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LARVAL REARING TEMPERATURE AFFECTS MORPHOLOGY OF *ANOPHELES ALBIMANUS* (DIPTERA: CULICIDAE) MALE GENITALIA

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ABSTRACT. The structure of the male genitalia of *Anopheles albimanus* Wied. is affected by larval rearing temperature. Morphometric analyses of 50 specimens revealed significant variation of 5 genitalic characters. The gonocoxa, dorsal claspette lobes, and aedeagus were longer for males reared at 22°C. The parabasal seta was longer and the aedeagus was wider for males reared at 30°C. Extra parabasal setae and lateral projections of the aedeagus were seen on some specimens.

INTRODUCTION

Male genitalic characters are important in the taxonomy of *Anopheles* (*Nyssorhynchus*) Blanchard (Hill 1930, Gabaldón 1940, Galvão 1943, Levi-Castillo 1949, Cova Garcia 1961, Faran 1980, Faran and Linthicum 1981, Linthicum 1988). Many morphological features of mosquitoes used as taxonomic characters are affected by environmental conditions (Clements 1992). Gabaldón et al. (1941) described abnormalities of the accessory setae on 2 male *Anopheles albimanus* Wied. These 2 specimens each had 3 accessory setae instead of the usual 2. Although they found extra parabasal setae on 2 *Anopheles nuneztovari* Gabaldón (one as *Anopheles goeldii* Rozeboom and Gabaldón), no abnormalities of this sort were found on *An. albimanus*. The purpose of this study was to determine whether the morphology of male genitalia is affected by larval rearing temperature of *An. albimanus*.

MATERIALS AND METHODS

Female *An. albimanus* were obtained from a colony maintained at the Florida Medical Entomology Laboratory. This colony was started from mosquitoes taken from a colony maintained at the USDA Insects Affecting Man and Animals Laboratory (now the Medical and Veterinary Entomology Research Laboratory), Gainesville, FL. The origin of that colony was mosquitoes collected in El Salvador (Bailey et al. 1980). All females were held individually, provided with a blood meal, and permitted to oviposit. Eggs were hatched under standard conditions. After first instars had hatched, progeny of each female were divided in half and reared in enamel pans at one of 2 temperatures. Progeny of 10 female *An. albimanus*, designated 1 to 10, were reared at 22°C and 30°C, thus 20 pans of larvae were used. Larvae were counted individually such that half of the progeny of a particular female were reared at each temperature. The maximum number of larvae in any pan was 51. Larvae from each isofemale progeny brood were divided as evenly as possible between the 2 temperatures; broods with an odd number of larvae obviously could not be divided exactly equally. This was done to avoid effects of differing larval density on adult morphology (Kitthawee et al. 1992). This rearing regimen was used because rearing isofemale progeny broods minimizes the confounding of genetic and environmental influences (Thorpe 1976). Larvae were fed daily a constant ration of 15 mg per pan of a 50:50 mixture of liver powder and brewer's yeast. Pupae were removed daily and kept separate by mother and temperature until adult emergence. Adults were killed with chloroform and point mounted. Five male progeny of each of 5 females were selected from each rearing temperature, making a total of 50 genitalia examined. Genitalia were prepared in the same manner as those in an earlier study (Hribar 1994). Specimens were examined and illustrations made by using a phase-contrast microscope fitted with a drawing tube. Measurements were made from illustrations by using a Summagraphics® digitizing tablet and SigmaScan® software. Ten characters were digitized for each specimen: lengths in mm of the gonocoxa (GC), gonostylus (GS), gonostylar claw (GSC), aedeagus (AEL), basal apodeme (BAD), parabasal seta (PBS), the larger of the 2 accessory setae (ASL), ventral lobes (VLL), dorsal lobes (DLL), and width of aedeagus (AEW). Occasionally, fewer than 10 characters were measured for a given specimen, due to damage to one or more structures. Prior to statistical analysis, measurements were transformed by $X' = \ln(X + 1)$ (Zar 1984). Differences between rearing temperatures were analyzed by using 2-tailed t-tests conducted with the SAS TTEST procedure (SAS Institute 1985). Results are reported as backtransformed means ± SEM.

Table 1. Differences of genitalic morphology between male *Anopheles albimanus* reared at 22°C and 30°C.

Character[1]	Rearing temperature (°C)	*n*	Mean ± SEM[2]	*t* value	df	*P*
GC	22	25	0.354 ± 0.003	2.622	48	0.0117
	30	25	0.341 ± 0.003			
PBS	22	25	0.070 ± 0.001	−3.381	48	0.0014
	30	25	0.078 ± 0.002			
DLL	22	25	0.102 ± 0.001	3.574	48	0.0008
	30	25	0.095 ± 0.001			
AEL	22	25	0.200 ± 0.002	3.976	48	0.0002
	30	25	0.183 ± 0.003			
AEW	22	25	0.034 ± 0.001	−4.205	48	0.0001
	30	25	0.043 ± 0.001			

[1] GC, gonocoxa length; PBS, parabasal seta length; DLL, dorsal lobe length; AEL, aedeagus length; AEW, aedeagus width.
[2] Measurements in mm.

RESULTS

Five characters differed between males reared at 22°C and 30°C: GC, PBS, DLL, AEL, and AEW. Three characters, GC, DLL, and AEL, were larger for males reared at 22°C, whereas 2 characters, PBS and AEW, were larger for males reared at 30°C (Table 1). The remaining 5 characters (GS, GSC, ASL, BAD, and VLL) did not differ between rearing temperatures.

Some morphological differences between males reared at different temperatures were apparent without measurement. Extra parabasal setae (i.e., 2 setae where one normally is found) were present on 5 males reared at 30°C, but on only one male reared at 22°C. Small lateral projections of the aedeagus were seen on 14 males reared at 30°C, but again, on only one male reared at 22°C.

DISCUSSION

The differing variation of genitalic characters at different temperatures raises some interesting questions about the taxonomy of *Anopheles* mosquitoes. In the subgenus *Nyssorhynchus,* the relative lengths of the aedeagus and ventral lobes, and of the gonocoxa and ventral lobes, are important for identifying species. Obviously, if the lengths of the aedeagus and gonocoxa are subject to temperature-induced variation, but the length of the ventral lobes is not, then these ratios will change according to larval rearing temperature. Relative width of the tip of the aedeagus also is used in available keys to *Nyssorhynchus,* and the fact that this character may vary with larval rearing temperature introduces some uncertainty. Fortunately, there are other, better characters that are used in species descriptions and in keys, but the point must be made that some genitalic characters are subject to change based on larval rearing temperature.

The genitalia of male *An. albimanus* are distinct enough from those of other *Nyssorhynchus* species that confusion or misidentification by individuals experienced with this subgenus is unlikely. The absence of setae on the fused ventral claspette lobes, the rounded apices of these lobes, and the shape of sternite IX serve to distinguish this species from any others (Faran 1980). However, the greater question remains of whether morphometric characters are suitable for identification keys if those characters are in fact subject to environmentally induced variation. This study illustrates just one of the many aspects of anopheline biology that are poorly understood, in spite of the massive amount of work that has been conducted on these mosquitoes during this century.

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