

## Short Communication

# Assessment of the correlation between wing size and body weight in captive *Culex quinquefasciatus*

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### Abstract

**Introduction:** Mass production of mosquitoes under laboratory conditions allows implementing methods to control vector mosquitoes. Colony development depends on mosquito size and weight. Body size can be estimated from its correlation with wing size, whereas weight is more difficult to determine. Our goal was to test whether wing size can predict the weight. **Methods:** We compared dry weight and wing centroid size of *Culex quinquefasciatus* reared at different temperatures and four diets. **Results:** Weight and wing size were strongly correlated. The diets did not influence wing size. **Conclusions:** Wing centroid size is a good predictor of *Cx. quinquefasciatus* body weight.

**Keywords:** Dry weight. Wing geometric morphometrics. Body size.

*Culex quinquefasciatus* Say is a vector of etiological agents of filariasis and encephalitis such as West Nile virus disease<sup>(1)</sup> and lymphatic filariasis<sup>(2)</sup>. It is widely accepted that the spread of these diseases can be prevented by the use of mass-produced, genetically engineered mosquitoes to control the mosquito vector<sup>(3) (4)</sup>.

The production and maintenance of mosquito colonies requires knowledge of the factors that contribute to and influence mosquito fitness. One of the important factors to ensure satisfactory mass production is adequate feeding of the mosquitoes<sup>(4)</sup>. The fitness and health of colonized mosquitoes can be indirectly evaluated by measuring their body size and weight<sup>(5)</sup>. The possibility that body size and weight may be associated with the number of gametes produced, flight capacity, parity, and reproductive success has been extensively discussed<sup>(5) (6)</sup>.

Although an important piece of data, mosquito weight – with the exception of dry weight – can easily be measured incorrectly<sup>(3)</sup>. Drying a mosquito, however, takes a long time [between 24 and 48h according to Dominic et al.<sup>(7)</sup>] and requires an expensive analytical balance, making the procedure too costly and laborious for regular use.

Unlike body weight, insect body size can easily be estimated from wing size because of the correlation between these two variables in some mosquito species<sup>(8)</sup>. With the aim to identify a morphometric predictor of body weight, we tested the correlation between wing centroid size and dry body weight in *Cx. quinquefasciatus*, maintained on four types of food source at two different temperatures.

*Culex quinquefasciatus* colony was reared from samples collected along the banks of the Pinheiros River, São Paulo, Brazil. We used four plastic trays (24.5 × 14.5 × 6.0cm) for males and four identical trays for females. Each tray contained 500mL of water and 100 first instar larvae, which were separated with the aid of a pipette and a stereoscopic microscope (Motic® SMZ-168, Hong Kong, China). The larvae were reared in two groups at two different temperatures. The *hot* group was kept at 27 ± 2°C and the *cold* group at 20 ± 2°C. Both groups were fed for 10 consecutive days with 20mg of feed per day in each container. The following diets were used: Super Red® fish feed (FF) (Haifeng Feeds Co., Ltd., Nantou, Taiwan), Dog Chow® dog food (DF) (Purina, St. Louis, MI, USA), a 50:50 mix of fish feed and dog food (FDF), and a mix of these feeds with Ninho® powdered milk (FDF-PM) (Nestle, Vevey, Switzerland). Within each group there were eight subgroups (four male subgroups and four female subgroups), and the larvae in each of the four subgroups for a given gender and temperature were fed a different diet. During the pupal stage, the pupae were individually separated, and after the adults had emerged, these

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were killed by freezing (-20°C). The frozen adults were then placed in an oven at 60°C for 48h, and their dry weights were individually measured three times using an analytical balance (MC1-RC210-P Analytical Balance, Sartorius AG, Göttingen, Germany). The mosquitoes were placed individually in 2mL Eppendorf tubes (Hamburg, Germany) for the morphometric analysis and stored for later use. The samples are listed in **Table 1**.

