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

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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) and two from temperate regions (Buenos Aires City and San Bernardo) of Argentina. Eggs collected in the field were raised in three experimental colonies. F1 eggs were acclimated for 7 days prior to immersion at 7.6 or 22°C (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 2 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 among treatment levels, whereas after the second immersion we compared the hatching response among 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 Resistencia than for Buenos Aires and San Bernardo, whereas the hatching response at ambient temperature was lower for San Bernardo than for Buenos Aires and Resistencia. The results support the hypothesis that populations from colder regions show an adaptive inhibition of egg hatching.

Introduction

Invasive species are of great concern because of their effects on native species and ecosystems and/or human activities and health (Lounibos and 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 and Terblanche, 2007; Whitman and Agrawal, 2009), whereas, over longer time scales, another factor that may 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 and Kang, 2005; Chown and 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 through unfavourable conditions as dormant eggs (Vinogradova, 2007; Denlinger and Armbruster, 2014).

The yellow fever mosquito *Aedes aegypti* is the main vector of arboviruses such as dengue, Zika and Chikungunya viruses (Lounibos and 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 and Kramer, 2016), whose geographic 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

(Thomas *et al.*, 2012; 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 seasonal diapause (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 (fig. 1), including the provinces of Buenos Aires (Zanotti *et al.*, 2015), La Pampa (Rossi *et al.*, 2006; Diez *et al.*, 2014), Mendoza (Domínguez and 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 Azul (Carbajo *et al.*, 2019), 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 9.5°C, and the period of monthly average temperatures below 12°C 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 possible number of eggs for the next reproductive season should be observed.

In this study, we assessed two alternative hypotheses regarding the mechanisms leading to the range expansion of *Ae. aegypti* towards colder regions in Argentina: (1) that the range expansion is aided by an adaptive inhibition of the egg hatching response at low temperatures or (2) that the range expansion is aided by a high-phenotypic plasticity to environmental conditions, without a specific adaptation in populations from colder areas. Thus, the aim of the current 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. The 30-year climate data were obtained from climate-data.org, which interpolates data from different surrounding meteorological stations for a 30-year period (1982–2012). Absolute minima for each location were obtained from the National Meteorological Service (www.smn.gob.ar/observaciones), which corresponded to a 30-year period (1981–2010) for Re and BA, and to a 10-year period (2001–2010) for SB.

Resistencia (Re) (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 (fig. 1). The city covers an area of 562 km^2 and has nearly 290,700 inhabitants (INDEC, 2010). The climate is subtropical without a dry season, with annual mean temperature of 21.3°C, an absolute minimum temperature of −4.1°C and cumulative rainfall of 1324 mm. Mean monthly temperatures are above 15°C during the whole year (fig. 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 and Oria, 2002).

Buenos Aires City (BA) (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 (fig. 1). The city covers an area of 203 km^2, and has a population of approximately 3 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 16.8°C, an absolute minimum temperature of −2.1°C and cumulative rainfall of 1040 mm. Mean monthly temperatures are below 12°C from June to August (fig. 2). Thus, the period unfavourable for *Ae. aegypti* development lasts approximately 3 months, and, during this period, the overall average temperature is 11°C.

Finally, San Bernardo (SB) (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 (fig. 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 15.1°C, an absolute minimum temperature of −7°C and a cumulative rainfall of 897 mm. Mean monthly temperatures are near or below 12°C from May to September (fig. 2). Thus, in this case, the period considered unfavourable for *Ae. aegypti* development lasts approximately 5 months, and, during this period, the overall average temperature is 10.4°C.

Source of eggs for the experiment

The eggs used in the experiment were F1 obtained from three experimental colonies (one for each locality) maintained at 24°C 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 autumn (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 × 3 cm in diameter), which contained a wet 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 1 week to ensure the complete development

Figure 1. Location of the localities from which the *Ae. aegypti* populations studied were collected (black circles). Re, Resistencia; BA, Buenos Aires City; SB, San Bernardo. Grey circles represent localities where *Ae. aegypti* has been detected during the last decade.

