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Adaptation to temperate climates: Evidence of photoperiod-induced embryonic dormancy in *Aedes aegypti* in South America

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Abstract

Dormancy is a developmental arrest in arthropods, in response to unfavorable conditions in temporally varying environments. In Aedes aegypti, the supposed inability of eggs to inhibit hatching has been used to explain the restriction of this species to tropical and subtropical regions. However, the geographic range of Ae. aegypti is constantly expanding towards temperate regions. Thus, the aim of the present study was to assess the ability of Ae. aegypti individuals from a temperate region (Buenos Aires City, Argentina) to enter photoperiod induced dormancy. To this end, we exposed both the parental generation and the eggs to short-day (SD: 10L:14D) and long-day (LD: 14L:10D) photoperiods, and studied the temporal variation in egg hatching. The experiment consisted of 28 treatment combinations of three factors: parental photoperiod (SD or LD), egg storage photoperiod (SD or LD), and age of eggs (14, 28, 42, 56, 70, 91, and 112 days). The results showed a lower hatching response with the SD parental photoperiod, and a trend to higher hatching with longer egg storage time in all photoperiod treatment combinations. The egg storage photoperiod showed no effect on egg hatching. In both parental photoperiod treatments, egg replicates of most ages from different females showed a large variability, with some replicates with lowest hatching response and others with highest hatching response. Our results show the ability of Ae. aegypti to inhibit egg hatching in response to a short-day photoperiod, which could allow the further expansion of this species to regions with colder winters.

Key words: Culicidae; dormancy; photoperiod; overwintering; hatching; eggs age

1. Introduction

Some of the most important environmental changes that organisms have to cope with are seasonal cycles. Thus, to ensure their survival and reproductive success, organisms living in temperate regions have developed different strategies to determine the timing of the different stages of their life cycle (Tauber and Tauber, 1981). The primary mechanism through which insects synchronize their life history phases with appropriate seasonal conditions is dormancy (Tauber and Tauber, 1981). Dormancy is a general term that describes a state of developmental arrest which is adaptive, and usually accompanied with metabolic suppression. Dormancy includes different processes, such as quiescence and diapause (Kostal, 2006), which are associated to different physiological states, induction pathways, and tolerances to environmental extremes (Tauber and Tauber, 1981). In particular, diapause is defined as a profound interruption of the developmental pathway which is genetically regulated (Hahn and Denlinger, 2007), and induced previous to the beginning of the unfavorable conditions (Kostal, 2006.The ability of insects to enter dormancy (in particular diapause) varies among populations of the same species, depending on their evolutionary history. Populations that inhabit regions with more drastic winter conditions present a higher ability to enter diapause (Tauber and Tauber, 1972; Bradshaw and Holzapfel, 2001a; Schmidt et al., 2005; Bennett et al., 2005; Leisnham et al., 2011; Hou et al., 2016). The adaptive importance of diapause is supported by the rapid selection of this trait in species invading new geographic ranges (Urbanski et al., 2012; Bean et al., 2012; Pegoraro et al., 2017) and in those responding to environmental changes (Bradshaw and Holzapfel, 2001b; 2006).

In many organisms, including mosquitoes, the most important token stimulus that induces diapause is a short photoperiod, which is a predictable cue that anticipates unfavorable conditions before they begin (Denlinger and Armbruster, 2014). The stage during which

insects are sensitive to the token stimulus is species-specific, but often occurs during a developmental stage previous to that undergoing diapause (Tauber and Tauber, 1981). Therefore, in many cases, the environmental conditions experienced by the parental generation determine whether an individual enters diapause or not (Hahn and Denlinger, 2007). In mosquito species of the genera *Aedes* and *Psorophora*, diapause is commonly observed in the embryo stage (pharate larva) (Vinogradova, 2007; Denlinger and Armbruster, 2014). The stages sensitive to induce diapause may be different instars of the parental generation or the egg itself (Vinogradova, 2007). As a consequence of diapause, eggs do not hatch during the fall, preventing larval mortality when the temperature decreases below biologically viable thresholds at the beginning of the cold season (Armbruster, 2016). In some mosquito species, diapause also increases the survival of eggs by increasing cold hardiness (Thomas et al., 2012) and by preventing desiccation and thus premature mortality (Sota and Mogi, 1992; Urbanski et al., 2010).

