Effects of scarcity and excess of larval food on life history traits of *Aedes aegypti* (Diptera: Culicidae)

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ABSTRACT: Few studies have assessed the effects of food scarcity or excess on the life history traits of *Aedes aegypti* (L.) (Diptera: Culicidae) independently from larval density. We assessed immature survival, development time, and adult size in relation to food availability. We reared cohorts of 30 *Ae. aegypti* larvae from newly hatched to adult emergence with different food availability. Food conditions were kept constant by transferring larvae each day to a new food solution. Immature development was completed by some individuals in all treatments. The shortest development time, the largest adults, and the highest survival were observed at intermediate food levels. The most important effects of food scarcity were an extension in development time, a decrease in the size of adults, and a slight decrease in survival, while the most important effects of food excess were an important decrease in survival and a slight decrease in the size of adults. The variability in development time and adult size within sex and treatment increased at decreasing food availability. The results suggest that although the studied population has adapted to a wide range of food availabilities, both scarcity and excess of food have important negative impacts on fitness. ***Journal of Vector Ecology* 43 (1): 117-124. 2018.**

Keyword index: larval food; food scarcity; food excess; survival; development time; adult size.

INTRODUCTION

Aedes aegypti, which originated in the tropics, is epidemiologically important as the main dengue vector in the Americas, where the number of cases and the distribution range of this disease have increased every year. Its epidemiological importance is growing because *Ae. aegypti* is also the main vector of two viruses that have recently spread to America: Zika virus (Hennessey et al. 2016) and chikungunya virus (Escobar et al. 2016).

This mosquito species completes its immature development in small-volume artificial containers associated with the human environment, such as flasks, bottles, flower vases, and used tires (Focks et al. 1993). The main food sources for the larvae are microorganisms, detritus, and animal carcasses (Merritt et al. 1992), whose availability depends on the external supply of plant and animal organic matter to the containers.

It is generally assumed that mosquito populations that develop in container habitats are regulated by bottom-up processes, in particular by the availability of food resources (Washburn 1995). Field studies have shown the importance of food availability during the development of *Ae. aegypti*. In these studies, the amount of food had a positive relationship with: a) the number of larvae and pupae in the containers (Subra and Mouchet 1984, Barrera et al. 2006), b) the amount of larval nutritional reserves (Arrivillaga and Barrera 2004), and c) the size of adults (Strickman and Kittayapong 2003). In experimental studies, larval food showed a positive relationship with female fecundity (Briegel 1990, Naksathit and Scott 1998). On the other hand, since larval food availability has also been related to changes in longevity of females (Naksathit and Scott 1998, Reiskind and Lounibos 2009, Joy et al. 2010), the effects of larval nutrition might translate into the capacity of disease transmission.

Most studies of the effects of food availability on the development of *Ae. aegypti* analyzed conditions of food scarcity and compared them to optimal conditions. However, recent studies have shown larvae developing in water with high contents of organic matter such as septic tanks, and have suggested that these habitats contribute significantly to maintain dengue transmission in Puerto Rico (Barrera et al. 2008). Further studies have shown that the presence and abundance of immature stages and adults in septic tanks was negatively related to the content of total dissolved solids (Burke et al. 2010). This is consistent with previous laboratory studies that suggest that the excess of food represents suboptimal conditions for *Ae. aegypti* immature stages. The tolerance of larvae to high nutrient content exhibits variability among strains from different origins (Pope and Wood 1981). A higher tolerance has been attributed to selection pressures at the site of origin, such as the predominance of polluted larval sites, or the insecticide treatment of clean water containers (Pope and Wood 1981, Barrera et al. 2008).

Despite the important consequences of food on the life history traits of immature mosquitoes, few studies (Olivo et al. 1979, Agnew et al. 2002, Arrivillaga and Barrera 2004, Couret et al. 2014, Levi et al. 2014) have attempted to assess this effect separately from larval density of *Ae. aegypti*. Previous studies used two types of feeding regimes:

a) Larval food added only once at the beginning of the experiment (allowing for food deployment). In these studies, none of the individuals completed development below a certain

amount of food, while above that limit individuals that reached the adult stage completed development on average within six days regardless of the food level (Levi et al. 2014), and the size of adults increased with higher food availability (Agnew et al. 2002).

b) A fixed amount of food added daily. In these studies that represented more realistic conditions, food availability varied with time, depending on the amount added daily and on the consumption rate of the studied larvae. The most important effects of food scarcity were an extension in development time and a decrease in the size of adults, while an increase in mortality was observed only at very low food levels (Olivo et al. 1979, Arrivillaga and Barrrera 2004, Couret et al. 2014, Romeo Aznar et al. 2015).

These studies have generally focalized on average effects of food availability (Olivo et al. 1979, Arrivillaga and Barrrera 2004, Couret et al. 2014), and little is known about the effects on the variability in traits like development time or adult size within cohorts. Only two previous studies mentioned an increased variability of development times and adult size for individuals reared with low food availability (Couret et al. 2014, Romeo Aznar et al. 2015), despite the importance that such differential variability may have on populations dynamics.

In the present study, we attempted to assess the response of the life history traits of *Ae. aegypti* to different amounts of larval food, including treatments that represent scarcity and excess of food as compared to optimal conditions. Food solutions were reset daily to maintain constant food availability during the whole immature development and thus decouple the dynamics of food from the previous larval consumption.

MATERIALS AND METHODS

Eggs of *Ae. aegypti* were collected with ovitraps placed in Buenos Aires city, Argentina. All the collected eggs were assumed to correspond to *Ae. aegypti* because this is the only container-breeding Aedine mosquito species in this region (Rubio et al. 2012). Before the beginning of the experiment, eggs were induced to hatch by immersion in dechlorinated tap water.

