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## Journal of Insect Physiology

journal homepage: [www.elsevier.com/locate/jinsphys](http://www.elsevier.com/locate/jinsphys)

## Is bigger really bigger? Differential responses to temperature in measures of body size of the mosquito, *Aedes albopictus*

Michael H. Reiskind\*, Ali A. Zarrabi

Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK 74078, United States

## ARTICLE INFO

## Article history:

Received 29 February 2012

Received in revised form 14 April 2012

Accepted 17 April 2012

Available online 25 April 2012

## Keywords:

Temperature-size growth rule

Allometry

Insects

Bergmann's rule

Reaction norm

Plasticity

## ABSTRACT

When confronted with variation in temperature, most ectotherms conform to a growth rule that “hotter is smaller”. This phenomenon can have important implications on population dynamics, interactions with other species, and adaptation to new environments for arthropods. However, the impact of other environmental factors and genetics may affect that general rule. Furthermore, most studies measure a single body part, and do not examine how temperature and other factors alter the allometric relationship between measurements of growth. In this study, we test the hypothesis that temperature and nutrition, while strongly affecting growth, do not change the allometric relationship between body mass and wing length in the mosquito *Aedes albopictus*. We tested this hypothesis by growing larval mosquitoes from two populations at five temperatures with three food levels. Contrary to our hypothesis, we find that temperature has a profound effect on allometry, with higher temperatures resulting in mosquitoes with shorter wings and greater body mass, and that the effects of temperature are dependent upon available food and population origin. We therefore reject our hypothesis and propose several testable mechanisms that will provide greater insight into the relationship between temperature, food, and measures of growth.

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### 1. Introduction

The response of ectotherms, particular invertebrates, to variation in temperature during growth phases has generally been summarized as the temperature size rule, in which “hotter is smaller”, such that ectotherms exposed to warmer temperatures achieve a smaller adult size (Atkinson, 1994; Kingsolver and Huey, 2008). Indeed there is tremendous empirical support for this “rule”, with over 80% of studies supporting this finding, the majority of which are from insects in both terrestrial and aquatic habitats (Atkinson, 1994, 1995). Exceptions to this rule are also often insects from a diverse set of orders, including Diptera, Ornithoptera, Lepidoptera and most especially, Ephemeroptera (Atkinson, 1994, 1995). Adherence to this rule, or the degree of adherence, is plastic within a species, reflecting local adaptation of thermal reaction norms (Gilchrist and Huey, 2004) and the effects of other ecological conditions, such as diet and nutrition (Diamond and Kingsolver, 2010).

Most studies of the effects of temperature on adult size in ectotherms use a single measurement of growth (Atkinson, 1994). A major assumption of many studies comparing final size of adult holometabolous insects is a tight allometric correlation between

the measured body part (e.g., wing length, thorax width, etc.) and other measurements of size (e.g., other body parts, dry weight, total length, etc.) for each individual. The physiological mechanisms for this correlation are not well understood, and may include metabolic rate forcing, changes in the timing of hormone release, sensitivity of target tissues, or some combination of these mechanisms (Nijhout, 2003; Nijhout and Grunert, 2010; Nijhout et al., 2010). However, these physiological mechanisms of enforcing allometry may be sensitive to type of environmental variation, such as temperature or nutrition, which may change the allometric relationships between body parts (Diamond and Kingsolver, 2010; Shingleton et al., 2007). Consequently, any temperature-size rule may depend upon what growth measurement is being used and there may be differences in the temperature-size rule depending upon nutrition (Diamond and Kingsolver, 2010).

Many studies of mosquito growth use a single measurement of body size (often wing length) to compare individual growth or estimate population growth, based upon the assumption that this body part correlates with other aspects of mosquito bodies that determine fitness (Hawley, 1985; Juliano, 1998; Livdahl and Sugihara, 1984; Lounibos et al., 1993). This is based on correlation studies comparing measurements of body size or wing length with weight and weight with fecundity in container inhabiting *Aedes* mosquitoes (Armbruster and Hutchinson, 2002; Blackmore and Lord, 2000; Briegel and Timmermann, 2001; Koenraadt, 2008;

\* Corresponding author.