In *Aedes albopictus* and *Aedes aegypti*, two medically important mosquito species, which are the main vectors of different arboviruses, including dengue, Zika and chikungunya viruses, the ability to produce diapausing eggs to survive winter conditions has been used as a plausible explanation for their different geographic ranges in the northern hemisphere (Paixão et al., 2017). *Aedes albopictus* is one of the most studied models of diapause in mosquitoes (Vinogradova, 2007). In this species, the variability in the incidence of diapause among populations has been widely studied, and a much higher incidence has been observed in populations from temperate regions than in those from tropical regions (Hawley et al., 1987; Lounibos et al., 2003; Lounibos et al., 2011). The ability of temperate region populations to enter diapause has been used to explain the ongoing expansion and success of this species in temperate areas of North America and Europe (Bonizzoni et al., 2013; Roche et al., 2015; Ruedas et al., 2018).

In contrast, according to that stated by different authors, *Ae. aegypti* lacks the ability to enter diapause (e.g., Hawley et al., 1989; Mitchell, 1995; Tsuda and Takagi, 2001; Mogi, 2011; Denlinger and Armbruster, 2014; Costanzo et al., 2016; Armbruster, 2016; Cunze et al., 2018), a statement that has been used to explain the restriction of this species to tropical and subtropical regions (Hawley et al., 1989; Mogi, 2011; Denlinger and Armbruster, 2018). However, none of these authors has reported results to support this statement. In fact, to our knowledge, no studies have evaluated the effect of the photoperiod on the egg hatching response of *Ae. aegypti* populations from temperate or tropical regions.

During recent decades, Ae. aegypti has increased its geographic range, colonizing temperate climate regions (Eisen et al., 2014), including cities with cold winter temperatures such as Washington, DC (USA), where successful overwintering, probably in subterranean habitats, has been demonstrated (Lima et al., 2016). In Argentina, after the continent-wide control program implemented between 1930 and 1960, this 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), and in the metropolitan area of Buenos Aires (Campos, 1983), i.e. more than 1000 km southwards, in 1991. In addition, an expansion of Ae. aegypti towards colder climate areas, including the provinces of 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), and San Juan (Carrizo Páez et al., 2016), has been recently documented. Within Buenos Aires province, the distribution has expanded to the south, currently covering localities such as 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 unfavorable for immature development of *Ae. aegypti* (Eisen et al., 2014). However, it should be noticed that temperature obtained from weather stations often neglect spatial heterogeneity caused by urban heat islands or microclimatic conditions within the urban habitat, and may lead to an underestimation of the favorability for mosquitoes (Murdock et al., 2017).

In Buenos Aires city, *Ae. aegypti* is well established and has increased its abundance during the last 20 years (Fischer et al., 2017). Its population dynamics is related to temperature, and reproduction and development occur mainly from late September to late June, during the warm season, while no adults and only few larvae are observed during the winter season (Vezzani et al., 2004, Fischer et al., 2017).

This suggests that this species might be adapted to the local climatic conditions, an adaptation that could include the evolution of diapause. The selection of this trait might have significantly increased the overwintering probabilities of these populations under harsh conditions, and might thus explain the ongoing expansion in the distribution range of this species towards colder regions in Argentina.

Based on all the above, the aim of the present study was to assess whether the *Ae. aegypti* population of Buenos Aires city is able to inhibit egg hatching in response to a short photoperiod. To this end, we exposed the parental generation and the eggs to different photoperiods and studied the temporal variation in the hatching response of eggs.

