Published in final edited form as:

J Med Entomol. 2011 September ; 48(5): 974–984.

Food Availability Alters the Effects of Larval Temperature on *Aedes aegypti* Growth

H. Padmanabha^{1,2,3}, B. Bolker⁴, C. C. Lord², C. Rubio¹, and L. P. Lounibos²

¹Instituto Nacional de Salud de Colombia, Vigilancia y Control en Salud Publica, Bogota, Colombia

²University of Florida, Florida Medical Entomology Laboratory, Vero Beach, FL 32962

⁴University of Florida, Department of Zoology, Gainesville, FL 32611

Abstract

Variation in temperature and food availability in larval habitats can influence the abundance, body size, and vector competence of the mosquito *Aedes aegypti*. Although increased temperature has energetic costs for growing larvae, how food resources influence the developmental response of this mosquito species to thermal conditions is unknown. We explored how rearing temperature and food affect allometric scaling between wing size and epidermal cell size in *Ae. aegypti*. Mosquitoes were reared at 22 and 28°C across a gradient of field-collected detritus designed to simulate commonly observed natural larval food resources. Overall, reduced temperature and increased food level increased wing size, but only temperature affected cell size. Females fed the least food had the longest time to maturation, and their increases in wing size induced by cold temperature were associated with larger, rather than more, cells. By contrast, males fed the most food had the shortest time to maturation, and their increases in wing size induced by cold temperature were associated with more, rather than larger, cells. Therefore, food levels can alter the underlying physiological mechanisms generating temperature-size patterns in mosquitoes, suggesting that the control of development is sensitive to the combination of nutrient and thermal conditions, rather than each independently. Conditions prolonging development time may favor increased cell division over growth. We suggest that understanding the effects of climate change on *Ae. aegypti* vectorial capacity requires an improved knowledge of how water temperature interacts with limited food resources and competition in aquatic container habitats.

Keywords

dengue; *Aedes aegypti*; temperature; resource limitation; climate change

Variation in the wing size of the dengue and chikungunya vector, *Aedes aegypti* (L.), has been associated with numerous components of its vectorial capacity, including the rate of production (Strickman and Kittayapong 2003, Barrera et al. 2006), longevity (Reiskind and Lounibos 2009), biting habits (Klowden et al. 1988, Nasci 1991), dispersal (Maciel-De-

Freitas et al. 2007), and susceptibility to infection (Alto et al. 2008, Westbrook et al. 2010). Rather than a direct consequence of wing size, these associations are likely to be outcomes of the larval environmental factors that generate size variation. As in most mosquitoes, colder water temperature and abundant larval food are the primary environmental determinants of larger *Ae. aegypti* adults (Gilpin and McClelland 1979, Tun-Lin et al. 2000, Barrera et al. 2006). Nonetheless, surprisingly little is known about the underlying mechanisms through which temperature and nutrient availability generate size variation in adults (Arendt 2007).

Biologists have long searched for mechanistic explanations as to why lower temperature and increased nutrients increase ectotherm size, but have opposite effects on development rate and growth trajectory (Atkinson and Sibly 1997, Angilletta and Dunham 2003). In a warming climate, understanding the basis of this trade-off is particularly important in species whose population dynamics are governed by variation in resource availability, as ectotherms require more energy to develop faster at higher temperatures (Lafferty 2009). In *Ae. aegypti*, for example, the domestic vessels that larvae inhabit in disease endemic climates (20–30°C) often experience episodic and irregular food inputs, which commonly generates effects of food limitation and density dependence (Gilpin and McClelland 1979, Subra and Mouchet 1984, Arrivillaga and Barrera 2004, Padmanabha et al. 2010). Accordingly, a potential interaction between food and temperature conditions in *Ae. aegypti* development could alter the prediction that rising temperature will increase the rate of vector production (Focks et al. 1993), which is based on the assumption that temperature effects on development rate, growth, and mortality are independent of food conditions (Gilpin and McClelland 1979). For example, we recently showed that heightened development rate at increased temperatures can increase the mortality rate of early-instar *Ae. aegypti* (Padmanabha et al. 2011). Because larval mortality as a result of resource competition is known to play a major role in the regulation of adult *Ae. aegypti* abundance (Southwood et al. 1972, Gilpin and McClelland 1979, Dye 1982), temperature-food interactions in the larval environment could have consequences for vectorial capacity.