E-mail address: [michael.h.reiskind@okstate.edu](mailto:michael.h.reiskind@okstate.edu) (M.H. Reiskind).

Leisnham et al., 2009; Nasci, 1990). These studies find a high degree of correlation between weight and wing length across many species, with explained variance ranging from 59% to 92%, and considerably less explained variation between measurement of body size and fecundity ( $r^2$  between 0.12 and 0.83). However, most of these studies generated diversity in sizes by varying food or initial larval density and none examined the effects of temperature on these allometries or on fecundity. As in other ectotherms, increased temperature has generally been associated with shorter development time and smaller adults in a wide array of mosquito species (Briegel and Timmermann, 2001; Brust, 1967; Lyimo et al., 1992; Ragland and Kingsolver, 2008; Rueda et al., 1990; Van den Heuvel, 1963; Westbrook et al., 2010). However, there have been some notable exceptions to the hotter is smaller rule for mosquitoes. Lounibos et al. (2002) found a significant increase in male dry mass in adult *Aedes albopictus* reared at 30 °C relative to those reared at 24 °C and a non-significant increase in female dry mass. Wing length data were not reported. Additionally, in one of the few studies that report both weight and wing length, Nayar (1969) found an increase in dry weight at intermediate temperatures (a “hump-shaped” relationship) while wing length decreased linearly at increasing temperatures in *Aedes taeniorhynchus*. In *Anopheles* there have been a couple of studies examining how temperature affects wing length and weight, both of which hint at a change in allometry across temperatures, although do not directly analyze an allometric variable (Koella and Lyimo, 1996; Lanciani and Le, 1995).

We tested the hypothesis that two measurements of total growth, wing length and adult dry weight of *A. albopictus* vary in the same manner in response to variation in larval temperature and nutrition, such that the allometry is insensitive to changes in environmental conditions. To avoid confounding effects of competition, we reared each mosquito individually. To determine whether these responses were conserved across different genotypes, we examined two populations of *A. albopictus* collected from widely separated areas in North America.

## 2. Methods

### 2.1. Mosquitoes

Mosquitoes used in this experiment were  $F_1$  mosquitoes from Tulsa, OK, collected from eggs in the summer of 2009 and  $F_4$  mosquitoes from an original population of *A. albopictus* eggs collected in the summer of 2008 in Palm Beach County, FL. The original collection of Florida mosquitoes included over 1500 eggs collected from 30 sites in urban Palm Beach County. Florida mosquitoes were maintained through three generations in the following conditions: 100 larvae/l of water infused with 4 g of live oak leaves (*Quercus virginiana*, collected in Vero Beach, FL) and 0.3 g of 1:1 yeast:albumin with a 14:10 L:D photoperiod at 26 °C. Adults were kept in the same conditions, at  $\approx$ 95% humidity. Eggs were generated by providing blood from a human volunteer (MHR, Oklahoma State University, IRB Exemption, August 24, 2008). The Oklahoma population was derived from over 3000 eggs collected throughout the summer in 45 sites spread throughout Tulsa County, OK. These eggs were allowed to hatch and reared to adults on a diet of ground fish food (Tetra-min ©, Tetra Werke, Melle, Germany). Field derived adults were added to the colony over the course of the summer. For both populations, eggs used in this experiment were laid in November of 2009 from adults housed in separate chambers held at 26 °C, 14:10 L:D, provided 20% sugar water *ad lib* and given human blood from a volunteer (MHR, Oklahoma State University, IRB Exemption, August 24, 2008).