Recently hatched larvae (less than 24 h old) were separated in cohorts of 30 individuals. Each cohort was randomly assigned to one food treatment. The food used was dry Baker´s yeast (Levex®) diluted in 800 ml of dechlorinated tap water. Yeast cells (*Saccharomyces cerevisiae*) constitute an optimal source of food for *Ae. aegypti* larvae and are also easy to manipulate and quantify (Souza et al. 2016). Treatments used were defined according to the results of a preliminary experiment (Romeo Aznar et al. 2015) in order to include treatments with food scarcity but low mortality (2.3438 mg/800 ml), the presumed optimal food (75 mg/800 ml), and eight times the presumed optimal food (600 mg/800ml), which might be considered excess of food. Three replicate cohorts for each food treatment (2.3438, 4.688, 9.375, 18.75, 37.5, 75, 150, 300, and 600 mg yeast) were reared in plastic cylindrical containers (1,000 ml, diameter 105 mm, height 125.5 mm), covered by a plastic cap to avoid contamination.

During the duration of the experiment, each container was inspected daily, larvae were counted, their instar recorded, and then transferred to a new container with the corresponding food amount to maintain relatively constant food availability. The new food solutions were prepared 24 h before use to stabilize temperature conditions. Although no measures were taken, the growth of yeast cells was expected to be minimal because no further nutrients were added. Pupae were transferred individually to containers conditioned for adult emergence. These consisted of a small plastic cap containing 1 ml of water and the pupa, placed inside a larger cylindrical container (diameter 39 mm, height 55 mm), and covered by a nylon mesh to prevent the escape of the adult mosquito. Temperature conditions were 25.4 $\pm$ 1.0º C, and a 12:12 (light:dark) photoperiod was maintained.

For each individual, time to pupation, time to adult emergence, and sex were recorded. Both wings were removed and measured (from the alular notch to the distal margin excluding the fringe scales) to the nearest 0.001 mm by using a dissecting microscope equipped with a digital camera. Measurements were performed on digital photographs with the Leica Application Suite V 4.0.0.

For each cohort of 30 larvae, total survival was calculated as the number of individuals that reached the adult stage divided by the initial number of larvae, and instar specific survival was estimated as the number of individuals that successfully completed the instar divided by the number of individuals that initiated that instar.

For each adult, total development time (from hatching to adult emergence), duration of the pupal stage (from pupation to adult emergence), sex, and wing length were recorded. To estimate the duration of each larval instar, the first step was to calculate the percentage of larvae within each instar (*i*) for each day. These percentages were then plotted against time on log x probability paper, to estimate the time at which 50% of the larvae reached each successive instar (t_{50}) (Enfield and Pritchard 1977). The duration of each instar (*i*) in days was calculated by subtracting $t_{50\ (i)}$ values from $t_{50\ (i+1)}$. The proportion of females was calculated for each treatment as the number of females divided by the total number of individuals that reached the adult stage.

The differences among treatments of total survival, survival of each larval instar and pupal stage, and proportion of females were analyzed with pairwise comparisons using the Fisher exact test, adjusting the significance of the test with the Benjamini-Hochberg correction for multiple comparisons (Benjamini and Hochberg 1995). Cohorts were pooled for these analyses to avoid loss of power because of the small numbers of individuals in some treatments. The effects of the treatments on duration of each larval instar and the pupal stage, on total development time, and on wing length were analyzed with Generalized Linear Mixed Models (GLMM), using the R software, Version 3.2.3 (R Core Team 2015), accessed through a user-friendly interface in Infostat software (Di Rienzo et al. 2014). Models were fitted and parameters were estimated using the Maximum Likelihood method (Pinheiro and Bates 2000). In all cases, post-hoc multiple comparisons were performed with Fisher´s Least Significant Difference test on ranks (Conover 1999), adjusting the significance of the test with the Benjamini-Hochberg correction for multiple comparisons (Benjamini and Hochberg 1995).

The effect of the treatments on the duration of each larval instar was analyzed using the normal family and the identity link function, and treatment, larval instar, and treatment x instar interaction were included as fixed effects. The container identity was included as a random effect to account for the lack of independence among instars from the same container. The

temporal correlation of successive instars was accounted for with a type 1 autocorrelation term, and the variances were stabilized among treatments with the varIdent function (Pinheiro and Bates 2000).

The effects of treatment and sex on total development time, duration of the pupal stage, and wing length were analyzed with GLMM, using the gamma family and the log link function. In these models, food treatment, sex, and their interaction were included as fixed effects, and container identity was included as a random effect to account for the lack of independence between individuals from the same container.

To assess the effect of larval food on the variability in development time and in wing length, the coefficient of variation (CV = standard deviation/mean) of each variable within each treatment was calculated for females and males separately. To assess the sex specific responses of development time and wing length to different food treatments, the percent differences between sexes in development time and wing length were calculated as 100 x (M-F)/[(M+F)/2] (Wormington and Juliano 2014). Cohorts were pooled for these analyses to avoid overestimation of the variability owing to a small number of individuals in some treatments. The relationships of variability measures (CV of development time for males and females, CV of wing length for males and females, and percent differences between sexes of development time and wing length) with food availability were analyzed with Spearman rank correlations, appropriate for non-linear relationships. The relative importance of food availability on different life history traits was analyzed together with a principal component analysis on the correlation matrix of total survival, proportion of females, mean development time, and mean wing length in each container.

RESULTS

Immature development was completed by at least some individuals in all treatments, although large differences in life history parameters were observed along the food availability gradient. No statistical differences in the survival of 1st and 2nd instar larvae were detected among treatments with the Fisher exact test. In contrast, a trend to significantly higher mortality of the 3rd and 4th larval instars was observed in the treatments with highest and lowest food availability. In the pupal stage, significantly higher mortality was observed at the highest food availability (Table 1).

