response (replicates with a proportion of hatched eggs higher than 0.5) at immersion temperatures equal to or higher than 10.3°C for Re, equal to or higher than 14.1°C for BA, and equal to 16°C for SB.

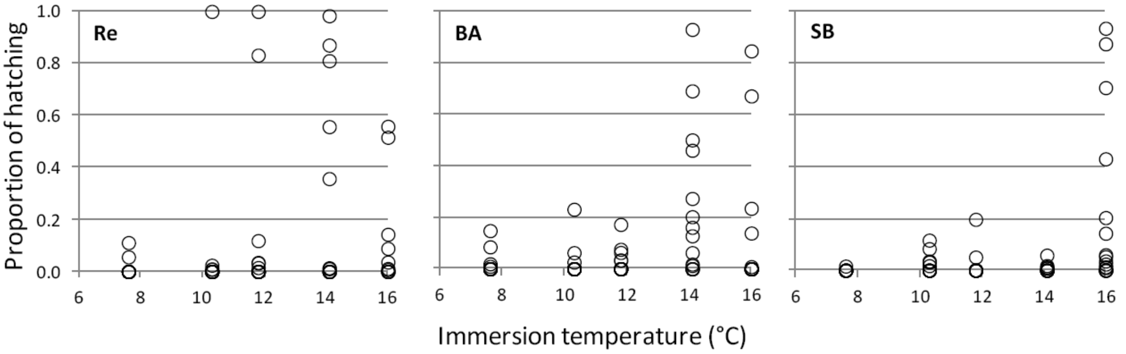

Figure 3: Hatching response of different replicates as a function of immersion temperature. Re: Resistencia, BA: Buenos Aires City, SB: San Bernardo.

The results showed significant effects of the population (GLM Wald test: $F_{2,242} = 5.64$, $p < 0.01$) and the immersion temperatures (GLM Wald test: $F_{1,242} = 25.57$, $p < 0.001$) on the hatching response, but not of the acclimation temperature or the two-way interactions. The immersion temperature had positive effects on the hatching response (estimate = 0.35; SE = 0.07; t = 5.06; $p < 0.001$), i.e. more hatching was detected at higher temperatures in the three populations. Furthermore, a significant positive effect of Re (estimate = 0.74; SE = 0.35; t = 2.11; $p < 0.05$), and a non significant negative effect of SB (estimate = -0.54; SE = 0.43; t = -1.28; p = 0.2) were detected when compared with BA. The post-hoc test confirmed a significantly higher hatching response for Re than for SB and BA, with no differences between the latter two populations.

Larval mortality was marginally significantly affected by the population (GLM Wald test: $F_{2,79} = 2.97$, $p = 0.0572$), but not by the acclimation or immersion temperature or the two-way interactions. Larval mortality was highest for Re, intermediate for BA, and lowest for SB (Table 1), and post-hoc comparisons showed that differences were significant between Re and SB ($p < 0.05$).

A high variability among replicates was observed for the immersion at ambient temperature. For all populations, some replicates showed complete hatching, some partial hatching, and others no hatching of viable eggs (Figure 4). The curves of rank-ordered hatching predisposition of replicates at ambient temperature showed two patterns: one with the populations of Re and BA, which presented an approximately sigmoid curve, and the other with the population of SB, which showed an exponential form (Figure 4). The curves of Re and BA were similar, and showed approximately 60% of the replicates with a hatching inhibition of 50% of their eggs. In contrast, SB showed a higher frequency of replicates with low hatching predisposition, and a lower frequency of replicates with high hatching predisposition. The proportion of replicates with null hatching was relatively low (less than 15%), and similar for the three populations analyzed (Figure 4).