The wings of the male and female mosquitoes were mounted and analyzed according to a procedure described previously<sup>(9)</sup>. The Kolmogorov-Smirnov normality test was used to verify the Gaussian distribution of the data, and Tukey's test was performed to test the statistical significance between the treatment means, both tests were performed using GraphPad InStat version 3.1 (GraphPad Software, San Diego, CA, USA). The correlations between biometric variables (weight and centroid size) were assessed by the Pearson correlation test.

A total of 351 individuals were analyzed, consisting of 94 females and 118 males from the *hot* group and 70 females and 69 males from the *cold* group. The dry weight and wing size of the mosquitoes in both groups combined ranged from 0.19mg to 1.13mg and 1.2mm to 2.7mm, respectively.

We observed a highly significant positive correlation ( $p = 0.001$ ) between weight and wing size for males and females in the *cold* group ( $r = 0.75$  for both) and for males and females in the *hot* group ( $r = 0.68$  and  $0.59$ , respectively), as shown in **Figure 1**.

The results of the correlation analysis between wing size and body weight in each of the four food-source subgroups (supplementary graph 1) were consistent with those obtained when the mosquitoes fed on the different food sources were combined (positive correlation between wing size and body weight,  $p < 0.001$ ). The exception was females from the *hot* group fed on FF, for which there was no statistically significant correlation between wing size and body weight ( $p = 0.109$ ).

No statistically significant differences ( $p \geq 0.05$ ) in wing size were observed between the mosquitoes in each of the four food-source subgroups (either grown at 20°C or 27°C), as shown in **Figure 2**.

Wing size can be used to estimate *Cx. quinquefasciatus* body weight. Wing morphometrics therefore eliminates the need to

weigh mosquitoes individually, saving time and money and potentially even making precision weighing scales unnecessary.

Unlike our findings, previous studies have shown that the correlation between body weight and wing length in culicids was non-linear<sup>(3) (10)</sup>. Jirakanjanakit et al.<sup>(11)</sup> showed that wing length (unidimensional variable) and wing centroid size (multidimensional variable) correlate in *Aedes aegypti*. Despite this correlation, we believe that wing centroid size is more likely to linearly correlate with body weight owing to the multidimensional nature of these latter variables. It is coherent with our observation in *Cx. quinquefasciatus*.

As mentioned previously, size and body weight are important biological variables for mass production of insects because they are probably linked to fitness. Although this causal relationship is not well understood, its importance is undeniable, as large female mosquitoes, for example, have high fecundities compared to smaller mosquitoes<sup>(12)</sup>. Furthermore, larger and consequently heavier mosquitoes can fly higher and distribute more eggs<sup>(6)</sup>. They also have greater longevity and are more likely to spread the etiologic agents of parasitic infections<sup>(13)</sup>. However, the relationship between mosquito size, body weight, and fitness remains the subject of controversy. A study on *Aedes* and *Psorophora* failed to find an association between body size and increased reproductive capacity<sup>(14)</sup>, whereas a correlation between wing length and adult dry weight was quadratic in some Culicidae<sup>(10)</sup>.