Figure 2. Mean monthly temperatures for Resistencia (Re), Buenos Aires City (BA) and San Bernardo (SB). The data were obtained from climate-data.org, and represent 30 year averages (1982–2012).

of the embryos, and the females were separated and killed by freezing. All the eggs were collected within 2 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 of the immersion at low temperatures of eggs from each population analysed, previously acclimated to low temperatures. For acclimation, paddles with eggs were stored for 7 days prior to immersion in a cold chamber (commercial fridge) at average temperature of 7.6°C (range: 6.6–8.6°C). Control eggs were stored for the same period of time at room temperature of 22°C. After this, five immersion temperatures were tested: 7.6, 10.3, 11.8, 14.1 and 16°C, which aimed to represent approximately the range of the mean winter temperatures of the three localities from where the mosquito eggs were collected. To obtain these temperatures, non-commercial thermal baths were used, which have an approximate range of ±0.5°C around the mean temperatures. For each population, treatments consisted of population, and acclimation and immersion temperatures. Between 6 and 10 replicates (substrate with 7–135 eggs from the same female) were assigned to each treatment combination.

For immersion, paddles with eggs were individually placed in hatching tubes (50 ml Falcon® tubes), containing 40 ml of reverse osmosis filtered water. In this immersion, the hatching medium used was water, in order to simulate as much as possible the
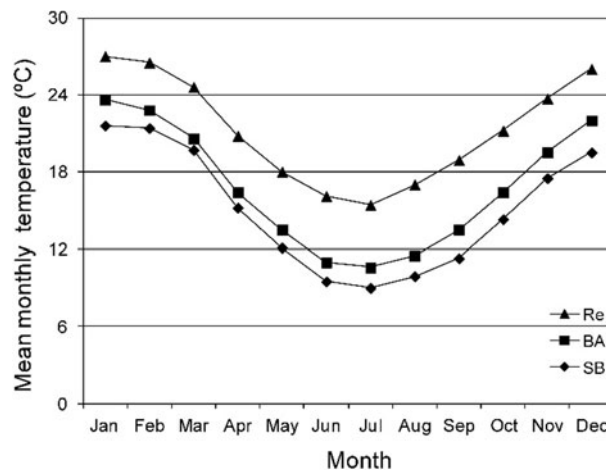
natural conditions, i.e. containers filling up with rainwater (Vitek and Livdahl, 2006). The hatching tubes were located in thermal baths at the corresponding temperature prior to immersion to stabilize the experimental temperature. After 48 h, the paddles were transferred to a dry tube placed at room temperature, and the live and dead larvae in each tube were counted.

To assess the viability of the remaining eggs, they were first subjected to second immersion at 22°C 2 weeks later, and after that the intact eggs were bleached to allow direct observation of the embryos. To this end, the eggs were immersed in a new hatching tube, containing a 40-ml solution of 47 mg powdered baker's yeast per l of filtered water. The addition of yeast aimed to generate a stronger stimulus than water only in order to stimulate egg hatching as much as possible, as previous experiments have shown that a larger number of eggs hatch under these conditions (Byttebier et al., 2014). After 48 h, 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. After this, on each substrate, the number of intact eggs was counted, and the intact eggs were bleached with a solution of sodium hypochlorite and observed under a stereoscopic microscope. Creamy-white embryos with visible eyes, abdominal segmentation and a hatching spine were considered viable, whereas 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 analyse

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 of the second immersion 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 second immersion.

For the immersion at low temperatures, the effects of acclimation, immersion temperature, population, all the possible two-way interactions and the three way interaction on the hatching response were analysed 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 analyse 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 analysed graphically. To this end, the replicates of each population were classified into one of the following categories according to their hatching response: $r \le 0.1$, $0.1 < r \le 0.2$, $0.2 < r \le 0.3$, $0.3 < r \le 0.4$, $0.4 < r \le 0.5$, $0.5 < r \le 0.6$, $0.6 < r \le 0.7$, $0.7 < r \le 0.8$, $0.8 < r \le 0.9$, $0.9 < r \le 1$, with r standing for each replica. The histograms of the frequencies of the different categories of hatching response were visually compared among the three populations.