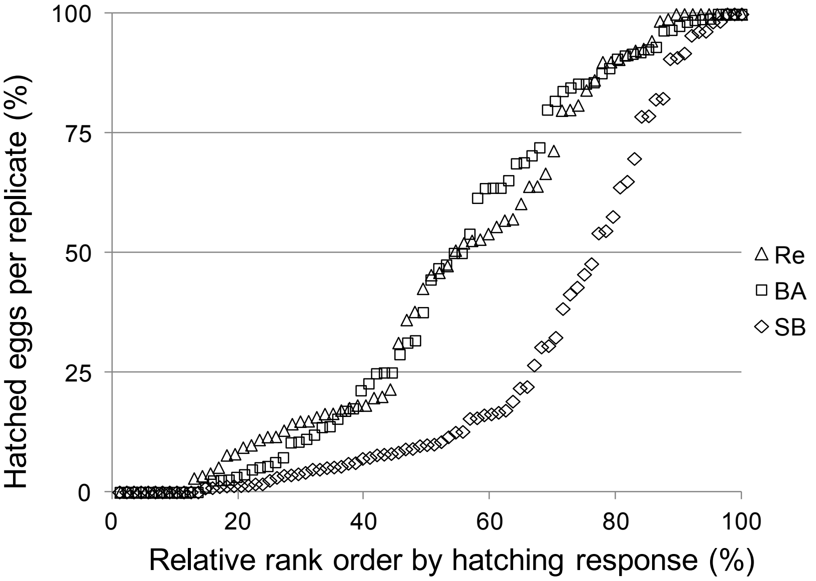

Figure 4: Cumulative hatching response after two immersions for each replicate within each population, ordered from lower to higher hatching. Re: Resistencia, BA: Buenos Aires City, SB: San Bernardo.

Discussion

This study provides evidence of an adaptive response of the populations from colder climates to low temperatures. This adaptation is evidenced by the lower hatching response of the eggs and the lower initial mortality of the larvae at low temperatures of the two populations from the temperate region (BA and SB), as compared to the population from the subtropical region (Re).

Eggs should hatch when the cost/benefit ratio is more favourable outside the egg shell than inside it (Warkentin, 2011). Thus, an increase in the hatching response is expected when thermal conditions are more favourable for larval development. This was observed in our study, evidenced by the positive relationship of the hatching response with the immersion temperature, and also in previous studies both in field (Bond, 1970; De Majo et al., 2017) and laboratory conditions (Byttebier et al., 2014).

This phenotypic plasticity of the egg hatching response as a function of temperature differed between the three populations studied, as shown by the lower hatching responses of BA and SB at all immersion temperatures, and some replicates with a high hatching response only at 14.1ºC and 16ºC respectively, which are temperatures at which larvae can successfully complete development (De Majo et al., 2019). In contrast, for the Re population, replicates with high hatching responses were observed at temperatures close to or lower than 12ºC, which is the lowest temperature for which larval development could be completed, with a relatively high mortality (De Majo et al., 2019). Thus, it could be considered that the hatching response at these temperatures would be inadequate, suggesting that the population that does not experience these temperatures naturally shows a less adjusted response to low temperatures. This is also consistent with the differences in initial mortalities observed between the three populations, which suggest that the risk of hatching at low temperatures also differs between populations, being higher for the population from the subtropical region and lower for the populations naturally exposed to lower winter temperatures.

As a consequence of the low hatching response of the SB population, in addition to the lower larval mortality, a higher survival of eggs during the winter period should occur. This strategy might be one of the keys that have allowed *Ae. aegypti* to colonize and establish in colder temperate areas such as the centre and south of Buenos Aires province, in Argentina. In contrast, the population from Re, with the highest hatching response at low temperatures, was harmed by the high initial mortality of individuals that hatched, suggesting that this population of subtropical origin has not developed the same ability as those from the temperate region. In other insect species, differences in the tolerance to low temperatures have been demonstrated to be associated with the temperature regimes of the place where they live, and are thus supposed to be adaptive (Chen & Kang 2005).

The lack of effect of the acclimation temperature on the hatching response differs from previous observations in both laboratory (Byttebier et al., 2014) and field studies (De Majo et al., 2017), and might be related to the duration of the acclimation period in our study, which might not have been long enough to induce differences in the hatching response.