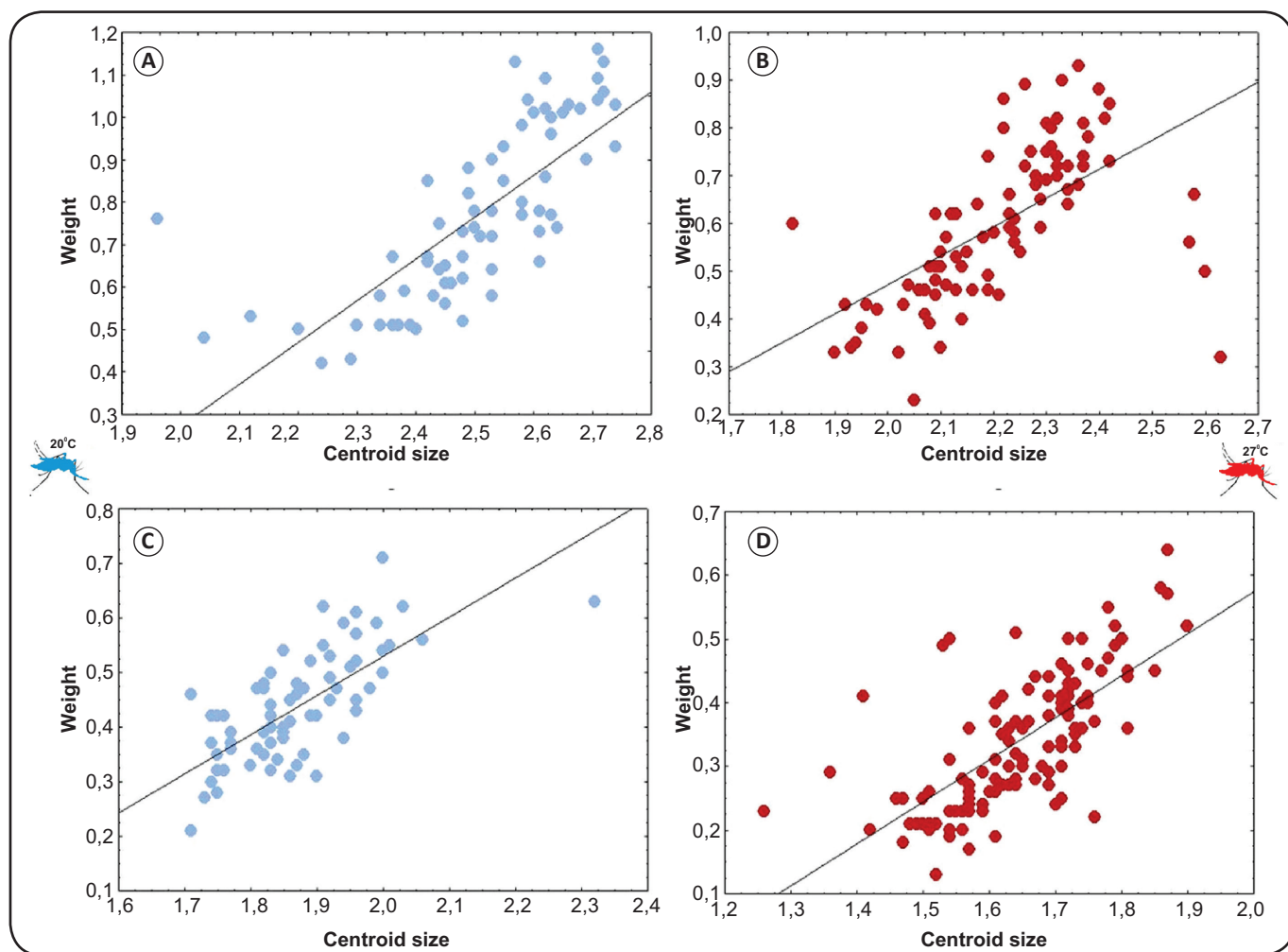
The positive correlation in the present study between wing size and body weight for two distinct temperatures and four nutritionally distinct diets indicates that these biological traits are closely related and that this correlation is reproducible. Although colonization methods vary substantially between laboratories and *mosquito factories*, our findings suggest that body weight can be predicted from wing-size data independently of the conditions under which a colony is reared.

The different food sources (FF, DF, FDF, and FDF+PM) used in this study did not influence wing size (or body weight), although the diets were not nutritionally equivalent. This is in contrast to the results reported by Damiens et al.<sup>(15)</sup>. We believe that the nutritional variations in this study did not have a significant influence on global size because the feeds used supplied the basic needs of the mosquitoes. It is thus