Similar results were obtained for the survival to adulthood, which showed significant differences among food treatments. Multiple comparisons showed highest survival at intermediate food availability (between 9.375 and 75 mg of yeast/day), and significant reductions at the high and low end of the food treatments analyzed (Table 1). The proportions of females among adults showed a trend to higher proportions of females at intermediate food concentrations, in coincidence with treatments where higher survival was observed. The differences were significant only between the lowest and two of the intermediate food treatments (Table 1).

The results of the GLMM analysis showed that the duration of larval instars was significantly affected by the food treatment ($p < 0.001$, $F = 167.1$, $df = 8$), instar ($p < 0.001$, $F = 917.0$, $df = 3$), and the instar by treatment interaction ($p < 0.001$, $F = 67.2$, $df = 24$). Within each food treatment, longer durations were recorded for the 4th instar, followed by the 1st instar and the 3rd instar, while the shortest duration was recorded for the 2nd instar. For all instars, a trend towards an extended duration with lower food levels was observed. This effect increased in significance in later larval instars, as shown by the significant instar by treatment interaction term. In comparison with optimal food conditions (75 mg of yeast/day), the duration of the 1st larval instar increased significantly at the two lowest food levels, the duration of the 2nd and 3rd larval instar increased significantly at the three lowest food levels, and the duration of the 4th larval instar increased significantly at the four lowest food levels (Table 2). The duration of the pupal stage was significantly ($p < 0.05$) shorter for males (2.19 days) than for females (2.26 days), and no significant differences were detected among treatments (Table 2).

The GLMM analysis showed that total development time was significantly affected by food ($p < 0.001$), sex ($p < 0.001$) and the food by sex interaction ($p < 0.001$). Development times were shorter with high food levels, and although males generally completed development earlier, differences were significant with 75 mg yeast/day or less, while no differences between sexes were detected above that food level. For males, the shortest development times (8.4 days) were observed with the 37.5 and 75 mg yeast/day treatments and were significantly different from those with 18.75 mg yeast/day or less as well as from 600 mg yeast/day. For females, the shortest development times (9.1 days) were observed with 75 to 150 mg yeast/day, which differed significantly from those with 18.75 mg yeast/day or less. Below 18.75 mg yeast/day, all categories differed from each other in development time,

Table 1. Instar specific survival, total survival, and proportion of females for different food treatments. Similar letters indicate groups without significant differences within the corresponding row. (*) value not shown because only one individual survived.

Food (mg yeast/day/container)	2.344	4.688	9.375	18.75	37.5	75	150	300	600
Survival of 1st larval instar	0.98^{a}	0.93^{a}	1.00^{a}	0.99^{a}	0.99^{a}	0.99^{a}	1.00^{a}	1.00^{a}	1.00^{a}
Survival of 2nd larval instar	0.99^{a}	0.98^{a}	1.00^{a}	0.97^{a}	1.00^{a}	1.00^{a}	1.00^{a}	1.00^{a}	0.99^{a}
Survival of 3rd larval instar	0.92^{b}	0.99^{ab}	1.00^{a}	1.00^{a}	0.99^{a}	1.00^{a}	0.74^{c}	0.20^{d}	0.55^{e}
Survival of 4th larval instar	0.74^{c}	0.94^{b}	0.99^{ab}	1.00^{a}	0.99^{ab}	1.00^{a}	0.70^{c}	0.06^{e}	0.49^{d}
Survival of pupal stage	0.95^{abc}	0.96^{abc}	0.99^{ab}	0.99^{ab}	0.98^{ab}	1.00^{a}	0.89^{bc}	1.00^{abc}	0.79^{c}
Survival from 1st larval instar to adult	0.62^{c}	0.80^{b}	0.98^{a}	0.94^{a}	0.94^{a}	0.99^{a}	0.47^{c}	0.01^{e}	0.21^{d}
Proportion of females	0.30^{a}	0.49^{ab}	0.57^{b}	0.54^{ab}	0.56^{b}	0.49^{ab}	0.44^{ab}	*	0.42^{ab}

Table 2. Instar specific development time for different food treatments. Time is expressed in days. Similar letters indicate groups without significant differences within the larval or pupal stage. (**) indicates treatment not statistically analyzed because of total mortality of individuals.

Food (mg yeast/day/container)	2.344	4.688	9.375	18.75	37.5	75	150	300	600
Duration of 1st larval instar	3.7^{d}	3.4^{d}	2.4^{ef}	2.5^{ef}	2.0^{f}	2.4^{ef}	2.2^{f}	2.4^{ef}	2.7^{e}
Duration of 2nd larval instar	1.9^{fg}	1.1^{ghij}	1.0^{ij}	0.7^{jkl}	0.7^{jkl}	0.5^{l}	0.6^{kl}	0.5^{l}	0.6^{kl}
Duration of 3rd larval instar	2.9^{de}	1.5^{gh}	1.6^{g}	0.9^{jk}	1.0^{ij}	1.0^{ij}	1.1^{hij}	0.6^{l}	1.2^{ghi}
Duration of 4th larval instar	14.1^{a}	8.0^{b}	6.1^{c}	3.3^{d}	2.7^{e}	2.6^{e}	2.3^{ef}	2.0^{f}	3.1^{d}
Duration of pupal stage (males)	2.2^{a}	2.4^{a}	2.1^{a}	2.0^{a}	2.2^{a}	2.3^{a}	2.3^{a}	2.0^{a}	2.1^{a}
Duration of pupal stage (females)	2.5^{a}	2.4^{a}	2.4^{a}	2.2^{a}	2.2^{a}	2.3^{a}	2.2^{a}	**	2.1^{a}

with maximum mean values of 27.1 and 22.3 days for females and males respectively at the lowest food availability (Figure 1).