2. Methods

2.1. Experimental design

The eggs used in the experiment were obtained from two experimental colonies maintained at 21°C: one under a short-day photoperiod (SD: 10:14 - L:D) and the other under a longday photoperiod (LD: 14:10 - L:D). The light cycle was generated with a 18W LED light

tube (TBCin model LT8-1218-W-E), and controlled with a timer, without transition between light and dark conditions. These photoperiods were selected to represent the natural conditions of the fall-winter transition and the spring-summer transition respectively, in Buenos Aires city.

Both colonies were initiated simultaneously, starting from about 1000 field-collected eggs each. The eggs were collected with ovitraps in early fall (March and April) of the same year in Buenos Aires city. Once hatched, the larvae were fed *ad libitum* with a solution of powdered baker's yeast. Daily pupation in each colony was recorded to ensure the simultaneity of the processes between both treatments.

Five 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 humid paper conditioned for egg laying and a raisin as a source of sugar. The individual cages were inspected daily, and papers with eggs maintained under the same conditions of photoperiod and temperature for 9 days to ensure the complete development of the embryos.

The eggs from both colonies used in the experiment (F1 generation) were collected within a single week to ensure a homogeneous age of eggs in the experiment. During the following week, all intact eggs laid by each female were counted, and separated into subgroups (replicates) containing 15-34 intact eggs each. These replicates were placed individually into Petri dishes (45 mm in diameter, 10 mm in height) containing a wet cloth covered with white tissue paper. Every replicate was named with a code that included the identity of the mother and the parental photoperiod. During this period, all eggs were stored under their parental photoperiod at 21°C.

After separating the eggs into subgroups as explained above, the eggs (9 days old on average) were randomly assigned and stored under either a SD or a LD photoperiod at

room temperature (21°C on average) until immersion. All replicates from the same female were assigned to the same storage photoperiod. Hourly temperatures for each egg storage treatment were recorded with a HOBO® Pendant Temperature Datalogger placed beneath the eggs. The experiment consisted of 28 treatment combinations of three factors: parental photoperiod (SD or LD), egg storage photoperiod (SD or LD), and age of eggs (14, 28, 42, 56, 70, 91, and 112 days). Thirty replicates were used for each treatment. Replicates were randomly assigned to one storage time (each storage time corresponding to a different age of eggs), with the restriction that all replicates within one immersion corresponded to different females, to ensure that the 30 observations from each treatment constituted independent responses.

Once the egg storage time for each treatment was completed, the number of intact eggs in each replicate was counted again. All replicates with fewer than 15 intact eggs were replaced. Then, the eggs from every replicate were immersed in a plastic tube containing 10 ml of a hatching solution (46.8 mg of dry baker's yeast/liter of filtered water). The tubes were placed in a thermal bath to ensure constant temperature of 21°C, and the photoperiod during immersion was 12:12 (L:D). Twenty-four hours after immersion, the eggs were recovered from the solution and the number of larvae in each tube was counted. Chorions of the unhatched eggs were bleached with a commercial 50% 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 alive, while those without these characteristics were considered nonviable (Farnesi et al., 2009).

2.2. Data analyses

The hatching responses to each treatment were analyzed with Generalized Linear Mixed Models (GLMM), using the R software, Version 3.4.0 (R Core Team, 2017), accessed through a user-friendly interface in Infostat software (Di Rienzo et al., 2017). Models were

fitted and parameters were estimated using the Maximum Likelihood method (Pinheiro and Bates, 2000).

Two separate analyses were performed, to gain insights on the effects of the treatments on different aspects of the hatching response. First, the qualitative hatching response was analyzed, to assess the frequency of a complete inhibition of hatching in response to the treatments. Second, the quantitative response of those replicates that showed no complete inhibition of hatching was analyzed, to assess the change in the hatching propensity of individuals with some predisposition to hatching.