The hatching responses observed for the three populations analyzed in the present study were unexpectedly low as compared to those previously recorded for the *Ae. aegypti* populations from Buenos Aires city (25%, 41%, and 56% at 12ºC, 14ºC and 16ºC respectively) (Byttebier et al., 2014). This could be caused by the low age of the eggs used in our study (less than a month), which might have a lower predisposition to hatch. We have previously found that the hatching response at 21ºC for *Ae. aegypti* from Buenos Aires is lowest for recently laid eggs and increases gradually in eggs of increasing age (Fischer et al., 2019). This would explain the higher hatching response of 4-month-old eggs previously observed at temperatures similar to those used in the present study (Byttebier et al., 2014). Although these patterns differ from those observed in other laboratory studies where a decrease in the hatching response with time was observed (Zheng et al., 2015, Brown et al., 2017), the differences might be related to the fact that, in these latter works, the hatching rate was calculated without considering the non-viable eggs.

Our results show that different individuals respond to environmental stimuli in different ways, which is clear from the heterogeneity in the hatching response among replicates of each treatment and between treatments. This heterogeneity showed no clear differences between the three populations analyzed in our study. In previous studies, Gillett (1995a,b) also observed a large variability in the hatching response between eggs laid by different females of *Ae. aegypti* from two tropical African populations (Nigeria and Tanganyika), and this pattern has also been recently confirmed for the population from Buenos Aires City previously studied (Fischer et al., 2019). Although this seems to be a frequent phenomenon, it has been poorly documented, probably because most experimental studies on hatching group eggs from different females (e.g. Weissman Strum & Kindler, 1963; Ponnusamy et al., 2011; Thomas et al., 2012; Byttebier et al., 2014).

An unexpected result of the present study was the differences between the three populations in the second immersion at favourable temperatures. The lower hatching of the eggs from SB suggests that, besides the plasticity in response to thermal conditions, this population has an additional mechanism of inhibition. This mechanism might be the induction of a deep dormancy (probably diapause) induced by parental photoperiods that anticipate unfavourable thermal conditions, as we have recently documented for the *Ae. aegypti* population from Buenos Aires City (Fischer et al., 2019). Considering the higher latitude and the colder climate of SB as compared to BA, and that BA is the most probable source of colonizers for SB, it would be expected that the population of SB has the same deep dormancy mechanism induced by the photoperiod. If so, it could be hypothesized that the lower response of SB as compared to that of BA might be related to differences in the critical photoperiod to inhibit hatching in both populations, an issue that should be assessed in future studies. Other mosquito species, such as *Wyeomyia smithii* (Bradshaw, 1976), *Ae. sierrensis* (Vinogradova, 2007), and *Ae. albopictus* (Urbanski et al., 2012), have also been shown to present variations in the critical photoperiod to induce diapause, with a general pattern of longer photoperiods in populations from higher latitudes.

In conclusion, the differences here observed in the hatching response of the three populations studied during the first immersion support the hypothesis that the populations of *Ae. aegypti* from colder areas are better adapted to survive the low temperature season by a lower hatching and a lower larval mortality. The other side of this hypothesis would be to expect that the cold climate populations would show higher hatching at favourable temperatures, to take the most possible advantage of the warm periods to complete the development and reproduction cycles. However, our results showed the opposite pattern, with a lower hatching response of the most austral population under favourable thermal conditions, whose causes are not yet known. In addition, the results of the present study provide evidence of two possible mechanisms by which *Ae. aegypti* populations are adapting to colder climates: a more efficient inhibition of the hatching response under thermal unfavourable conditions and the inhibition of the hatching response when anticipating unfavourable thermal conditions. This issue should be further assessed in future studies.

Acknowledgements

We thank the students from the Mosquito Study Group for their assistance during the experiment. We thank María Victoria Gonzalez Eusevi for the editing and revision of the English of the manuscript. This study was supported by grants PICT-2012-1254 (FONCYT), and UBACyT 20020130100778BA (Universidad de Buenos Aires, Argentina). During the development of this work, Zanotti G. was doctoral fellow from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Di Battista C.M. and Giménez J. O. were doctoral fellows of CONICET. Fischer S. and Campos, R.E. are researchers of CONICET.

The authors declare no conflicts of interest.

References

Bond H.A., Keirans J.E. & Babbitt M.F. (1970) Environmental influences on the viability of overwintering *Aedes aegypti* (L.) eggs. *Mosquito News*, **30**, 528-533.