Because different environmental variables do not affect all body parts in the same way, the study of how different experimental treatments affect size allometry has been proposed as a tool for separating the effects of temperature and food availability on insect growth and development (Shingleton et al. 2009). In *Ae. aegypti*, thermal and nutritional constraints affect attainment of two developmental milestones: critical mass required for pupation and asymptotic or final mass, both of which are lower in males than in females. The interval to cessation of growth (ICG) is the period between attainment of the critical and final mass, during which up to 50% of total growth may occur under optimal feeding conditions in *Ae. aegypti* (Davidowitz and Nijhout 2004, Nishiura et al. 2007, Telang et al. 2007). Higher temperature (within thermal limits) increases growth efficiency (Rashed and Mulla 1989) and reduces both critical and final masses in *Ae. aegypti* (Chambers and Klowden 1990, Rueda et al. 1990); this generates a crossing of growth trajectories such that larvae growing in warm conditions reach the critical mass sooner than larvae in cooler conditions, but the latter larvae eventually reach a larger asymptotic mass (Atkinson and Sibly 1997). In contrast, higher food levels increase both size and development rate such that larvae reared

with more food reach the critical mass faster and grow to a larger final mass (Angilletta et al. 2003). Thus, increased temperature and decreased food, while both reducing the final mass of *Ae. aegypti*, have different effects on the larval growth trajectory, particularly during the ICG. Because not all body components have the same growth trajectory, it is likely that morphological indicators of allometry may identify interactive effects between temperature and nutrition (Shingleton et al. 2009).

Body size in multicellular organisms can be viewed as determined by two components: the size of cells and their number. In insects, body size is determined by the size of the surface epidermis that secretes the exoskeleton; thus, epidermal cells are the key determinants of overall size (French et al. 1998). In Diptera, wing cell size is conveniently estimated as the inverse of the density of trichomes, or hairs, each of which represents a single cell on the wing. The total number of cells in the wing can then be estimated by dividing the overall area by estimated cell size. Arendt (2007) reviewed a total of 14 studies comparing cell size and number relationships in *Drosophila melanogaster*, all of which analyzed size and number of epidermal wing cells. In general, these studies showed that colder temperature affects female size through larger effects on cell size than on cell number, whereas in males colder temperature increases both cell size and number (DeMoed et al. 1997, French et al. 1998, Azevedo et al. 2002, Arendt 2007). By contrast, food effects on female *D. melanogaster* wing size are generated largely through cell number (DeMoed et al. 1997). While these studies show that cell size and number can respond independently to environmental variation (Nijhout 2003), it is unknown whether the relationships observed in *D. melanogaster* hold true in mosquitoes. The only other Dipteran studied, the dungfly *Scathophaga stercoraria*, differs from *D. melanogaster* in that lower temperature has larger effects on cell number than on cell size (Blanckenhorn and Llaurens 2005).

In *Ae. aegypti*, up to 80% of growth may occur in the fourth-instar larva (Telang et al. 2007), including the thoracic proliferation of imaginal discs, which give rise to the wing epidermis (Christophers 1960, Nishiura 2002). Therefore, wing morphology is likely to be highly sensitive to larval environmental conditions that contribute to size variation in field-collected adults. For example, allometric effects of food and density treatments on the geometry of wing veins were similar between sexes in mid-range food/density treatments, but not at extremely high- and low-density conditions (Jirakanjanakit et al. 2007). However, to our knowledge, no studies have investigated the interactive effects of food and temperature variations on mosquito growth and morphology. In this study, we describe how temperature and food in the larval environment affect the relationships between cell size and wing size in *Ae. aegypti*. In particular, we reared larvae under variable food conditions in two separate feeding experiments designed to simulate resource conditions encountered in typical domestic habitats of this species and temperatures reflective of the altitudinal range of endemic dengue transmission in Colombia. We determined the independent and interactive effects of food and temperature treatments on wing size and cell size, as well as their relative effect on cell size versus number.