To assess the qualitative response, each replicate was classified either as responder (coded as 1), when at least one larva was observed, or as nonresponder (coded as 0), when no hatching was observed, and the proportion of responders was calculated for each treatment. The relationship between the qualitative hatching response and the parental photoperiod (LD: 1, SD: 0), egg storage photoperiod (LD: 1, SD: 0), egg age, and their interactions was analyzed with GLMM, using the Bernoulli distribution and the logit link function. The identity of the mother was included in the model as a random effect to account for the lack of independence in the responses of the eggs laid by the same female in different immersions. The average storage temperature for the 120 h prior to each immersion (Table 1) was also included as a random effect to account for the variability in the storage conditions between treatments and during the study. Nonsignificant terms were sequentially deleted from the full model, and the model with the lowest Akaike information criterion (AIC) value was selected.

Table 1. Mean temperature (± standard deviation) for the periods previous to each immersion in the two egg storage conditions

Immersion	1	2	3	4	5	6	7
Egg age (days)	14	28	42	56	70	91	112
Short-Day	19.37	20.65	20.89	21.55	20.90	21.41	22.68

storage	± 0.99	± 0.36	± 0.33	± 0.22	± 0.08	± 0.37	± 0.57
Long-Day	18.79	20.83	21.05	21.78	20.93	21.53	22.75
storage	± 0.92	± 0.34	± 0.32	± 0.27	± 0.09	± 0.34	± 0.40

To assess the quantitative response, the proportion of hatched eggs in each replicate was calculated as the number of observed larvae divided by the number of viable eggs potentially able to hatch, where the number of viable eggs was estimated as the number of larvae plus the number of viable embryos. For those replicates with at least one hatching, the relationship between the quantitative response and the parental photoperiod (LD: 1, SD: 0), egg storage photoperiod (LD: 1, SD: 0), egg age, and their interactions was analyzed with GLMM, using the binomial distribution and the logit link function. The mother identity and average storage temperature prior to each immersion were included as random effects. Nonsignificant terms were deleted with the same criterion as in the previous analysis.

To analyze the variability in the induction of dormancy among eggs from different females within each treatment, we assessed the frequency of replicates that attained different hatching responses. Replicates were classified in one of the following categories according to their hatching response: $0 \ge r > 0.1$, $0.1 \ge r > 0.2$, $0.2 \ge r > 0.3$, $0.3 \ge r > 0.4$, $0.4 \ge r > 0.5$, $0.5 \ge r > 0.6$, $0.6 \ge r > 0.7$, $0.7 \ge r > 0.8$, $0.8 \ge r > 0.9$, $0.9 \ge r$, with r standing for each replica. For each treatment, we also calculated the variability statistic H (Coffey et al., 1988), which is appropriate for variables bounded between 0 and 1. This statistic calculates the observed variability in relation to the maximum possible value for a given average among replicates, and takes values from 0 to 1, with 0 indicating that all replicates are equal and 1 indicating the maximum possible variability, which occurs when at most one replicate takes a value different from 0 or 1 (Coffey et al., 1988).

3. Results

The parental photoperiod and the age of eggs had a significant effect on the qualitative hatching response, while the egg storage photoperiod and the interaction terms were not significant. The best model obtained included only the two significant variables (Table 2). Table 2. Parameter estimation of the fixed effects in the model for the qualitative hatching response of *Aedes aegypti* eggs

	Estimate	Std. Error	z value	Pr(> <u> z</u>)
(Intercept)	-1.4458	0.4235	-3.4137	<0.001
Parental photoperiod	3.5115	0.4377	8.0234	<0.001
Egg age	0.0458	0.0074	6.1874	<0.001

Fewer than 25% of replicates from the SD parental photoperiod showed some hatching at 14 days of age, a percentage that increased over time, reaching more than 90% of replicates with some hatching after 90 days. In contrast, more than 80% of the replicates from the LD parental photoperiod showed some hatching at 14 days of age, a percentage that increased slightly, reaching 100% after 70 days (Figure 1).