Bradshaw W.E. (1976) Geography of photoperiodic response in diapausing mosquito. *Nature*, **262**, 384-385.

Brady O.J., Golding N., Pigott D.M., Kraemer M.U.G., Messina J.P., Reiner R.C.Jr., Scott T.W., Smith D.L., Gething P.W. & Hay S.I. (2014) Global temperature constraints on *Aedes aegypti* and *Ae. albopictus* persistence and competence for dengue virus transmission. *Parasites & Vectors*, **7**, 338.

Brown H.E., Smith C. & Lashway S. (2017) Influence of the length of storage on *Aedes aegypti* (Diptera: Culicidae) egg viability. *Journal of Medical Entomology*, **54**, 489-491.

Byttebier B., De Majo M.S. & Fischer S. (2014) Hatching response of *Aedes aegypti* (Diptera: Culicidae) eggs at low temperatures: effects of hatching media and storage conditions. *Journal of Medical Entomology*, **51**, 97-103.

Campos R.E. (1993) Presencia de *Aedes* (*Stegomyia*) *aegypti* L. 1762 (Diptera: Culicidae) en la localidad de Quilmes, Provincia de Buenos Aires, República Argentina. *Revista de la Sociedad Entomológica Argentina*, **52**, 36.

Carrizo Páez R.E., Carrizo Páez M.A. & Murúa A.F. (2016) First record of *Aedes aegypti* (Diptera: Culicidae) in San Juan, Argentina. *Revista de la Sociedad Entomológica Argentina*, **75**, 93-95.

Chen B. & Kang L. (2005) Insect population differentiation in response to environmental stress. *Progress in Natural Science* **15 (4)**, 289:296.

Chown S.L. & Terblanche J.S. (2007) Physiological diversity in insects: ecological and evolutionary contexts. *Advances in Insect Physiology*, **33**, 50-152.

Conover W.J. (1999) Practical nonparametric statistics. 3rd ed. John Wiley & Sons, New York, NY.

Curto S.I., Boffi R., Carbajo A.E., Plastina R. & Schweigmann N. (2002). Reinfestación del territorio argentino por *Aedes aegypti*. Distribución geográfica (1994-1999). In O. D. Salomón. Actualizaciones en Artropodología Sanitaria Argentina, Fundación Mundo Sano, Buenos Aires, p. 127-137.

Davis N.C. (1932). The effects of heat and of cold upon *Aedes (Stegomyia) aegypti*. Part I The survival of *Stegomyia* eggs under abnormal temperature conditions. *American Journal of Epidemiology* **16 (1)**, 177-191.

De Majo M.S., Montini P. & Fischer S. (2017) Egg hatching and survival of immature stages of *Aedes aegypti* (Diptera: Culicidae) under natural temperature conditions during the cold season in Buenos Aires, Argentina. *Journal of Medical Entomology*, **54**,106-113.

De Majo M.S., Zanotti G., Campos R.E. & Fischer S. (2019) Effects of constant and fluctuating low temperatures on the development of *Aedes aegypti* (Diptera: Culicidae) from a temperate region. *Journal of Medical Entomology*, **56 (6)**, 1661-1668.

Denlinger D.L. & Armbruster P.A. (2014). Mosquito Diapause. *Annual Review of Entomology,* **59**, 73-93.

Diez F., Breser V.J., Quirán E.M. & Rossi G.C. (2014) Infestation levels and new records of *Aedes aegypti* (Diptera: Culicidae) in La Pampa Province, Argentina. *Revista Chilena de Entomología,* **39,** 67-72.

Di Rienzo J.A., Casanoves F., Balzarini M.G., Gonzalez L., Tablada M. & Robledo C.W. (2019) InfoStat versión 2019. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar

Domínguez C. & Lagos S. (2001) Presencia de *Aedes aegypti* (Diptera: Culicidae) en la provincia de Mendoza, Argentina. *Revista de la Sociedad Entomológica Argentina,* **60**, 79-80.