According to the GLMM analysis, wing lengths were significantly affected by sex ($p < 0.001$) and by food treatment ($p < 0.001$), but no interaction between sex and food level was detected. Males always had smaller wings than females, and for both sexes the longest wing lengths were obtained with 75 and 150 mg yeast/day, which is significantly longer than those from the highest food treatment. Shorter wing lengths were detected as food availability decreased, with significant reductions among consecutive treatments in most cases (Figure 2).

Variability in development time showed a negative relationship with larval food (inset in Figure 1) and was significant both for females ($Rs = -0.71$, $p < 0.05$, $n = 8$) and for males ($Rs = -0.80$, $p < 0.02$, $n = 8$). On the other hand, the variability in wing length of adults also showed a negative relationship with larval food (inset in Figure 2), although the relationship was significant only for females ($Rs = -0.90$, $p < 0.005$, $n = 8$) but not for males ($Rs = -0.64$, $p = 0.08$, $n = 8$). Percent differences among sexes in development time were largest at low food treatments and decreased significantly with increasing food ($Rs = 0.93$, $p < 0.001$, $n = 8$). In contrast, percent differences among sexes in wing length were relatively constant (Figure 3), and no significant trend across food treatments was detected ($Rs = 0.43$, $p = 0.29$, $n = 8$).

The two principal components explained 84% of the variability among cohorts in the four variables analyzed. The first principal component explained 51.7% of the variability among containers and had a negative correlation with development time ($r = -0.92$, $p < 0.001$), and a positive correlation with wing length ($r = 0.81$, $p < 0.001$) and proportion of females ($r = 0.65$, $p < 0.002$). The second principal component explained 32.6% of the variability and was positively correlated with survival ($r = 0.79$, $p < 0.001$) and with the proportion of females ($r = 0.59$, $p > 0.005$) and negatively correlated with wing length ($r = -0.49$, $p < 0.15$).

According to their location on the two principal components, two gradients of treatments could be differentiated (Figure 4). The first gradient along the first axis (and values near to zero on the second axis) related mainly to development times and wing lengths, ranges from food scarcity (low food treatments from 2.34 to 4.67 mg yeast/day) associated with slow development, small adults, and a lower proportion of females to optimal food (intermediate food treatments from 37.5 to 75 mg yeast/day) associated with fast development, large adults, and a higher proportion of females. The gradient along the second axis (and positive or near to zero values on the first axis), related mainly to survival and to the proportion of females, ranges from optimal food, associated with high survival, a high proportion of females and large adults, to excess of food (highest food treatments from 150 to 600 mg yeast/day), associated with a low survival, a lower proportion of females, and intermediate-sized adults.

DISCUSSION

The ability of some individuals to complete development in all treatments suggests that the population studied is well adapted to a wide range of food availability, although with different responses of life history traits to extreme conditions of scarcity or excess of food, both of which have been demonstrated to represent suboptimal conditions.

The extension of the development times and the reduced size of adults observed under conditions of food scarcity are consistent with results of previous studies (Bar Zeev 1957, Olivo et al. 1979, Arrivillaga and Barrera 2004, Couret et al. 2014). The ability to survive long times with very little food suggests that food scarcity might be a selective force in this population, because food availabilities in larval habitats are usually low, at least in tropical and subtropical climates (Subra and Mouchet 1984, Barrera et al. 2006). On the other extreme, some effects of excessive food on adult size (smaller adults) and survival (lower survival) have also been observed in previous studies (Olivo et al. 1979, Arrivillaga and Barrera 2004).

The abrupt increases in mortality in the highest food treatments shows that these conditions are not well tolerated by the population studied and are probably avoided in natural conditions. In fact, experimental studies have shown that although *Ae. aegypti* females are attracted to oviposit on water with microorganisms, infusions prepared with low plant biomass are preferred, while those with high plant biomass are not (Ponnusamy et al. 2010). This preference might partly explain the widely distributed concept that *Ae. aegypti* larvae develop in clean water (Clements 1992), although this idea should be revised considering that with a moderate excess of food immature stages develop faster and adults attain larger sizes than under food-limiting conditions (Olivo et al. 1979, Arrivillaga and Barrera 2004).

The effects of both scarcity and excess of food seem to be more important for later larval instars, as suggested by the higher