Materials and Methods

Experimental Rearing of *Ae. aegypti*

Experiments were carried out at the Universidad Nacional de Colombia, Bogotá, Colombia (elevation 2,640 m; latitude 4°N; mean temperature 13.0[C]), in rearing conditions designed to simulate typical domestic habitats at different elevations in Colombia. In the highest altitude, cities with endemic dengue transmission ($\approx 1,500$ m) water temperatures in typical *Ae. aegypti* habitats range between 21 and 23°C, as compared with 28–30°C in low-lying endemic cities (Padmanabha et al. 2010). Twenty newly hatched *Ae. aegypti* larvae (F₂) from Barranquilla, Colombia (altitude 5 m, latitude 10°N, mean monthly temperatures 26.8–28.5°C), were provided different levels of standardized household detritus (see below) as a nutrient base in 20-liter buckets (filled with at least 19.5 liters of water). This water volume is lower than that of typical water storage vessels in which *Ae. aegypti* production concentrates in dengue endemic areas of Colombia (Padmanabha et al. 2010). A 5W aquarium heater (Aquarios S.A. Bogotá, Colombia) was submerged at the bottom of each bucket to heat the water $\approx 3^\circ\text{C}$ above the ambient and prevent a temperature gradient from forming within each vessel. Buckets were placed in one of two ambient temperatures: cold buckets were exposed to Bogotá indoor temperatures (no climate modification, 12–18°C), and warm buckets were placed in a noninsulated room (3 $\times$ 4 m) maintained at 25°C with 80% ($\pm 10\%$) relative humidity, using an electric space heater and a humidifier. These treatments were standardized before experiments so that the cold group water would fluctuate between 21 and 23°C and the warm group between 27 and 29°C.

Food administered was a detritus mixture collected in the vicinity of water storage vessels of a dengue endemic neighborhood of Colombia. Details of preparation are previously described (Padmanabha et al. 2011). To simulate a range of food conditions that *Ae. aegypti* may experience in household vessels, two consecutive experiments were carried out in each temperature condition. In experiment 1, five groups of three replicate vessels received one of the following treatments every 3 d (beginning on the day of larval hatching): 50, 100, 200, 400, and 800 mg of sediment (hereby referred to by the respective daily application rates: 16.7, 33.3, 66.7, 133.3, 266.7 mg/d). Based on the pupation results of experiment 1 (called the high food experiment), we sought to induce food scarcity in experiment 2 (called the low food experiment). However, we observed that 40 mg was the minimum amount of food that contained matter from both of the layers described above. Accordingly, in the low food experiment, we varied food input frequency instead of amount. Groups of four replicate vessels per temperature were each assigned one of the following daily probabilities of addition of 40 mg of sediment: 0.1, 0.25, 0.5, and 0.75, giving expected mean food application rates of 4, 10, 20, and 30 mg/d, respectively. A random number generator determined the days in which each replicate received a food treatment. We used this method instead of food application because regular food input under food-limiting conditions may stall development in the L4 stage (Gilpin and McClelland 1979). Food application rates ($\pm$ SE) for each group were as follows: 2.9 (± 10.5), 11.4 (± 18.4), 20.0 (± 20.4), and 27.8 (± 18.8) mg/d. Experiments 1 and 2 gave a total of 62 vessels. Fourth-instar larvae (L4) and pupae were counted daily until all larvae died or pupated. Because experimental rearing conditions of roughly 22 and 28°C were achieved by heating the water in buckets placed in

colder ambient conditions, all pupae were removed and placed in emergence cups in ambient temperature of the warm chamber (25°C). This allowed us to readily retain and anesthetize eclosed adults for wing mounting. We note that wing development occurs in L4, rather than in the pupal stage (Christophers 1960).

Wing Photography and Image Analysis

The right wing of each adult mosquito with undamaged wings was mounted on a cover slip. Digital cameras and Optika Vision Pro software were employed to produce two images of each wing: 1) a $2,048 \times 1,536$ pixel image of the entire wing using a dissecting microscope ($\times 2.5$) (Fig. 1a), and 2) a 480×640 pixel image of a 0.0108 mm^2 area in the third posterior wing cell between the first and second anal veins using a compound microscope ($\times 40$) (Fig. 1b).

Image J software (National Institutes of Health) was used to calculate the area enclosed by a trace of the perimeter of the wing (Fig. 1a). It also performed an automated hair count by dichotomizing the color spectrum and counting the number of black points (Fig. 1b). Because wing veins, split hairs, or discontinuities in the background color as a result of mounting imperfections were all potential sources of error, counts were conducted on a smaller 0.0032 mm^2 square area within Fig. 1b. For each image we located the square that maximized the visualization of hairs and minimized blurriness and debris. Images with noticeable errors in dichotomization of hairs were discarded. Mean cell size was estimated by the reciprocal of hair counts, and an index of wing cell number was defined as cell number = wing size/cell size.