Eisen L., Monaghan A.J., Lozano Fuentes S., Steinhoff D.F., Hayden M.H. & Bieringer P.E. (2014) The impact of temperature on the bionomics of *Aedes* (*Stegomyia*) *aegypti*, with special reference to the cool geographic range margins. *Journal of Medical Entomology*, **51**, 496-516.

Farnesi L.C., Martins A.J., Valle D. & Rezende G.L. (2009) Embryonic development of *Aedes aegypti* (Diptera: Culicidae): influence of different constant temperatures. *Memorias do Instituto Oswaldo Cruz,* **104**, 124-126.

Fischer S., De Majo M.S., Quiroga L., Paez M. & Schweigmann N. (2017) Long-term spatio-temporal dynamics of the mosquito *Aedes aegypti* in temperate Argentina. *Bulletin of Entomological Research*, **107**(2), 225-233.

Fischer S., De Majo M.S., Di Battista C.M., Montini P., Loetti V. & Campos R.E. (2019) Adaptation to temperate climates: Evidence of photoperiod-induced embryonic dormancy in *Aedes aegypti* in South America. *Journal of Insect Physiology*, **117**, 103887.

Gillett J.D. (1955a) Variation in the hatching response of *Aedes* eggs (Diptera; Culicidae). *Bulletin of Entomological Research,* **46**, 241-254.

Gillett J.D. (1955b) The inherited basis of variation in the hatching response of *Aedes* eggs (Diptera: Culicidae). *Bulletin of Entomological Research,* **46**, 255-265.

Grech M., Visintin A., Laurito M., Estallo E., Lorenzo P., Roccia I., Korin M., Goya F., Ludueña Almeida F. & Almirón W. (2013) New records of mosquito species (Diptera: Culicidae) from Neuquén and La Rioja provinces, Argentina. *Revista de Saude Publica*, **46**, 387-389.

Huey R., Gilchrist G. & Hendry A. (2005) Using invasive species to study evolution: case studies with *Drosophila* and salmon. In: Species Invasions: Insights into Ecology, Evolution, and Biogeography (ed. By D. Sax, J. Stachowicz and S. Gaines), pp 139-164. Sinauer, New York.

INDEC (2010) Censo 2010 Provincia de Buenos Aires. Resultados definitivos por partido. Available in: http://www.estadistica.ec.gba.gov.ar/dpe/Estadistica/CENSO2010%20REVISION/librocenso2010.pdf)

Kraemer M.U.G., Sinka M.E., Duda K.A. *et al.* (2015) The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus*. *eLife*, **4**, e08347

Kraemer M.U.G., Reiner R.C., Brady O.J. *et al.* (2019) Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nature Microbiology* **4,** 854–863.

Lee C.E., Remfert J.L. & Chang Y-M (2007) Response to selection and evolvability of invasive populations. *Genetica*, **129**, 179-192.

Lima A., Lovin D.D., Hickner P.V. & Severson D.W. (2016) Evidence for an overwintering population of *Aedes aegypti* in Capitol Hill Neighborhood, Washington, DC. *American Journal of Tropical Medicine and Hygiene,* **94**: 231-235.

Lounibos P.L. & Kramer L.D. (2016) Invasiveness of *Aedes aegypti* and *Aedes albopictus* and vectorial capacity for Chikungunya virus. *The Journal of Infectious Disease* **2016: 214 (Suppl. 5)**, 453-458.

Mayer S.V., Tesh R.B. & Vasilakis N. (2017) The emergence of arthropod-borne viral diseases: a global prospective on dengue, chikungunya and Zika fevers. *Acta Tropica*, **166**, 155-163.

Medlock J.M., Hnasford K.M., Versteirt V., Cull B., Kampen H., Fontenille D., Hendrickx G., Zeller H., Van Bortel W. & Schaffner F. (2015) An entomological review of invasive mosquitoes in Europe. *Bulletin of Entomological Research* **105**: 637-663.

Ponnusamy L., Böröczky K., Wesson D.M., Schal C. & Apperson C.S. (2011) Bacteria stimulate hatching of yellow fever mosquito eggs. *PLos One*, **6(9)**, e24409.