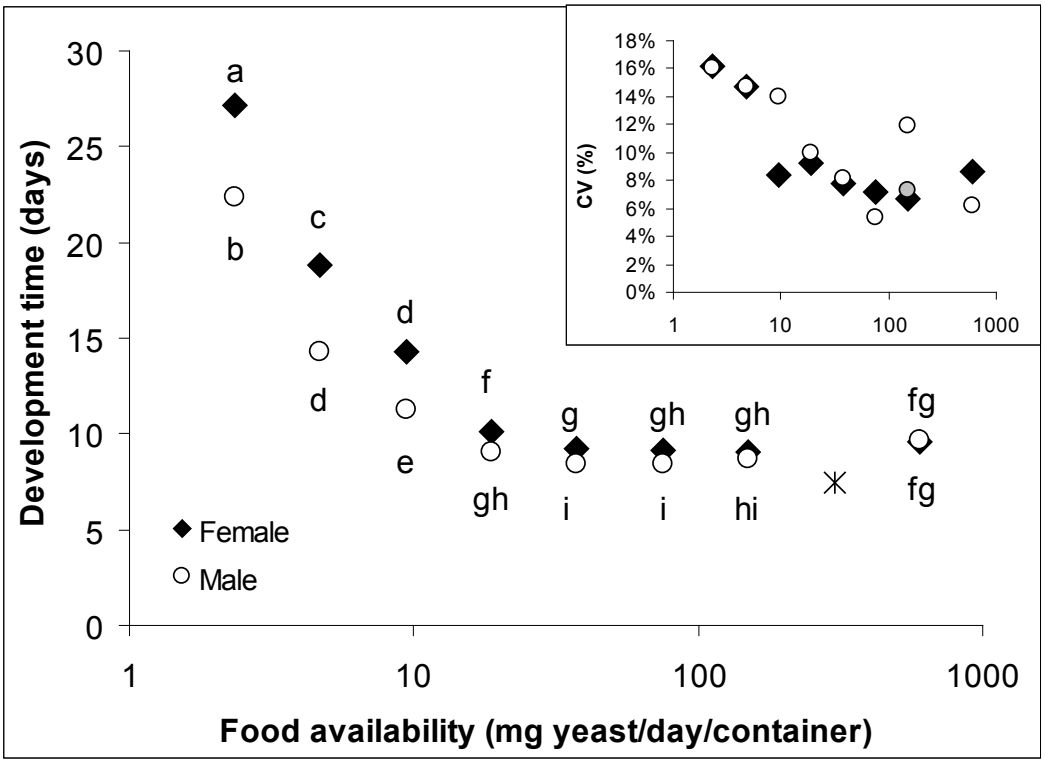

Figure 1. Development time for females and males reared with different food treatments. (*) not included in the statistical analysis because only one male survived. Similar letters indicate treatments without significant differences. Inset: coefficient of variation of development times for females and males. The grey symbol indicates the CV calculated eliminating one outlier individual.

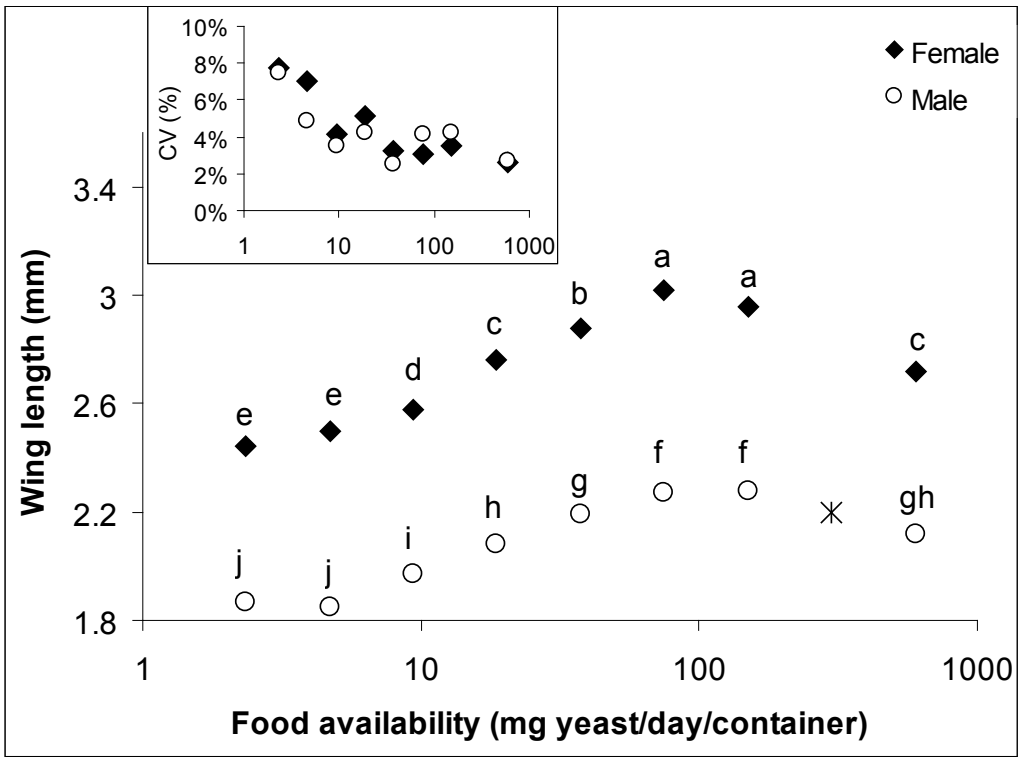

Figure 2. Wing length of females and males reared with different food treatments. (*) not included in statistical analysis because only one male survived. Similar letters indicate groups without significant differences. Inset: coefficient of variation of wing lengths for females and males.