To evaluate whether the small area analyzed may have generated unrepresentative hair counts, counts were performed in a 2- to 3-fold larger area in a subset of 50 females (Supp. Fig. S1 [online only]). Proportional increase in hair counts with increased area, in addition to a slightly reduced correlation between wing area and the cell size when estimated in the larger area, led us to conclude that increasing the size of the hair count area would not have improved, and may have potentially reduced, the precision of our cell size estimates (see Supp. Fig. S2 [online only]). Therefore, in the absence of evidence of heterogeneities in epidermal cell size across mosquito wings, we considered that our procedure achieved an appropriate balance between including a representative number of hairs in each count (≈ 45 –85) and maximizing image quality.

Data Analysis

As a result of the variation in food input schedules and contrasting developmental strategies between sexes, data were analyzed separately for males and females and in the high and low food experiments. This allowed for qualitative, but not quantitative comparisons of results across sexes and feeding experiments. We first determined the effects of food input rate, temperature treatment, and the food-temperature interaction on wing size and cell size. Subsequently, we analyzed whether treatments had a larger impact on cell size or number.

Because of the large difference in scaling of wing and cell size, we used Z-scores to normalize wing area (Z_{wing}), mean cell size (Z_{size}), and cell number index (Z_{number}), where z

$= (x_i - \bar{x})/\bar{\sigma}$ (Marquardt 1980). We determined whether treatments had a larger impact on cell size or cell number through the difference in Z-scores, defined for each mosquito i as $Z_{diff,i} = Z_{size,i} - Z_{number,i}$. Accordingly, mosquitoes with disproportionately larger cell size as compared with cell number would have an increased Z_{size} as compared with Z_{number} , and thus a large positive Z_{diff} value. Mosquitoes with a disproportionately greater cell number as compared with cell size, $Z_{diff,i}$, will be negative. Therefore, predictors positively associated with Z_{diff} will have a larger impact on cell size, and a negative association indicates a larger impact on cell number. No association with Z_{diff} means the treatment induced proportional changes in cell size and cell number.

We used multiple random-effects, maximum likelihood regression using the procedure *xtreg* in STATA 8.0 Statistical Software (StataCorp, College Station, TX). Container was the group variable assigned randomly. We quantified the combined effects of temperature (warm group = 27–29°C versus cool group = 21–23°C), the Z-score of feeding rate (hereby referred to as food), and the food by temperature interaction (hereby referred to as warm × food) on the dependent variables Z_{wing} , Z_{size} , and Z_{diff} . Wald tests ($\alpha = 0.05$) were employed to determine the significance of regression coefficients. A separate model was constructed for each sex and food input experiment for a total of four models for each dependent variable. By evaluating significant associations with Z_{wing} , Z_{cell} , and Z_{diff} , conclusions were drawn as to whether treatment effects on overall wing size were because of a larger effect on cell size or number. We also evaluated whether the size difference between sexes was associated with increased cell size or number (see Supp. Fig. S3 [online only]).

Results

Mean observed temperatures across containers were 22.2°C (range, across vessels: 21.7–22.5) and 28.2°C (range, across vessels: 27.9–28.6) in cool and warm treatments, respectively, which were underestimates, as temperature in each container was generally measured in the cooler morning hours. We observed no significant temperature differences among buckets of different feeding treatments within each chamber. In the containers that were monitored hourly, average daily minimum and maximum temperature over the two experimental trials were 22.0 and 22.9°C (mean 22.5°C) in the cold treatment and 27.9 and 29.1°C (mean 28.5°C) in the warm treatment. Totals of 128 and 216 mosquitoes were analyzed in the low and high food experiments, respectively. Mean duration of larval development, or time to pupation, was lower in males and in the warm treatment. Increased food reduced development times, both between experiments and across their respective feeding gradients (Fig. 2). Variation in development times between the 22 and 28°C treatments was less than the variation observed among food treatments within each temperature.