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's LSD rank test was used (Conover, 1999).

Results

A total of 17,348 viable eggs were used, 1442 (8.3%) of which hatched during the first immersion when analysing 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).

A large variability in the hatching response of different replicates was observed, with most replicates showing a low or null-hatching response during the first immersion (fig. 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.

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, i.e. more hatching was detected at higher temperatures in the three populations (table 2). Furthermore, a significant positive effect of Re, and a non-significant negative

effect of SB were detected when compared with BA (table 2). 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. In the three populations, a bimodal hatching response was observed, with a higher representation in the categories of high and low hatching, and a lower representation in the intermediate categories (fig. 4). Furthermore, in the three populations a higher frequency of low hatching (0.2 or less) was observed as compared to the frequency of high hatching (more than 0.8). The histograms of hatching predisposition of replicates at ambient temperature showed two patterns: one for the populations of Re and BA, which were similar, and showed approximately 60% of the replicates with hatching below 0.5. In contrast, SB showed a higher frequency of replicates with low-hatching predisposition (84% of the replicates with hatching below 0.5), and a lower frequency of replicates with high-hatching predisposition as compared to Re and BA (fig. 4). The proportion of replicates with null hatching was relatively low (<15%), and similar for the three populations analysed.

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 under field (Bond *et al.*, 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 among 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 14.1 and 16°C, 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 12°C, 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

Table 1. Proportions of hatching responses and larval mortality for three populations of *Ae. aegypti* of Argentina

	Resistencia	Buenos Aires City	San Bernardo
Annual mean temperature per location (visual)			
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)
Hatching success (visual)			

In parentheses, the number of hatched or dead larvae from the total analysed.
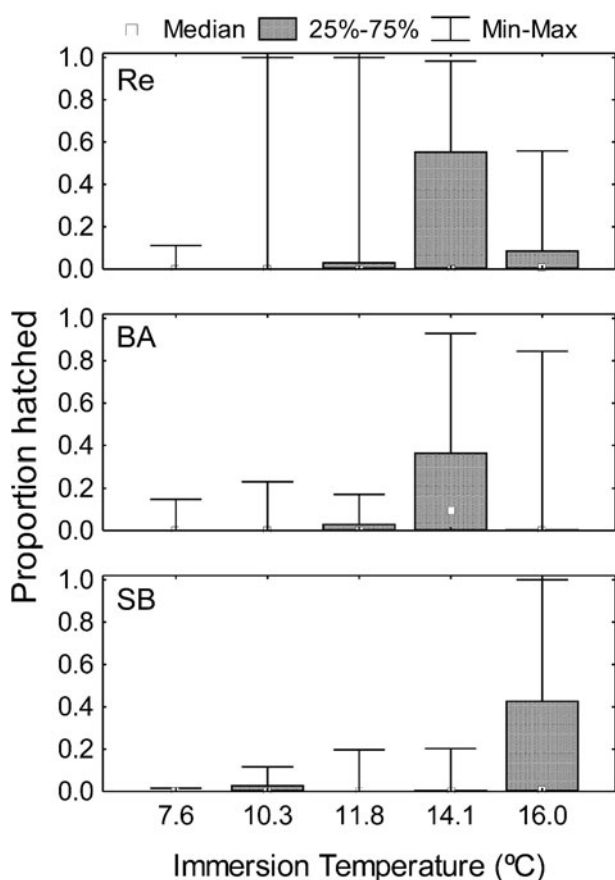

Figure 3. Hatching response of different replicates within populations during the first immersion as a function of immersion temperature. Re, Resistencia; BA, Buenos Aires City; SB, San Bernardo.