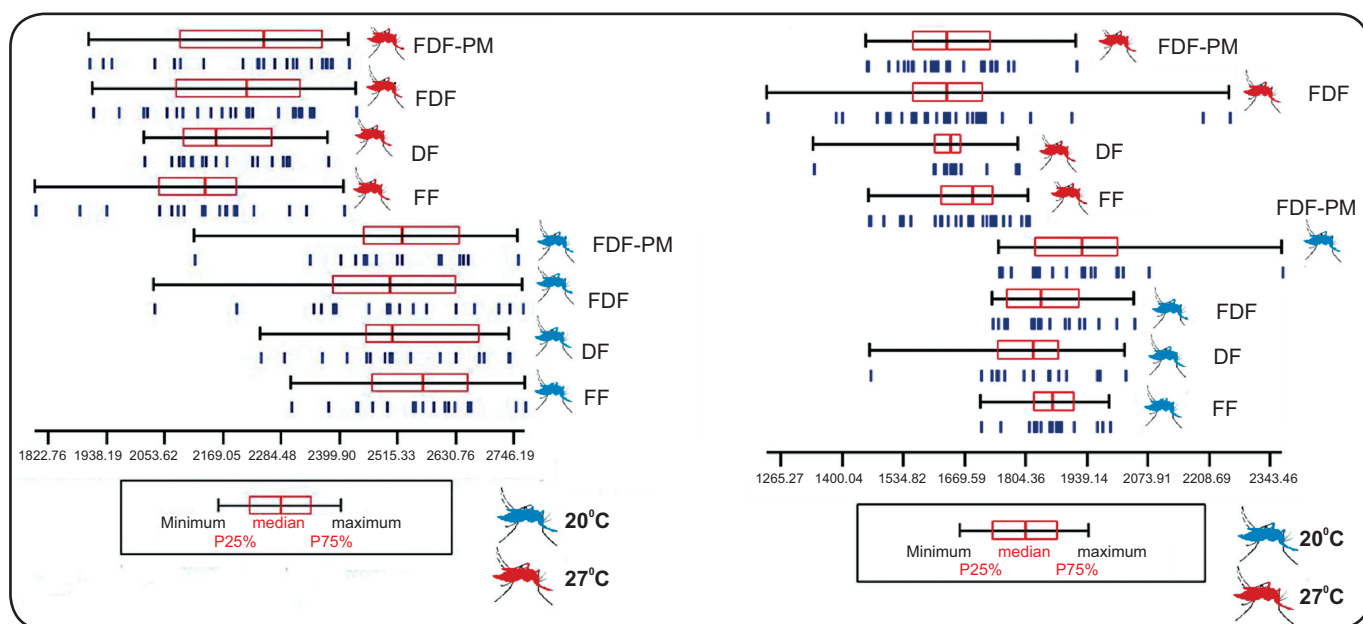
**TABLE 1**  
Number of individuals in the *hot*\* and *cold*\* groups according to the type of food tested.

Type of food	Female hot group	Female cold group	Male hot group	Male cold group
FF	19	20	32	15
DF	25	17	18	16
FDF	27	17	40	19
FDF-PM	23	16	28	19
<b>Total</b>	<b>94</b>	<b>70</b>	<b>118</b>	<b>69</b>

FF: fish feed; DF: dog food; FDF: a mixture of FF and DF; FDF-PM: a mixture of FF and DF with powdered milk. \*Hot and cold groups were reared at 27 °C and 20 °C, respectively.



**FIGURE 1.** Correlation between dry weight and wing length in *Culex quinquefasciatus* males maintained at (A) 20°C and (B) 27°C. Correlation between dry weight and wing length in *Culex quinquefasciatus* females maintained at (C) 20°C and (D) 27°C. Each dot corresponds to an individual.  $P \leq 0.001$ .



**FIGURE 2.** Centroid size of *Culex quinquefasciatus* females and males bred and kept at 27°C and 20°C. Four types of diet were used: 1. fish feed (FF); 2. dog food (DF); 3. a mixture of FF and DF (FDF); and 4. a mixture of FF and DF plus powdered milk (FDF-PM).

reasonable to suppose that the choice of one particular diet from the four tested is not a limiting factor in the control of *Cx. quinquefasciatus* body size.

#### Conflict of Interests

The authors declare that they have no conflict of interests.

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