Total wing area decreased in the warm treatment in both the high and low food experiments and increased with heightened food application only in the high food experiment (Figs. 3a, 3b, 4a, and 4b). The effect of temperature on wing area (Z -) was much larger than that of food variation, with no significant interaction and very similar results between sexes (Fig. 5). Epidermal cell size was less sensitive than wing size to the effects of food and temperature treatments (Fig. 5). Warmer temperature reduced cell size in both sexes and

food experiments, whereas food variation did not influence cell size (Figs. 3c, 3d, 4c, 4d). However, cells were larger in the warm group in the highest food levels (Figs. 3c, 3d, 4c, and 4d). This occurred in males in the high food experiment and in females in the low food experiment, both generating significant warm $\times$ food interaction terms (Fig. 5).

A positive association between a treatment and Z_{diff} defined for each mosquito as the Z-score of cell size minus the Z-score of cell number, indicates a larger effect on cell size than on cell number, whereas a negative association indicates a larger effect on cell number. In males in the low food experiment, regression coefficients were close to zero for all treatments (Fig. 5), indicating that temperature effects on wing area (Fig. 4a) were not produced by differential increases in cell size and number. By contrast, in the high food experiment, the warm treatment in males had a positive, but not statistically significant, effect on Z_{diff} (Fig. 5). This result indicates that temperature effects on wing area were brought about through both increased cell size and cell number, with slightly larger increases in the latter. Food effects on wing area were brought about by larger increases in cell number (Fig. 5). However, in the lower food applications, the colder temperature in males was associated with larger rather than more cells; this pattern reversed in the higher food applications, with greater increases in cell number than size at 28°C as compared with 22°C (Fig. 6a). Given the consistent effects of colder temperature on increased wing area across all food levels (Fig. 4b), these results show that food level modified how males attained larger size in the cold.

In females in the high food experiment, temperature had a positive, although not significant, effect on Z_{diff} , and effects of food or interaction were not detectable. This indicates that food and temperature effects on wing area were generated through both increased cell and wing size, although the colder treatment increased cell number slightly more than size. A similar pattern was observed in females in the low food experiment, but with a significant food $\times$ temperature interaction. At lower food levels, females in the cold treatment had an increased cell size relative to cell number, but at higher food levels, they had more, rather than larger cells (Fig. 6b). As with males in the high food experiment, this result shows that at low food levels cold temperature increased wing area (Fig. 3b) through larger increases in cell size, but at high food levels, the cold-induced size increase was the result of increased cell number.

Discussion

Although both temperature and food resources in their aquatic stages are key processes shaping the body size, abundance, and distribution of *Ae. aegypti* adults, surprisingly little is known about the underlying mechanisms through which larval temperature and food availability influence growth and development. Allometric scaling between organ and cell size has proven useful for separating the effects of environmental conditions in ectotherms (Arendt 2007, Shingleton et al. 2009); however, to our knowledge, it has not been used to investigate potential interactions between temperature and nutrient conditions during development. In this study, we demonstrate that independent, interactive, and sexually dimorphic impacts of food and temperature in the larval environment can be observed on adult morphology of a disease vector. While both reduced temperature and more food

increased wing size, only temperature had significant effects on cell size. Moreover, the allometric effect of temperature on wing versus cell sizes changed at extreme levels of food resources in both sexes. Thus, food levels can alter the underlying physiological mechanisms generating temperature-size patterns in mosquitoes. These results suggest that temperature may modify the food-driven dynamics of urban *Ae. aegypti* populations (Southwood et al. 1972, Gilpin and McClelland 1979).

Physiologically, the sizes of insect body parts are a product of their growth rate and the duration of the growing stages (Nijhout 2003, Davidowitz and Nijhout 2004). Lowered temperature and nutrients both reduce growth rate and increase the length of growing stages in *Ae. aegypti*, with egg-laying females often delaying metamorphosis longer than males to maximize growth (Rashed and Mulla 1989, Bedhomme et al. 2003). Accordingly, females reared at 22°C in the 4 and 10 mg/d groups (low food experiment) had the longest development times (Fig. 2b) of all experimental treatments and likely had the lowest nutrient uptakes. We found that in these low food treatments, cell size was comparatively larger than cell number (high Z_{diff}), but as food level rose, lower temperature caused more, rather than bigger, cells (low Z_{diff}) (Fig. 6b). Similarly, cells were larger at 22°C in these low food groups, but at higher food levels cells were similar in size at each temperature or even slightly larger at 28°C (Fig. 3c). This resulted in significant food-temperature interactions on both cell size and Z_{diff} (Fig. 5). Given that cell division in insects is linked to threshold nutrient concentrations in the hemolymph (Nijhout 2003), we interpret that reduced temperature had a larger impact on cell growth at extremely low nutrient levels; however, in the presence of sufficient nutrients, prolonged development time at 22°C increased the time available for cell division. The marked effects of temperature on overall female wing size underscore how food availability modified the underlying mechanisms through which lower temperature increased wing area.