### 2.2. Growth conditions

Mosquitoes were hatched in distilled water and placed singly in 50 ml conical tubes to avoid confounding effects of competition. Tubes contained 35 ml of nutrient broth. Full strength nutrient broth was made in a single batch and then diluted to half and quarter strength for three food levels:  $x$ ,  $x/2$ ,  $x/4$  (hereafter high, medium, low). The formula for full-strength nutrient broth was 2 g of live oak leaves and 0.15 g of 1:1 yeast:albumin per liter, aged for 3 days, based upon previous experiments (Alto et al., 2008). We tested five temperatures in five incubators set to: 9, 14, 19, 24, and 29 °C that would capture a range of growth conditions. Twenty-four neonate larvae (replicates) were used for each population (2: TUL and FL), food level (3: high, medium, low) and temperature (5: 9, 14, 19, 24, 29 °C) in a full factorial design, for a total of 720 experimental units ( $2 \times 3 \times 5 \times 24$ ). Temperatures were monitored every 10 min for the duration of the experiment using iButtons© (Maxim Corporation, Dallas, TX), and the actual average temperatures and standard deviations were:  $8.63 \pm 0.41$  °C,  $14.302 \pm 0.43$  °C,  $19.47 \pm 0.44$  °C,  $23.62 \pm 0.23$  °C, and  $27.39 \pm 0.74$  °C. As such, we refer to each temperature treatment in the results by the mean measured temperature, rounded to the nearest first decimal place.

### 2.3. Variables measured

Tubes were monitored daily for adult emergence. Emergence date was noted, and the number of days from hatching to emergence recorded. After emergence, nutrient broth was drained from the tube, and adults were allowed to live for 2 days without access to water or any food. After 2 days, adults were placed in a drying oven at 50 °C for at least 48 h before measurement of dry weight. Each adult was sexed and weighed to the nearest microgram on an ultra-microbalance (Sartorius M P 3 balance, Data Weighing Systems, Inc. Elk Grove, IL). After weighing, one wing (usually the left wing, unless damaged) of each adult was removed and measured using imaging software (iSolution Lite, IMT technologies, Vancouver, BC, Canada) and a dissecting microscope.

### 2.4. Statistical analysis

Differences in mortality between treatments was determined by the binomial test of proportions using an approximation of a z-score. Development time data were not normal, nor could be transformed to meet the assumption of normality. Therefore, the effects of population origin, temperature and food were analyzed separately using a Kruskal–Wallis non-parametric rank test (Sokal and Rohlf, 1995). Measurements of body size (dry weight and wing length) were normally distributed by Kolmogorov–Smirnov’s test ( $p > 0.05$  for both measurements). As we were interested in the effects of temperature, food and population origin on relative growth measures and to quantify whether our treatments affected the allometry between mass and wing length, we generated normalized mass and wing length measurements, and took the difference for each individual within each sex:  $Z_{\text{wing}} = (\text{wing}_i - \text{wing}_{\text{avg}})/\sigma$ ;  $Z_{\text{mass}} = (\text{mass}_i - \text{mass}_{\text{avg}})/\sigma$ ;  $Z_{\text{diff}} = Z_{\text{wing}} - Z_{\text{mass}}$ . Normalization allowed meaningful comparisons of allometry by putting mass and wing length on the same scale and taking the difference between the scores. Alternative approaches, such as the ratio of wing length to mass, generate non-normal data that present analytical difficulties. We then used these z-scores as our outcome variable in a GLM analysis of the relative effects of population, food and temperature, using Type III sum of squares to account for unequal group sizes. In the interest of space, we only present female data in our figures; male data are available as supplementary data from the corresponding author. Creating the variable  $Z_{\text{diff}}$  allows us to directly

analyze the effect of temperature, food and population origin on the allometry between mass and wing length. All analyses were run in SAS 9.2 (SAS Inc. Cary, NC).

### 3. Results

#### 3.1. Mortality

No mosquitoes survived to pupation in the 8.6 °C treatment. At 19.5, 23.6 and 27.4 °C there was high survival, with little variation by population or food regime (survival to adulthood across both populations and all food regimes: 87.5%; data available in [supplementary data](#)). However, at 14.3 °C there was lower survival than at higher temperatures (Table 1; survival to adulthood across both populations and all food regimes: 45.83%). This lower survival was similar across the two populations, and was driven by significantly lower survival in low and medium food regimes (data pooled from both populations; High vs. Medium:  $z = 6.142, p < 0.0001$ ; Medium vs. Low:  $z = 5.282, p < 0.0001$ ; High vs. Low:  $z = 7.841, p < 0.0001$ ; Table 1).