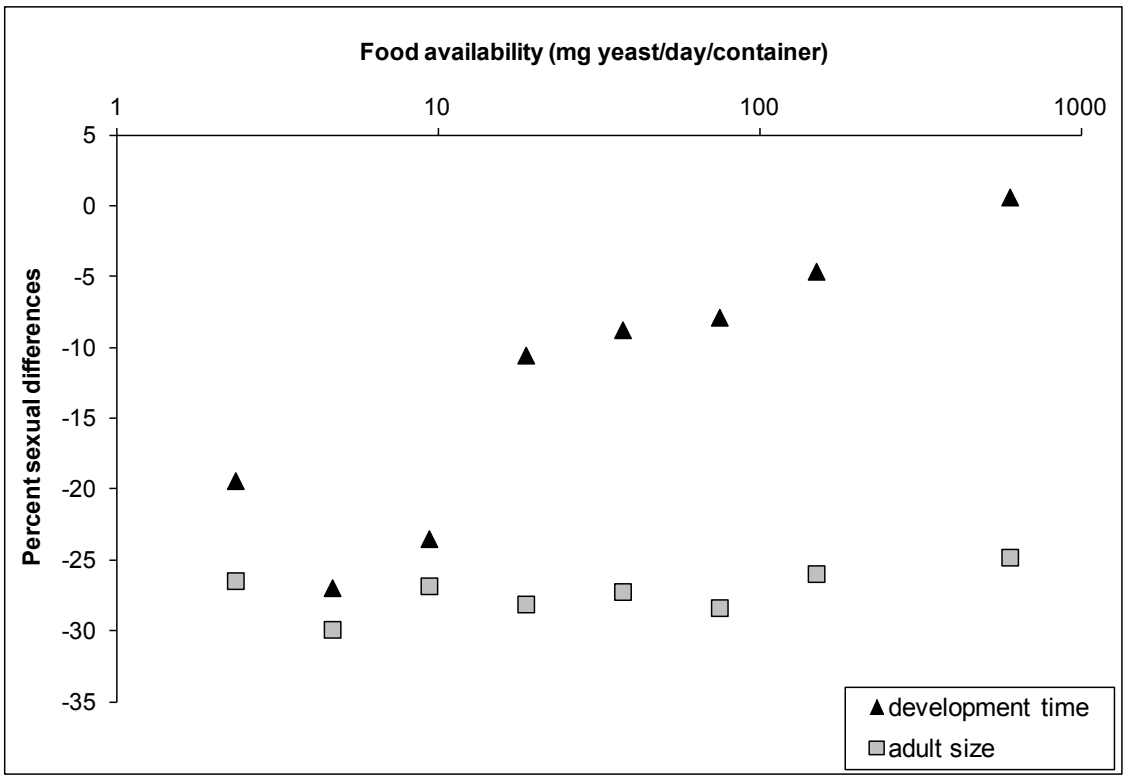

Figure 3. Percent sexual differences in development time and adult size of individuals reared with different food treatments. For each food level and variable, the percent difference between males and females was calculated as = 100 x (M-F)/[(M+F)/2].

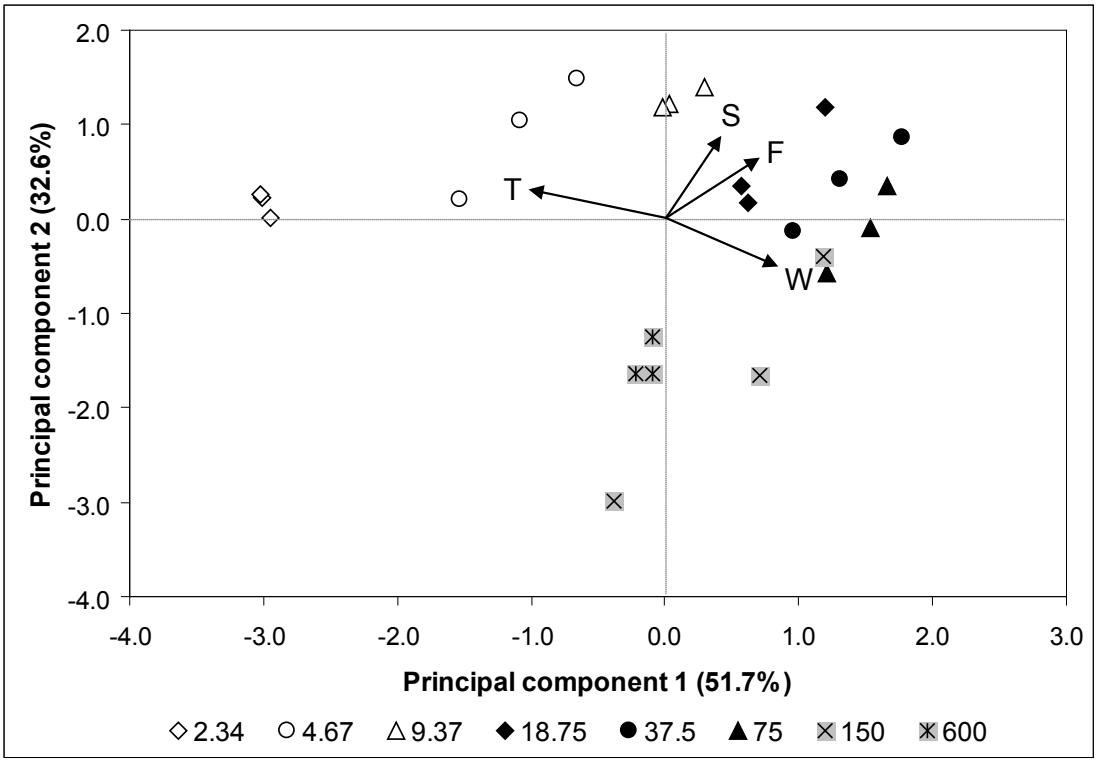

Figure 4. Principal Component Analysis results. Symbols show coordinates of cohorts on the two principal components; Arrows indicate correlation with each of these principal components. S: survival, W: wing length, T: development time, F: proportion of females.

variability in survival and duration of the 3rd and 4th larval instars in different food treatments compared with those of the 1st and 2nd larval instars. The effects of food scarcity on early instars were detected only with the most extreme treatments, while those on later instars were significant at slightly suboptimal conditions. These findings are coincident with studies that reported that development time, adult size, fecundity, and longevity are determined mainly by the feeding regime of later instars, regardless of the amount of food received by individuals during early instars (Zeller and Koella 2016). The similar duration of the pupal stage in different food treatments is consistent with the fact that no feeding occurs during this stage, and thus the completion of this stage depends on the nutritional reserves accumulated in the larval stage. An extended duration of this stage would deplete the nutritional reserves necessary to complete the emergence of the adults.