Among replicates with some hatching, the proportion of hatched eggs was significantly affected by the parental photoperiod, the egg age, and the interaction between these two variables (Table 3). The parental photoperiod affected egg hatching, with the LD parental photoperiod leading to a higher proportion of eggs hatching, regardless of the egg age. In both parental photoperiod treatments, the youngest eggs showed the lowest hatching response whereas the oldest eggs showed the highest hatching response. This increase in hatching with egg age was higher for eggs from the LD parental photoperiod than for those from the SD parental photoperiod, as shown by the positive significant interaction term (Table 3).

 Table 3. Parameter estimation of the fixed effects of the model for the quantitative hatching response of Aedes aegypti

 eggs

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	-2.109	0.346	-6.10	<0.0001
Parental photoperiod	1.767	0.196	9.03	<0.0001
Egg age	0.021	0.005	4.41	<0.0001
Parental photoperiod x egg age	0.004	0.0017	2.41	0.0158

On average, more than 50% of the eggs from the LD parental photoperiod, except for 14day-old eggs, hatched in all immersions, whereas fewer than 50% eggs from the SD parental photoperiod, except for 112-day-old eggs, hatched in all immersions (Figure 2). Based on the results of the qualitative and quantitative analyses, where the egg storage photoperiod showed no significant effect, the frequency of the different hatching response ranges was analyzed in relation to the parental photoperiod for each egg age (Figure 3). For replicates from the SD parental photoperiod treatment, the most frequent response was low hatching during the first three months, and a change towards higher hatching after this period. In contrast, for replicates from the LD parental photoperiod treatment, the predominance of low hatching was observed only within the first two weeks, and after that a high hatching response was observed in most replicates.

Replicates from different females from both parental photoperiod treatments and for most egg ages showed a large variability, with some replicates showing the lowest hatching response and others showing the highest hatching response. In general, a sort of bimodal distribution was observed, with the categories with intermediate hatching response showing a lower representation than that of the categories with low or high hatching response. This was accompanied by high values of the variability statistic H, which in most cases exceeded 0.6 of the maximum possible value for the given average hatching. Exceptions for this were the youngest eggs from the SD parental photoperiod treatments (where in all replicates fewer than 30% of the eggs hatched) and the oldest eggs from the

LD parental photoperiod treatments (where in all replicates more than 20% of eggs hatched). Both of these treatment combinations showed the lowest variability as indicated by their respective H statistic.

4. Discussion

The geographic range expansions of different insects have been favored by the adaptation to the local conditions (e.g., Huey et al., 2000; Broennimann et al., 2007; Urbanski et al., 2012). In recent years, the distribution of *Ae. aegypti* has greatly expanded towards temperate regions in America (Eisen et al., 2014; Zanotti et al., 2015), a fact that might have been facilitated by an adaptation of the populations to local conditions, although no studies on this subject have been performed so far. In the case of the *Ae. aegypti* population from Buenos Aires city, the results of our study showed the inhibition of egg hatching in response to a short-day photoperiod.

Although in this study we did not investigate the physiological or genetic pathways related to the inhibition to hatch, our methods to assess the hatching response are similar to those used to determine the presence of diapause in other mosquito species such as *Ae. albopictus* (Pumpuni et al., 1992; Lounibos et al., 2003; 2011; Urbanski et al., 2012) and *Ae. triseriatus* (Kappus and Venard, 1967). The response to the short photoperiod in our study, in which egg hatching was inhibited even when the temperature and flooding conditions were favorable for immature development, is consistent with the definition of diapause, although other explanations might also be possible, such as an effect of the parental photoperiod on the physiology of the embryos affecting hatching propensity, without a formal diapause. These alternative possibilities and their genetic and physiological bases should be examined in further molecular studies such as those performed on *Ae. albopictus* (Poelchau et al. 2013). In the case it is confirmed, this would

be the first evidence worldwide that *Ae. aegypti* is able to enter photoperiod-induced diapause.