#### 3.2. Development time

Temperature and food had significant effects on days to emergence for both females and males, with faster development at higher temperatures and lower food levels (Kruskal–Wallis test; females: temperature:  $\chi^2 = 170.18, df = 3, p < 0.0001$ ; food:

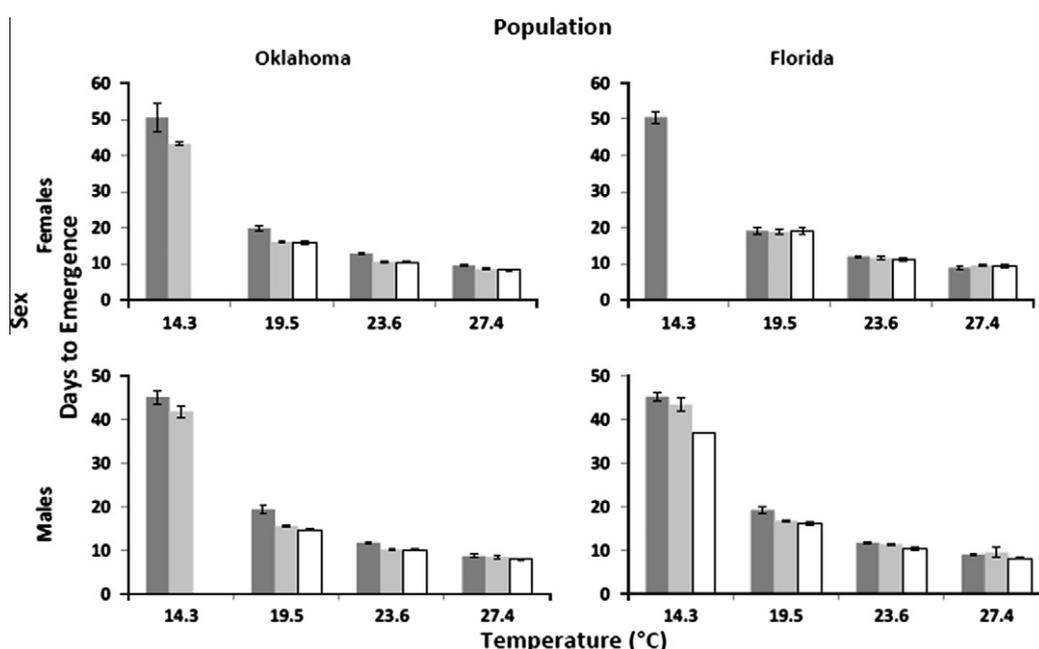
$\chi^2 = 19.04, df = 2, p < 0.0001$ ; males: temperature:  $\chi^2 = 223.55, df = 3, p < 0.0001$ ; food:  $\chi^2 = 28.04, df = 2, p < 0.0001$ ). Population origin did not significantly affect development time for either sex. For both sexes, higher temperatures profoundly decreased development time, while increased food concentration slightly increased development time (Fig. 1). At 14.3 °C no females emerged from the Florida population at medium food.

#### 3.3. Allometry between mass and wing length

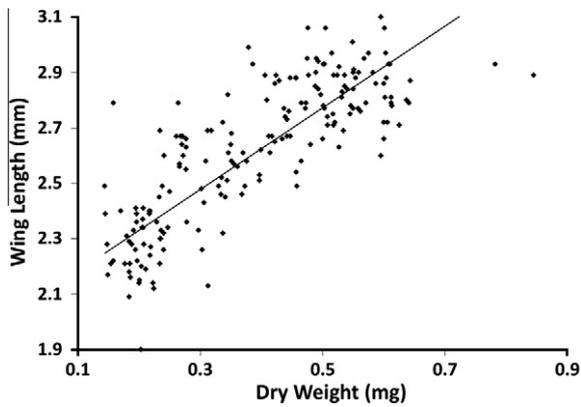
Over all treatments, wing length and weight were highly correlated (Fig. 2, females:  $n = 191, r = 0.8054, p < 0.0001$ ; males not shown:  $n = 249, r = 0.6749, p < 0.0001$ ; correlations by population origin and temperature are available in [supplementary data Table S1](#)). Both normalized wing length ( $Z_{wing}$ ) and mass ( $Z_{mass}$ ) were significantly affected by temperature, food and population origin and some interactions between these effects in males and females (Fig. 3, Tables 2 and 3). The allometry between the normalized mass and wing length measures ( $Z_{diff}$ ) for each individual female was significantly affected by food availability, temperature and population origin (Fig. 3, Table 4). At higher temperatures and increased food availability, females were heavier with shorter wings ( $Z_{diff}$  was more negative) while at lower temperatures and decreased food availability, individuals were lighter with longer wings ( $Z_{diff}$  was more positive). Higher temperatures were associated with lower  $Z_{diff}$  (shorter wings relative to body mass) at all