Similar to females in the low food experiment, growth of males in the high food experiment was affected by a significant interaction between food level and temperature on Z_{diff} . Whereas in the lower food groups cell number and cell size were relatively similar ($Z_{diff} \approx 0$), in the higher food groups, increased size at lower temperature (Fig. 4b) was clearly an outcome of increased cell number rather than size (Fig. 6a). In contrast to females in the cooler, lower food regimes, males reared at 28°C at feeding levels of 133.3 and 266.7 mg/d experienced the shortest development times (Fig. 2a). We also note that reduced size in males as compared with females was overwhelmingly the result of reductions in cell number more than cell size (Supp. Figs. S3 and S4 [online only]). These results suggest that shortened growing time in conditions of warm temperatures and high food availability reduced the time available for cell division as compared with cell growth in area. Interestingly, male cell size in the 133.3 and 266.7 mg/d treatments was slightly larger at 28 than 22°C. A number of studies suggest that in holometabolic insects, and in *Ae. aegypti* in particular, factors that affect development rate have disproportionate effects on the ICG in the final larval instar (Gilpin and McClelland 1979, DeMoed et al. 1999, Nishiura et al. 2007, Telang et al. 2007). Moreover, in the tobacco hornworm, *Manduca sexta*, experimental deprivation of nourishment during the ICG has been shown to reduce the number of wing cells in direct proportion to the reduction in body size (Nijhout and Grunert

2010). Based on our results, we speculate that cell division occurs more readily than cell growth in *Ae. aegypti* larvae that have physiologically committed to pupation; therefore, factors that reduce the ICG would have larger effects on cell division.

It is well established that wing size in *Ae. aegypti* increases with lower rearing temperatures and increased food, but to our knowledge no studies have investigated the combined effects of food and temperature on wing size, nor their effects on cell size. In a study of the geometric relationships in *Ae. aegypti* wing veins, the allometric effects of resource availability on wing geometry changed at extremely low and high larval densities/food inputs (Jirakanjanakit et al. 2007). Understanding the effects of extreme resource conditions may be highly relevant given that resource limitation and competition in larval stages have been shown to regulate the rate of adult production and population dynamics in field *Ae. aegypti* (Southwood et al. 1972, Subra and Mouchet 1984, Barrera et al. 2006, Southwood et al. 1972). Moreover, as heterogeneity in the food particles available to *Ae. aegypti* affects larval nutrition (Rashed and Mulla 1989), heterogeneous resource inputs that larvae encounter in nature are likely to bear little resemblance to standardized laboratory diets (Barrera 1996). Thus, in our experimental design, we sought to maximize the similarity of our experiments with the wide range of resource conditions experienced by *Ae. aegypti* in the field, and simultaneously minimize the potentially large stochastic effects of administering small quantities of heterogeneous detrital food. This led us to vary the frequency instead of quantity of food input, as in the high food experiment, and, to maximize adult production, offer the food at random intervals (Gilpin and McClelland 1979). This made it impossible to combine the analysis of two experiments to better isolate the independent effects of food abundance. Nonetheless, the design generated nutritionally stressed mosquitoes, as evidenced in the long development times (Fig. 2), and the wide range of food abundance was essential for detecting interactive effects of heightened feeding rate and temperature. Moreover, the overall lack of food effects on cell size across the array of feeding treatments, in contrast to the more consistent effects of temperature, clearly shows distinct effects of each of these variables on mosquito development (Figs. 3 and 4).