The higher proportions of females in treatments with higher survival suggest that the negative effects of food scarcity or excess might be acting stronger on females than on males. Sex-specific reaction norms in response to food availability could be inferred for development time, meaning that females exhibit a larger extension of development time in response to food scarcity than males. These results are similar to those obtained under competition conditions (Bedhomme et al. 2003) and might be related to the fitness advantage (the relative ability of an individual to survive, reproduce, and propagate genes) that males obtain when emerging early and accessing to copulate with virgin females. On the other hand, females are expected to maximize their fitness by attaining the maximum possible body size, thus increasing fecundity. This has been supported experimentally by a lower reduction in body size of females compared with that of males under competition (Bedhomme et al. 2003), and under moderate food scarcity (Wormington and Juliano 2014), although in the latter experiment a very small range of food conditions was analyzed. However, our results do not support this kind of response, as indicated by the relatively constant relationships in size among sexes. Thus, our results suggest that the proportionally larger extension in development time experienced by females compared to males at low food availabilities does not translate to a proportionally increase in size.

Interesting results were obtained regarding within treatment variability in development time and adult size among individuals. The increase in variability of both traits at the lowest food treatments is consistent with a widespread trend of higher variation in the presence of environmental stress (Badyaev 2005). Increased variability of life history traits has been studied mainly in *Drosophila* in response to stress conditions such as larval crowding (Imasheva and Bubliy 2003), extreme temperatures (Sisodia and Singh 2009), and nutritional limitation (Bubliy et al. 2000). It has been suggested that the increase in variability might be related to either an increase in the rate of mutation and recombination in response to stress, or by the expression of genetic variation that is hidden under a normal range of conditions. In any case, this variation is thought to be adaptive when it facilitates the development of novel adaptation to changed environments (Badyaev 2005).

In the case of our experiment, the larger variability of the analyzed traits under food scarcity conditions may support the hypothesis that the population of *Ae. aegypti* in Buenos Aires is subjected to a variety of food conditions. In this scenario, frequent food stress conditions maintain or even increase genetic variation and select for small size individuals, and periodic exposure to optimal conditions favors the maintenance of the large size variants within the population. Similar conclusions were reached in a study of a natural population of *Ae. aegypti* from Trinidad, that demonstrated genotype differences in the response of adult size to larval food scarcity, which provide the population with a wide phenotypic plasticity to environmental effects (Schneider et al. 2011).

The differential variability in development time under food scarcity conditions should be included in populations dynamics models, especially for females, since generally a certain synchronicity in development is assumed. On the other hand, changes in size variability (especially for females) related to food conditions deserve further study, since this might be a useful indicator of the nutritional quality of containers in the field.

In conclusion, food availability during larval development has an important influence on fitness, affecting the development time, survival, and size of the emerged adults. Although not measured here, other traits such as adult nutritional reserves (Briegel 1990), longevity, blood feeding pattern, and fecundity (Zeller and Koella 2016) are also known to be affected.

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REFERENCES CITED

Agnew, P., M. Hide, C. Sidobre, and Y. Michalakis. 2002. A minimalist approach to the effects of density-dependent competition on insect life-history traits. Ecol. Entomol. 27: 396-402.

Arrivillaga, J. and R. Barrera. 2004. Food as a limiting factor for *Aedes aegypti* in water storage containers. J. Vector Ecol. 29: 11-20.

Badyaev, A.V. 2005. Stress-induced variation in evolution: from behavioural plasticity to genetic assimilation. Proc. R. Soc. B 272: 877-886.

Barrera, R., M. Amador, and G.G. Clark. 2006. Ecological factors influencing *Aedes aegypti* (Diptera: Culicidae) productivity in artificial containers in Salinas, Puerto Rico. J. Med. Entomol. 43: 484-492.

Barrera, R., M. Amador, A. Diaz, J. Smith, J. L. Munoz-Jordan, and Y. Rosario. 2008. Unusual productivity of *Aedes aegypti* in septic tanks and its implications for dengue control. Med. Vet. Entomol. 22: 62-69.

Bar Zeev, M. 1957. The effect of density on the larvae of a mosquito and its influence on fecundity. Bull. Res. Counc. Isr. 6: 220-228.

Bedhomme, S., P. Agnew, C. Sidobre, and Y. Michalakis. 2003. Sex-specific reaction norms to intraspecific larval competition in