**Table 1**  
Survival to adulthood in Florida and Oklahoma mosquitoes at 14.3 °C at three food levels.

Food	Florida			Oklahoma		
	Number		% Survival to adult	Number		% Survival to adult
	Male	Female		Male	Female	
High	12	8	83.3	14	7	87.5
Medium	12	0	50	7	6	54.167
Low	1	0	4.167	0	0	0



**Fig. 1.** Average days to emergence for female and male mosquitoes from Oklahoma and Florida. Missing bars means no individuals of that population and sex emerged from that treatment. Error bars are  $\pm 1$  s.e.m.

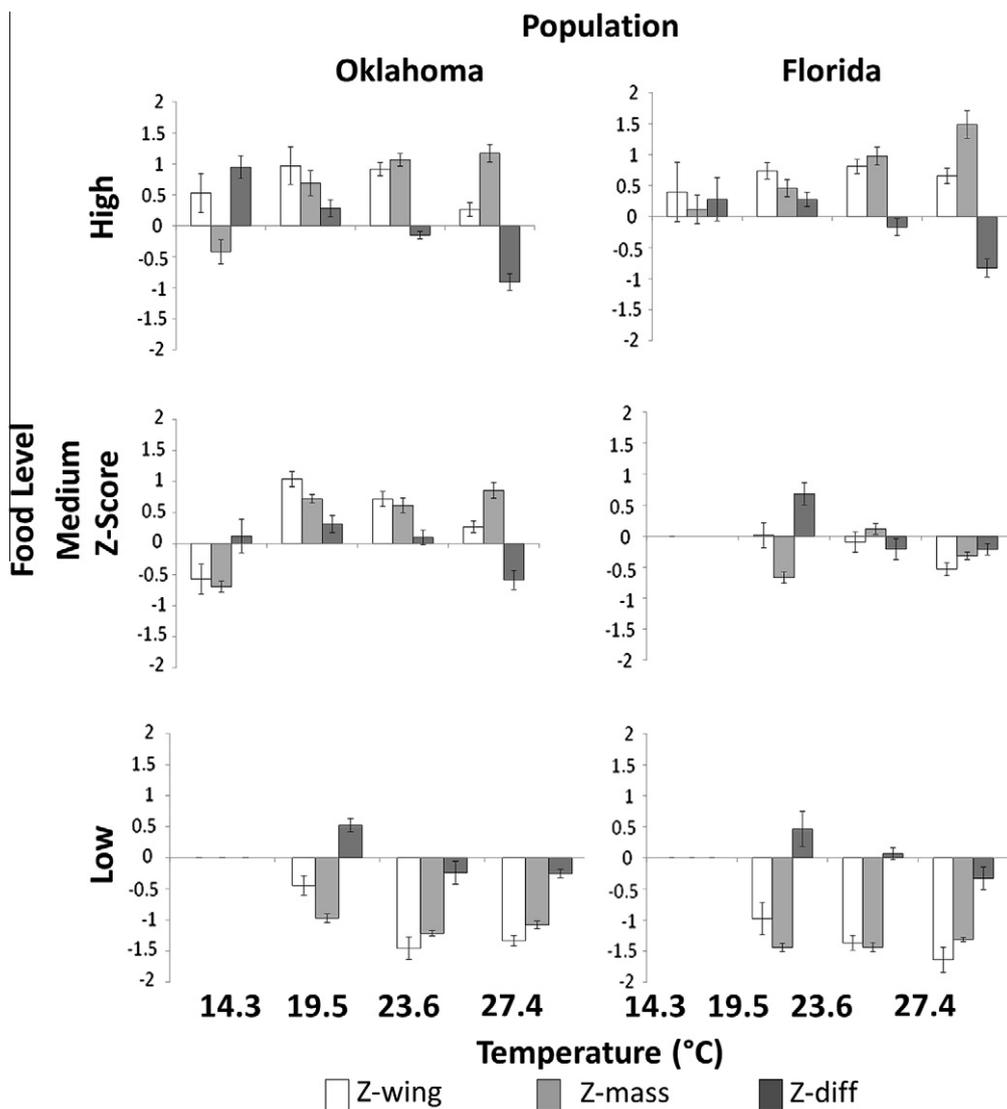


**Table 2**

Generalized linear model of the effects of rearing temperature, food level and population origin on  $Z_{wing}$ , the normalized wing length of mosquitoes by sex.

Sex	Source	$F_{num}$ df	P-value
Females:	Temperature (T)	20.55 <sub>3</sub>	<0.0001
	Food level (F)	193.96 <sub>2</sub>	<0.0001
	Population (P)	16.31 <sub>1</sub>	<0.0001
	T × F	3.49 <sub>5</sub>	0.005
	T × P	0.84 <sub>3</sub>	0.4762
	F × P	10.39 <sub>2</sub>	<0.0001
	T × F × P	1.26 <sub>4</sub>	0.2867
Males:	Temperature (T)	25.78 <sub>3</sub>	<0.0001
	Food level (F)	114.86 <sub>2</sub>	<0.0001
	Population (P)	14.77 <sub>1</sub>	0.0002
	T × F	3.66 <sub>5</sub>	0.0033
	T × P	1.75 <sub>3</sub>	0.1576
	F × P	3.83 <sub>2</sub>	0.0231
	T × F × P	1.20 <sub>5</sub>	0.3078

