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Nota Científica
(Short Communication)

EFFICIENCY OF THE SEPARATION OF *AEDES* (*STEGOMYIA*) *ALBOPICTUS* (DIPTERA: CULICIDAE) MALE AND FEMALE PUPAE USING A SIEVING DEVICE

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RESUMEN. Después de medir el tamaño del cuerpo, abdomen y cefalotórax de la pupa macho y hembra de *Aedes albopictus*, decidimos usar el tamaño del cefalotórax de la pupa macho para construir un dispositivo para la separación de individuos por sexo en el estado pupal. De manera interesante, muchas pupas macho no pasaron a través del tamiz y fueron retenidos y sacrificados como pupas hembra; esto puede significar un desperdicio de recursos que no ha sido informado previamente. Por tal motivo se hace énfasis en que la eficiencia de retención de pupas macho debe ser confirmada si se planea su cría masiva.

Many experiments with mosquitoes (Diptera, Culicidae) require unmated individuals (Blackmore and Lord 2000, Koenraadt 2008), which are obtained by keeping them separated by sex in non-reproductive stages. Separation of unmated individuals by sex in the adult stage may be difficult because mating can pass unnoticed, forcing the researcher to be aware of the moment they molt into adults to be rapidly removed (Brazil & Brazil 2000). For small experiments this can be a manageable task, by placing larvae or pupae in separate vials (Dame *et al*. 1974). But if larger numbers are required, as in mark-release-capture experiments or in autocidal control of mosquito populations, the previous approach is merely inappropriate.

Size differences of both male and female mosquito pupae were pointed out by McCray (1961), Gerberg *et al*. (1969) and Sharma *et al*. (1972) who took advantage of this finding to build a sieving-based apparatus for rapid sexing of *Aedes* (*S.*) *aegypti* pupae. However, variables like larval density, temperature, food and others can affect pupae cephalothorax size, making it obligatory to follow strict culture conditions so the device can effectively separate males from females (Papathanos *et al*. 2009). Actually, the importance of rapid, automatic sexing was evident in a recent study: Bellini *et al*. (2007) used a sieving device to separate 20,000 male pupae of *Ae. albopictus* a week with a separation efficiency of 97-99%. However, these studies based the designs of their devices on trial and error strategies, instead of using morphological parameters of pupae, which could be a more appropriate approach. Here, we measured three morphological characteristics of female and male *Ae. albopictus* pupae and show how these characteristics proved to be useful to build a device for rapid sexing of pupae, ideal for research with few requirements of biological material or small laboratories with few resources.

Mosquitoes were collected as larvae in the city of Tapachula, Mexico (N14°53'11.85", W92°17'13.09") and reared following Medici *et al*. (2011) in laboratories of El Colegio de la Frontera Sur in November 2012; once in the pupal stage, 90 individuals were individually separated in vials and then transferred one at a time to a microscope slide. Cephalothorax, abdomen and body length were measured with a 10 mm scale in a stereoscopic microscope (Fig. 1, A). Next, these pupae were taken back to their respective vials to allow their development into adults to determine sex. Forty pupae corresponded to female and 50 to male pupae. Student's *t*-tests were applied to data to find differences between male and female pupae characteristics. Statistics were done in R for Mac OS X V2.15. Subsequently with this information we constructed a device with a 1.6 × 1.1 mm mesh size based on the male cephalothorax size.

According to our results, the separation of male and female pupae is a straightforward task, since the measurements of the three morphological characteristics is significantly different between sexes (Figure 1, B). The device designed here, we separated 61 individuals into 30 supposedly male pupae (SMP) and 31 supposedly female pupae (SFP), which were reared to adult to confirm sex. Thirteen percent of SMP were actually female mosquitoes; meanwhile 32% of SFP were confirmed as males.

With pupae morphometrics we were able to build a sieving device with good separation efficiency, but far from the one achieved by Bellini *et al*. (2007). In our study, despite the average larger size of female pupae, the smaller ones could pass trough the sieve. These small fe-

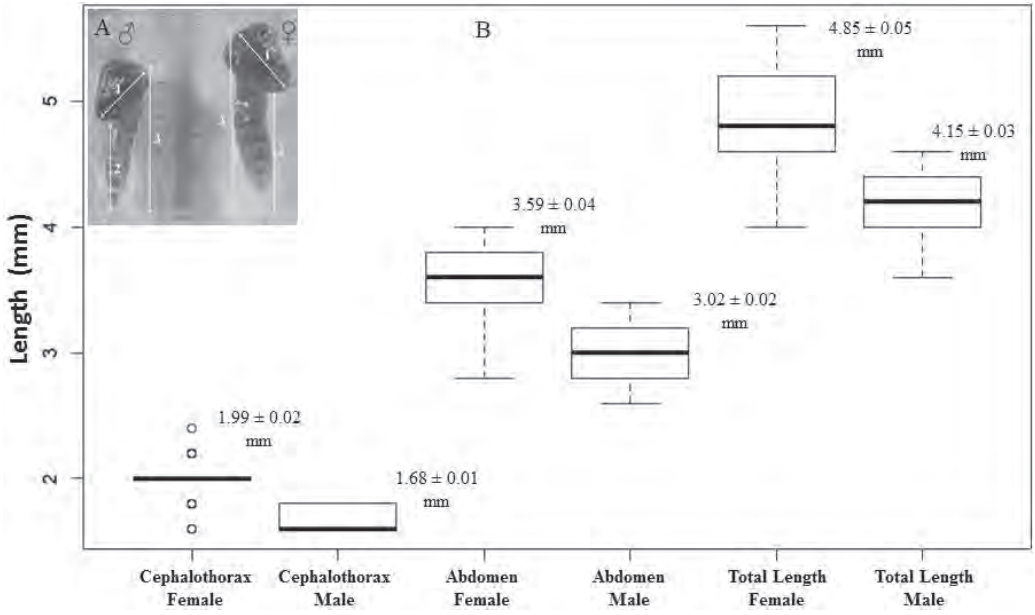

Figure 1. (A) Lateral view of the characteristics of *Ae. albopictus* male and female pupa that were measured: (1) cephalothorax, (2) abdomen, (3) body. (B) All three characteristics, cephalothorax (t = -9.99, df = 58.44, P < 0.001), abdomen (t = -11.22, df = 70.25, P < 0.001) and body (t = -10.37, df = 66.92, P < 0.001), were significantly different between sexes. Numbers in boxplots are mean ± S.E.

males are part of the variation in natural populations of mosquitoes, from which we obtained our biological material. Thus by selecting large females with a lower standard error in their morphometrics this problem could be minimized, a very important aspect not mentioned by Bellini *et al*. (2007). More important yet is the finding that many males did not pass the sieve, which can be considered a waste of resources. The utility of screening of large numbers of genetically modified or sterile males (Sterile Insect Technique, SIT) is important in biological control by augmentation programs of local populations of mosquitoes (Alphey *et al.* 2010). However, when considering measures of male standards, the aspect of male mating competitiveness in the field is neglected, where size is a crucial factor in terms of sexual selection in mosquitoes (Sawadogo *et al.* 2013).

This makes it evident that is not only important to observe the number of females in the SMP, but also the retention of males in the SFP that will be sacrificed for not being separated, which seems to be a larger number than expected. In addition, it should be noted that the selection of a standard size males, likely to result in deficiencies of control programs due to low competitiveness with large males in field.

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