The lower induction of the hatching response by the SD parental photoperiod was an expected result, and is in agreement with observations for many other species of the same genus, including Ae. albopictus, Ae. vexans, Ae. caspius, Ae. cinereus, Ae. triseriatus, and Ae. togoi (Vinogradova, 2007). The lack of previous records of photoperiod-induced dormancy in Ae. aegypti might be due to the lack of studies assessing this phenomenon, the failure to detect it, and/or the failure to publish such results. In fact, we found no studies evaluating the effect of the parental photoperiod on the induction of diapause in Ae. *aegypti*, and it might be that negative results have not been published because of a bias against negative results in scientific publications (Fanelli, 2012). Furthermore, it should be considered that most studies on Ae. aegypti have been performed either with highly inbred lab strains or with field-obtained individuals from tropical and subtropical regions, which makes it unlikely to discover an effect such as diapause induction even by accident. In many species, the ability to induce diapause is specific for temperate region populations, which are subjected to selection to tolerate or avoid harsh winter conditions. Such differences between temperate and tropical climate populations have been demonstrated for several other mosquito species, including Ae. albopictus (Hawley et al., 1987; Leisnham et al., 2011) and Ae. triseriatus (Shroyer and Craig, 1983), and differences in diapause-inducing conditions have also been observed along latitudinal clines in many other insect groups such as coleopterans (Bean et al., 2012), brachyceran dipterans (Tyukmaeva et al., 2011; Pegoraro et al., 2017), hymenopterans (Paolucci et al., 2013), and lepidopterans (Chen et al., 2013).

In our study, hatching was inhibited by the photoperiod conditions experienced by some stage of the parental generation or the first days of the egg stage, coincident with the

embryogenesis. Specific experiments are required to identify the stage that contributes most to the observed inhibition. In other mosquito species, such experiments have demonstrated that the photoperiod conditions experienced by late larval instars, pupae and adults of the parental generation can induce diapause in *Ae. albopictus* (Mori et al., 1981), Ae. atropalpus (Anderson, 1968), Ae. vexans (Wilson and Horsfall, 1970), and Ae. sollicitans (Anderson, 1970), whereas the photoperiod experienced by the eggs shortly after oviposition can induce diapause in Ae. canadensis (Pinger and Eldridge, 1977). Interestingly, the egg storage photoperiod after embryonic development completion has been shown to induce diapause in Ae. triseriatus (Kappus and Venard, 1967) and Ae. sollicitans (Anderson, 1970), in contrast to the results obtained during our study in Ae. aegypti, where the egg storage photoperiod had no effect on the hatching response. These differences among species in the stage that induces diapause seem to be independent of their phylogenetic relatedness, since species corresponding to the same subgenus (Ochlerotatus: i.e. Ae. canadensis and Ae. atropalpus) exhibit different sensitive stages, and in species corresponding to different subgenera, diapause is induced at the same stages (i.e. Ae. (Stegomyia) albopictus, Ae. (Ochlerotatus) atropalpus and Ae. (Aedinomorphus) vexans).

The variability in the response to the dormancy-inducing conditions (i.e. a short photoperiod), with some individuals showing a complete response, others a partial response (as shown by the lower hatching of responsive replicates), and others no response (i.e., almost complete hatching of some replicates within the first month), suggests that this population retains high heterogeneity for this trait, which points to the underlying variability in the plasticity of the response to photoperiod changes (Wennersten and Forsman, 2012). This could be explained by alternative but not mutually exclusive scenarios. One alternative might be that the relatively short time elapsed from the

reintroduction of *Ae. aegypti* to Buenos Aires from subtropical regions was not enough to select this trait in the whole population. In this case, we could consider that a part of the population is poorly adapted to the local winter conditions, and could expect that this trait might still be under selection and may continue changing in the next few years. Another alternative is that this kind of dormancy in the *Ae. aegypti* population of Buenos Aires is under weak selection. This means that although there is a certain benefit for not hatching during late fall (i.e. avoiding potentially lethal temperature conditions during the winter season), in some years, the winter conditions are not harsh enough to kill the developing larvae. In that case, the hatching, completion of development, and early emergence of nondormant individuals could have a fitness advantage by initiating reproduction earlier in the spring season. This hypothesis is partly supported by the experimental results of a recent study showing a survival of 30% of the immature stages raised under winter outdoor conditions, which suggests a tolerance of the population to the local winter conditions (De Majo et al., 2017). Further studies should look for evidence for each of these scenarios.