**Fig. 2.** Correlation between wing length and dry weight in females across all temperature and food regimes with both populations. The least-square means fit line is described by the equation: wing length (mm) = 2.089 + (mass(mg) × 1.3682), with an  $r^2 = 0.6487$ ,  $df = 190$ ,  $p < 0.0001$ .



**Fig. 3.** Average  $Z_{wing}$  (white bars),  $Z_{mass}$  (light grey bars), and  $Z_{diff}$  (dark grey bars) scores (range: 2 to -2) for individual females by food level, population origin and temperature. Error bars are  $\pm 1$  s.e.m. There were no female mosquitoes from either location produced at low food and 14 °C or Florida mosquitoes at medium food, 14 °C.

**Table 3**

Generalized linear model of the effects of rearing temperature, food level and population origin on  $Z_{\text{mass}}$ , the normalized weight of mosquitoes by sex.

Sex	Source	$F_{\text{num df}}$	P-value
Females:	Temperature (T)	69.92 <sub>3</sub>	<0.0001
	Food level (F)	475.05 <sub>2</sub>	<0.0001
	Population (P)	72.43 <sub>1</sub>	<0.0001
	T × F	6.08 <sub>5</sub>	<0.0001
	T × P	4.66 <sub>3</sub>	0.0037
	F × P	28.14 <sub>2</sub>	<0.0001
	T × F × P	3.28 <sub>4</sub>	0.0129
Males:	Temperature (T)	19.50 <sub>3</sub>	<0.0001
	Food Level (F)	352.92 <sub>2</sub>	<0.0001
	Population (P)	16.31 <sub>1</sub>	<0.0001
	T × F	12.96 <sub>5</sub>	<0.0001
	T × P	1.66 <sub>3</sub>	0.1755
	F × P	16.91 <sub>2</sub>	<0.0001
	T × F × P	3.86 <sub>5</sub>	0.0022

**Table 4**

Generalized linear model of the effects of rearing temperature, food level and population origin on  $Z_{\text{diff}}$ , the allometry of wing length and mass for female mosquitoes.