the mosquito *Aedes aegypti*. J. Evol. Biol. 16: 721-730.
Benjamini, Y. and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B 57: 289-300.
Briegel, H. 1990. Metabolic relationship between female body size, reserves, and fecundity of *Aedes aegypti*. J. Insect Physiol. 36: 165-172.
Bubliy, O.A., A.G. Imasheva, and V. Loeschcke. 2000. Half-sib analysis of three morphological traits in *Drosophila melanogaster* under poor nutrition. Hereditas 133: 59-63.
Burke, R., R. Barrera, M. Lewis, T. Kluchinsky, and D. Claborn. 2010. Septic tanks as larval habitats for the mosquitoes *Aedes aegypti* and *Culex quinquefasciatus* in Playa-Playita, Puerto Rico. Med. Vet. Entomol. 24: 117-123.
Clements, A.N. 1992. *The Biology of Mosquitoes: Development, Nutrition, and Reproduction.* Chapman & Hall, London, UK.
Conover, W.J. 1999. *Practical Nonparametric Statistics*. 3rd edn. John Wiley & Sons, New York, USA.
Couret, J., E. Dotson, and M.Q. Benedict. 2014. Temperature, larval diet, and density effects on development rate and survival of *Aedes aegypti* (Diptera: Culicidae). PLoS ONE 9: e87468.
Di Rienzo, J.A., F. Casanoves, M.G. Balzarini, L. Gonzalez, M. Tablada, and C.W. Robledo. 2014. InfoStat version 2014. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. (http://www.infostat.com.ar).
Enfield, M.A. and G. Pritchard. 1977. Estimates of population size and survival of immature stages of four species of *Aedes* (Diptera: Culicidae) in a temporary pond. Canad. Entomol. 109: 1425-1434.
Escobar, L.E., H. Qiao, and A. Townsend Peterson. 2016. Forecasting Chikungunya spread in the Americas via data-driven empirical approaches. Parasit. Vectors 9: 112.
Focks, D.A., D.G. Haile, E. Daniels, and G.A. Mount. 1993. Dynamic life table model for *Aedes aegypti* (Diptera: Culicidae): analysis of the literature and model development. J. Med. Entomol. 30: 1003-1017.
Hennessey, M., M. Fischer, and J.E. Staples. 2016. Zika Virus spreads to new areas — region of the Americas, May 2015–January 2016. MMWR Morb. Mortal. Wkly. Rep. 65: 55-58. doi: 10.15585/mmwr.mm6503e1
Imasheva, A.G. and O.A. Bubliy. 2003. Quantitative variation of four morphological traits in *Drosophila melanogaster* under larval crowding. Hereditas 138: 193-199.
Joy, T.K., A.J. Arik, V. Corby-Harris, A.A. Johnson, and M.A. Riehle. 2010. The impact of larval and adult dietary restriction on lifespan, reproduction and growth of the mosquito *Aedes aegypti*. Exp. Gerontol. 45: 685-690.
Levi, T., E. Ben-Dov, P. Shahi, D. Borovsky, and A. Zaritsky, 2014. Growth and development of *Aedes aegypti* larvae at limiting food concentrations. Acta Trop. 133: 42-44.
Merritt, M.R., R.H. Dadd, and E.D. Walker. 1992. Feeding behavior, natural food, and nutritional relationships of larval mosquitoes. Annu. Rev. Entomol. 37: 349-376.
Naksathit, A.T. and T.W. Scott. 1998. Effect of female size on fecundity and survivorship of *Aedes aegypti* fed only human blood versus human blood plus sugar. J. Am. Mosq. Contr. Assoc. 14: 148-152.
Olivo, R., G.P. Hoskin, and K. Frogner. 1979. Some effects on newly emerged mosquitoes (*Aedes aegypti*) resulting from food deprivation during larval development. J. Invertebr. Pathol. 33: 53-56.
Pinheiro, J.C. and D.M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer, New York, USA.
Ponnusamy, L., N. Xu, K. Böröczky, D.M. Wesson, L.A. Ayyash, C. Schal, and C.S. Apperson. 2010. Oviposition responses of the mosquitoes *Aedes aegypti* and *Aedes albopictus* to experimental plant infusions in laboratory bioassays. J. Chem. Ecol. 36: 709-719.
Pope, V. and R.J. Wood. 1981. Tolerance of *Aedes aegypti* larvae to synthetic sewage. Mosq. News 41: 732-736.
R Core Team. 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/).
Reiskind, M.H. and L.P. Lounibos. 2009. Effects of intraspecific larval competition on adult longevity in the mosquitoes *Aedes aegypti* and *Aedes albopictus*. Med. Vet. Entomol. 23: 62-68.
Romeo Aznar, V., M.S. De Majo, S. Fischer, D. Francisco, M.A. Natiello, and H.G. Solari. 2015. A model for the development of *Aedes* (*Stegomyia*) *aegypti* as a function of the available food. J. Theor. Biol. 365: 311-324.
Rubio, A., M.I. Bellocq, and D. Vezzani. 2012. Community structure of artificial container-breeding sites (Insecta: Diptera) in relation to the urbanization level. Landsc. Urban Plan. 105: 288–295.
Schneider J.R., D.D. Chadee, A. Mori, J. Romero-Severson, and D.W. Severson. 2011. Heritability and adaptive phenotypic plasticity of adult body size in the mosquito *Aedes aegypti* with implications for dengue vector competence. Infect. Genet. Evol. 11: 11-16.
Sisodia, S. and B.N. Singh. 2009. Variations in morphological and life-history traits under extreme temperatures in *Drosophila ananassae*. J. Biosci. 34: 263-274.
Souza, R.S., H.M. Diaz-Albiter, V.M Dillon, R.J., Dillon, and F.A. Genta, 2016. Digestion of yeasts and Beta-1,3 Glucanases in mosquito larvae: physiological and biochemical considerations. Plos ONE 11(3): e0151403.
Strickman, D. and P. Kittayapong. 2003. Dengue and its vector in Thailand: calculated transmission risk from total pupal counts of *Aedes aegypti* and the association of wing length measurements with aspects of the larval habitat. Am. J. Trop. Med. Hyg. 68: 209-217.
Subra, R. and J. Mouchet. 1984. The regulation of preimaginal populations of *Aedes aegypti* (L.) (Diptera: Culicidae) on the Kenya coast. II. Food as a main regulatory factor. Ann. Trop. Med. Parasitol. 78: 63-70.
Washburn, J.O. 1995. Regulatory factors affecting larval mosquito populations in container and pool habitats: implications for biological control. J. Am. Mosq. Contr. Assoc. 11: 279-283.
Wormington, J.D. and S.A. Juliano. 2014. Sexually dimorphic body size and development time plasticity in *Aedes* mosquitoes (Diptera: Culicidae). Evol. Ecol. Res. 16: 223-234.
Zeller, M. and J.C. Koella. 2016. Effects of food variability on growth and reproduction of *Aedes aegypti*. Ecol. Evol. 6: 552-559.