Considerable variability among individuals was observed even under the LD parental photoperiod, which might be attributable to a bet-hedging strategy. This type of strategy, which is associated with a maximization of the geometric mean fitness of the mother in stochastic environments where there are no cues that reliably predict the coming environmental conditions (Wennersten and Forsman, 2012), is well known for *Ae. aegypti* (Gillett, 1955a; b) and other mosquito species (e.g. Andreadis, 1990; Vitek and Livdahl, 2006; Khatchikian et al., 2009), as well as for other insects, crustaceans, and plants (Evans and Dennehy, 2005). In the case of mosquito species that use water bodies such as tree holes or artificial containers filled by rain as larval habitats, the complete drying of the habitat during unpredictable drought periods would result in the total mortality of the

developing larvae. Thus, the delay in the hatching of part of the eggs would prevent the loss of all of a parent's offspring to any single drought (Evans and Dennehy, 2005).

Considering that the present study was performed on the F1 generation, the results should reflect the response of the natural population of Buenos Aires, since the eggs from the parental generation were collected in the field and thus there was no chance for prior selection on hatching traits in the laboratory. The main conclusions are that the sensitive stage to cues anticipating unfavorable conditions is the parental generation, while no evidence for an effect of the photoperiod experienced by the eggs was obtained, at least under the temperature conditions analyzed. The low hatching in all treatments of 14-day-old eggs, which experienced lower temperature conditions prior to immersion, together with the improvement of the hatching models with the inclusion of previous storage temperatures, suggests that the effect of the photoperiod may be interacting with the temperature experienced by the eggs, as has also been observed for other mosquitoes (Kappus and Venard, 1967; Pumpuni et al., 1992).

Besides inhibiting hatching during the winter season, diapause can also reduce the mortality of eggs. In several mosquito species, including *Ae. albopictus*, *Ae. riversi*, *Ae. galloisi* and *Ae. flavopictus*, diapausing eggs stored at 20°C during extended periods show higher survival than non-diapausing eggs (Sota and Mogi, 1992). In *Ae. albopictus*, diapausing eggs also exhibit higher survival and tolerance to low temperatures, which increases the probabilities of survival during the cold season (Thomas et al., 2012). Future studies should determine whether this also happens in the *Ae. aegypti* population of Buenos Aires, which would imply a higher tolerance during the egg stage to the cold winter conditions and an increase in winter survival. The ability to enter diapause might allow *Ae. aegypti* to further expand to regions with colder winters.

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Figure captions

Figure 1. Proportion of responders as a function of egg age for the short-day (SD) and long-day (LD) parental photoperiod treatments. Symbols: average data; Lines: models estimated, with 95% confidence limits.

Figure 2. Proportion of hatched eggs in responsive replicates as a function of egg age in the short-day (left) and long-day (right) parental photoperiod treatments. Black symbols and error bars indicate median and quartiles for different replicates, solid lines indicate the values predicted by the model, and dotted lines indicate the 95% confidence bands of the models.

Figure 3. Number of replicates in each range of proportion of hatched eggs in relation to egg age (horizontal divisions) and the short-day (SD) and long-day (LD) parental photoperiod treatments (vertical division). Gray columns indicate replicates within different ranges of hatching proportions, and black columns highlight the number of replicates with zero hatching within the $0 \ge r > 0.1$ range. H values correspond to the measure of variability among replicates within each treatment combination.









- Short parental photoperiod inhibited the hatching response of Aedes aegypti eggs
- The hatching response increased with eggs age up to four months
- No effect of the storage photoperiod on the hatching response was detected
- nt The hatching response was highly variable among egg batches from different females •