Sex	Source	$F_{\text{num df}}$	P-value
Females:	Temperature (T)	48.81 <sub>3</sub>	<0.0001
	Food level (F)	4.61 <sub>2</sub>	0.0112
	Population (P)	5.49 <sub>1</sub>	0.0203
	T × F	1.52 <sub>5</sub>	0.1865
	T × P	2.41 <sub>2</sub>	0.0691
	F × P	0.45 <sub>3</sub>	0.6408
	T × F × P	1.99 <sub>4</sub>	0.0981
Males:	Temperature (T)	34.76 <sub>3</sub>	<0.0001
	Food level (F)	12.16 <sub>2</sub>	<0.0001
	Population (P)	0.43 <sub>1</sub>	<0.0001
	T × F	3.39 <sub>5</sub>	0.0057
	T × P	1.11 <sub>3</sub>	0.3460
	F × P	1.35 <sub>2</sub>	0.2621
	T × F × P	1.49 <sub>5</sub>	0.1933

food levels. Overall, individuals from Oklahoma had a lower  $Z_{\text{diff}}$  than individuals from the Florida collection.

#### 4. Discussion

We found strong evidence that our two measures of body size, wing length and dry weight do not vary in the same direction with respect to different temperatures and food levels. Food level generally increased both wing length and weight, as has been noted in numerous other studies (Briegel and Timmermann, 2001; Juliano, 2009). However, the allometric relationship between wing length and weight showed significant variation as a function of food level, with smaller wings relative to mass at lower levels of food. However, the effects of food had a two-way interaction with temperature and population for wing length (food × temperature; food × population), and a three-way interaction with temperature and population for weight (food × temperature × population).

The effects of temperature on wing length and mass did not support our hypothesis. Indeed, when mosquitoes were reared at higher temperatures, they were heavier with shorter wings. The temperature-size rule “hotter equals smaller” (Kingsolver and Huey, 2008) is violated in one measurement of body size (weight), but not another, wing length, over most conditions. There appeared to be lower wing length at the coolest temperature we had development (14.3 °C) relative to warmer temperatures for Oklahoma mosquitoes at medium food levels, but across the range of 19.5–27.4 °C, wing length showed the expected “hotter equals smaller” pattern. The coolest temperature treatment did have

lower survival, and thus a smaller sample size of adult measurements. Although the majority of studies of mosquitoes have shown either weight or wing length to decline with increasing temperatures (Atkinson, 1994, 1995; Brust, 1967; Westbrook et al., 2010), a few studies of mosquitoes have found differing results, with higher temperature associated with increased weight, at least over part of the range of temperatures tested (Lounibos et al., 2002; Nayar, 1969). Nayar (1969) also found a decline in wing length with temperature, suggesting a similar change in allometry between weight and wing length as a function of temperature in *A. taeniorhynchus*. In addition, our results for dry weight showed a similar pattern for *A. albopictus* as those reported by Lounibos et al. (2002).

There are a number of possible explanations for this observation. It is well known that mosquito larvae are dependent upon microorganisms in their environment for nutrition (Merritt et al., 1992; Walker et al., 1991, 1988). We varied the concentration of nutrients, but did not strictly control the abundance or dynamics of the microbes. Therefore, increased temperature could increase the speed of bacterial population growth (Precht et al., 1973), which in turn could affect growth of mosquitoes by providing more food. In addition, higher temperatures may allow more active foraging, which increases the intake of microorganisms, as is seen in other ectotherms (Ward and Stanford, 1982). In these circumstances, mosquito larvae may be able to put on more weight, particularly in non-structural energy storage (e.g., glycogen, fat body), while still being constrained in the number or size of cells due to heat induced faster growth (Nijhout, 2003; Shingleton et al., 2007). Nayar (1969) found that dry weight correlated with higher percentage of glycogen and lipids in *A. taeniorhynchus*. Briegel and Timmermann (2001) describe mostly linear relationships between glycogen and lipids and body size for *A. albopictus*, but did find different slopes for the relationship between wing length and the biochemical constituents at different rearing temperatures. They do not report dry weight. We did not assess our mosquitoes for components of energy storage, but if they follow the pattern reported for *A. taeniorhynchus*, higher dry weights are made up of higher amounts of glycogen and lipids.