A further limiting factor is our use of a single tropical, sea level mosquito strain from Barranquilla (mean annual temperature 26–29°C). In Colombia, *Ae. aegypti* thrives in urban environments with mean temperatures above 20–21°C, but no studies have examined the degree of adaptation of local populations to altitude-generated variation in temperature, nor the degree of genetic separation of populations from different altitudes. In *Drosophila*, for example, the plasticity of wing size to temperature variation was similar in six species spanning a latitude gradient whose evolutionary divergence dates 50 million years (Powell et al. 2010). Given that *Ae. aegypti* has inhabited South America for <500 yr, with extensive invasion of high altitudes only over the past 30 yr (Groot 1980), we consider that similar results would have been obtained had we used a high altitude Colombian strain.

In this study, we demonstrate for the first time in a mosquito that larval growth and development are subject to the interactions between food and temperature. The underlying morphological differences suggest that temperature may affect the capacity to develop under food-limited conditions. For example, given the metabolic effects of temperature, thermal conditions may influence the capacity of larvae to survive starvation or the efficiency of

food conversion into biomass. This may have consequences for the rate of dengue vector production, as food limitation and competition for resources in larval stages are considered critical processes in determining the dynamics of both immature and adult *Ae. aegypti* (Southwood et al. 1972, Subra and Mouchet 1984, Barrera et al. 2006). Moreover, field surveys consistently demonstrate large variation in larval nutrient levels among urban containers harboring *Ae. aegypti* (Tun-Lin et al. 2000, Strickman and Kittayapong 2003, Padmanabha et al. 2010). Current models that predict increased *Ae. aegypti* production with increased temperature in the 20–30°C range (Focks et al. 1993, Jetten and Focks 1997) are based on the assumption that intraspecific larval resource competition is independent of thermal conditions. We show that in both sexes the morphological effects of temperature variations depend on food levels, suggesting that the temperature-independence assumption may not hold. Ultimately, predicting the effects of temperature on the abundance of this important pathogen vector will require an improved understanding of how water temperature affects the dynamics of food limitation and competition in aquatic urban habitats.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Steve Juliano for insightful comments on the manuscript, Lucia Suarez for support in conducting experiments, and Marta Quiñonez for lending us laboratory space. This work was supported by the Global Environmental Facility/World Bank Integrated National Adaptation Pilot to Climate Change in Colombia, NIH grant R01 AI 044793 to L.P.L., and the Yale University Climate and Energy Institute.

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Fig. 1.

Images used to determine wing size and epidermal cells size in *Ae. aegypti*. (a) Image of wing used to measure wing area. Square indicates region in which hair counts were conducted. (b) Image in third posterior wing cell showing hairs counted to estimate cell size.

Fig. 2.

Mean time to pupation among experimental treatments of food and temperature. Numbers above error bars (95% CIs [confidence intervals]) indicate the number of mosquitoes in each treatment that were included in the wing analysis (n). (a) High food experiment. (b) Low food experiment. Note difference in y-axis scale between (a) and (b).

Fig. 3.

Wing measurements in female *Ae. aegypti* across food and temperature treatments. Gray circles and error bars (95% CIs) are 22°C group, and black triangles and bars 28°C group. (a) Mean wing area low food experiment. (b) Mean wing area high food experiment. (c) Mean epidermal cell area low food experiment. (d) Mean epidermal cell area high food experiment.

Fig. 4. Wing measurements in male *Ae. aegypti* across food and temperature treatments. Gray circles and error bars ($\pm 95\%$ CIs) are the 22°C group, and black triangles and bars are the 28°C group. (a) Mean wing area low food experiment. (b) Mean wing area high food experiment. (c) Mean epidermal cell area low food experiment. (d) Mean epidermal cell area high food experiment.

Fig. 5. Coefficient plot of random effects models of wing-area (Z-score), mean cell size (Z-score), and Z_{diff} (difference) on warm temperature treatment, increased food, and food-warm interaction. Confidence intervals (error bars) that do not include the x -axis indicate significance ($P < 0.05$) of the covariate.

Fig. 6. Significant interactive effects of food and temperature on Z_{diff} (Z-score of cell size minus the Z-score of cell number, labeled as difference in y-axis). (a) Males in high food experiment. (b) Females in low food experiment. Black lines are the linear regression of feeding rate in each temperature. Positive slope for warm treatment and negative slope for cold treatment indicate a food-temperature interaction. Red dots are mosquitoes from warm

treatment; blue dots are from cold. Z_{food} is the z -transformed food application level.
(Online figure in color.)