R Core Team (2019) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. (https://www.R-project.org/)

Rossi G.C., Lestani E.A. & D´Oria J.M. (2006) Nuevos registros y distribución de mosquitos de la Argentina (Diptera: Culicidae). *Revista de la Sociedad Entomológica Argentina*, **65**, 51-56.

Rubio A., Cardo M.V., Vezzani D. & Carbajo A.E. (2020). *Aedes aegypti* spreading in South America: new coldest and southernmost records. *Memórias do Instituto Oswaldo Cruz*, **115**, e190496.

Rusticucci M.M., Venegas S.A. & Vargas W.A. (2003) Warm and cold events in Argentina and their relationship with South Atlantic and South Pacific Sea surface temperatures. *Journal of Geophysical Research*, **108 (11)**, 1-10.

Sinclair B.J., Williams C.M. & Terblanche J.S. (2012) Variation in thermal performance among insect populations. *Physiological and Biochemical Zoology*, **85 (6)**, 594-606.

Soper F.L. (1967) Dynamics of *Aedes aegypti* distribution and density. Seasonal fluctuations in the Americas. *Bulletin of the World Health Organization,* **36**, 536-538.

Stein M. & Oria G.I. (2002) Identificación de criaderos de *Aedes aegypti* (Diptera: Culicidae) y cálculo de índices de infestación en la provincia del Chaco. In O. D. Salomón. Actualizaciones en Artropodología Sanitaria Argentina, Fundación Mundo Sano, Buenos Aires, p. 161-166.

Thomas S.M., Obermayr U., Fischer D., Kreyling J. & Beierkuhnlein C. (2012) Low-temperature threshold for egg survival of a post-diapause and non-diapause European aedine strain, *Aedes albopictus* (Diptera: Culicidae). *Parasites & Vectors*, **5**, 100.

Tauber M.J., Tauber C.A. & Masaki S. (1986) Seasonal adaptations of insects. Oxford University Press.

Urbanski J., Mogi M., O´Donnell D., DeCotiis M., Toma T. & Armbruster P. (2012) Rapid adaptive evolution of photoperiodic response during invasion and range expansion across a climatic gradient. *American Naturalist*, **179**, 490-500.

Vinogradova E.B. (2007) Diapause in aquatic insects, with emphasis on mosquitoes, pp 83-113. In Alekseev VR, De Stasio BT, Gilbert JJ (eds), Diapause in aquatic invertebrates: theory and human use. Springer, Dordrecht, The Netherlands.

Visintin A.M., Laurito M., Diaz L.A., Benítez Musicant G., Cano C., Ramírez R. & Almirón W.R. (2009) New records of mosquito species for central and Cuyo regions in Argentina. *Journal of the American Mosquito Control Association,* **25**, 208-209.

Warkentin K.M. (2011) Environmentally cued hatching across taxa: embryos respond to risk and opportunity. *Integrative and Comparative Biology*, **51**, 14-25.

Weisman Strum A. & Kindler S.H. (1963) Effect of low temperatures on development, hatching and survival of the eggs of *Aedes aegypti* (L.). *Nature*, **196**, 1231–1232.

Whitman D.W. & Agrawal A.A. (2009) What is phenotypic plasticity and why is it important? In Phenotypic plasticity in insects: Mechanisms and consequences. Whitman, D.W and Ananthakrishnan, T.N. Science Publishers. Enfield NH, USA.

Zanotti G., De Majo M.S., Alem I., Schweigmann N., Campos R. & Fischer S. (2015) New records of *Aedes aegypti* at the southern limit of its distribution in Buenos Aires province, Argentina. *Journal of Vector Ecology*, **40**, 408-411.

Zheng M.L., Zhang D.J., Damiens D.D., Lees R.S. & Gilles J.R.L. (2015) Standard operating procedures for standardized mass rearing of the dengue and chikungunya vectors *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) - II - Egg storage and hatching. *Parasites & Vectors*, **8**, 348.

Zuur A.F., Ieno E.N., Walker N.J., Saveliev A.A. & Smith G.M. (2009). Mixed effects models and extensions in ecology with R. Springer Science+Business Media, New York, NY.