Upon initial examination, it may seem maladaptive for mosquitoes to respond to higher temperatures by becoming heavier with shorter wings, or respond to cooler rearing temperatures by being lighter, but with long wings. Shorter wings are correlated with lower fecundity (Armbruster and Hutchinson, 2002; Blackmore and Lord, 2000; Koenraadt, 2008; Leisnam et al., 2009) and lower survival in some cases (Hawley, 1985; Maciel-De-Freitas et al., 2007). However, empirical studies have found higher rates of population growth at higher temperatures (Alto and Juliano, 2001; Lounibos et al., 2002), and there are several possible ways in which the change in weight and wing length allometry at a given temperature may be beneficial at that temperature. One possibility is that the plasticity in wing length and weight may lead to lower wing loadings at low temperatures, which may be beneficial at colder operating temperatures due to higher energy demands for a given flight output (Gilchrist and Huey, 2004; Petavy et al., 1997). Similar reasoning was suggested for a change in wing length-weight allometry observed in *Anopheles gambiae*, although not tested (Koella and Lyimo, 1996). Furthermore, shorter wings, although less efficient in terms of glide ratio, may convey more maneuverability, which could be important in dodging host defenses, mating, or avoiding aerial predation (Dudley, 2000). This maneuverability requires more wing beats, which spends energy (Dudley, 2000). However, if mosquitoes reared at high temperatures are heavier at emergence because of increased glycogen storage or fat, they have more resources to spend in flight, and short wings may be an advantage. Therefore, in order to gain the most benefit of shorter wings, larval mosquitoes may be able to put on more weight at

higher temperatures, possibly by adjusting their foraging behavior. There may be a further cost to these increased foraging needs at higher temperature: less resistance to starvation. This exact effect was noted in a recent study of the related *Aedes aegypti* (Padmanabha et al., 2011), in which larvae reared at higher temperatures were less resistant to starvation than those reared at lower temperatures.

Individuals from a population originally collected in Florida were different from individuals from a population originally collected in Oklahoma. As we did not make population replicates or collect along a latitudinal transect, we cannot make conclusions about the various abiotic or biotic factors that may influence response to temperature in these distant locations. However, we can conclude that both populations, although different from one another in our measurements of size, had similar shaped responses in mass and wing length to variation in temperature and food level, suggesting that these thermal reaction norms are likely conserved amongst two widely separated populations of *A. albopictus* in North America and may be a general response. Previous studies of invasive populations of this species in North America have noted geographic patterns in other traits not related to a thermal reaction norm, such as diapause frequency, competitive ability, and correlates of fitness (Leishnam et al., 2009, 2011; Lounibos et al., 2003; O'Donnell and Armbruster, 2009), and rapidly evolving clines in thermal reaction norms have been described for other Diptera, including culicids (Huey et al., 2000). More collections would need to be done to draw strong conclusions about local adaptation to thermal environment in *A. albopictus*.

## 5. Conclusions

We found a break in allometry between wing length and dry mass as function of temperature across different food regimes in two populations of *A. albopictus*. Specifically, mosquitoes were relatively heavier for their wing lengths at higher temperatures compared to lower temperatures, suggesting a plastic reaction norm to temperature, conserved over some environmental (food) and genetic variation (population origin). Our results suggest several proximate and ultimate questions for future research. First, are the heavier mosquitoes produced at higher temperatures due to increased energy storage, or increased structural weight? Second, is increased weight due to changes in the larval environment, e.g., bacterial dynamics, and/or changes in behavior, e.g., increased foraging? Finally, are these combinations of wing length and weight adaptive to a particular thermal environment, with consequences for mosquito fitness under laboratory and field conditions?

## Acknowledgements

The authors wish to thank Talan Klein for helping measure wings and Greg Ragland, Harish Padmanabha, George Opit and Jack Dillwith for reading earlier versions of this manuscript. This work was supported by a grant from the Oklahoma Center for the Advancement of Science and Technology (OCAS HR09-157), by the Oklahoma Agricultural Experiment Station (OAES: Hatch Project 2702), and by the Multistate Hatch Project NE 1034 "Mosquitoes and Public Health" (MSS Project 2712).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jinsphys.2012.04.006>.

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