Table 2. Parameter estimates for the fixed effects in the model for the hatching response at low temperatures

Source	Estimate	Std. Error	t value	Pr(>|t|)
Intercept	−7.16	1.02	−7	<0.0001
Population (Re)	0.74	0.35	2.11	0.0357
Population (SB)	−0.54	0.43	−1.28	0.2029
Immersion temperature	0.35	0.07	5.06	<0.0001

Re, Resistencia; SB, San Bernardo.

differences in initial mortalities observed among 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 and 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 analysed in the current study were unexpectedly low as compared to those previously recorded for the *Ae. aegypti* populations from Buenos Aires City (25, 41 and 56% at 12, 14 and 16°C, 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 21°C 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 current 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 studies, the hatching rate was calculated without considering the non-viable eggs.
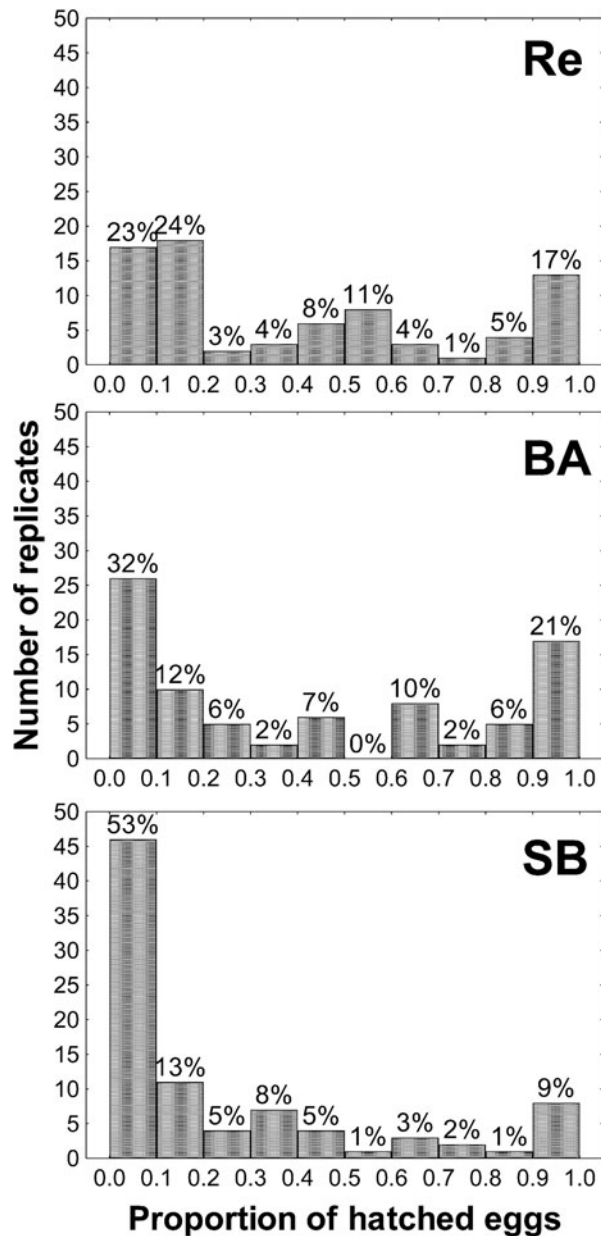
Figure 4. Number of replicates within populations in each range of hatching response during the second immersion. Re, Resistencia; BA, Buenos Aires City; SB, San Bernardo.

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 among the three populations analysed in our study. In previous studies, Gillett (1995a, 1995b) 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 did not differentiate eggs from different females (e.g.

Weissman-Strum and Kindler, 1963; Ponnusamy *et al.*, 2011; Thomas *et al.*, 2012; Byttebier *et al.*, 2014).

An unexpected result of the current study was the differences among 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), *Aedes 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.

The differences in the hatching response both at the first and the second immersion might also be related to other features of the climate from which each population came from. For example, the amount of rainfall determines the relative risk of desiccation of larval habitats, and in riskier regions it could be expected that eggs refrain from massive hatching, as it has been observed for the tree hole mosquito *Aedes triseriatus* (Khatchikian, *et al.*, 2009). The direct relationship of the hatching response with the total amount of rainfall at the location of origin observed in our study encourages further research on the effect of habitat desiccation risk on the hatching response of this species.

In conclusion, the differences 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 current 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.

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