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SCIENTIFIC NOTE

MORPHOLOGICAL SEXUAL DIMORPHISM IN THREE SPECIES OF ANOPHELINE MOSQUITO LARVAE

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ABSTRACT. Sexual separation at the larval stage in anopheline mosquitoes with the naked eye is difficult. We have identified distinguishing spots visible to the naked eye on the 6th abdominal segment of 3rd and 4th instars of *Anopheles stephensi, An. culicifacies*, and *An. subpictus*. Based on this feature, male and female larvae can be differentiated morphologically at 3rd and 4th instars of these species. This is the first report on these characteristic spots that may have a wide application for larval sexing in mosquito taxonomy, physiology, toxicology, genetics, and control.

KEY WORDS *Anopheles stephensi*, *Anopheles culicifacies*, *Anopheles subpictus*, sex identification, mosquito larva

Malaria, a major problem in India and other developing countries, is transmitted by primary vectors *Anopheles stephensi* Liston in urban areas and *Anopheles culicifacies* Giles in rural areas and by secondary vector *Anopheles subpictus* Grassi in both areas. There is a lack of simple convenient and efficient method for identification and separation of male and female mosquitoes at larval stage in the 3 anophelines. Sex separation in anophelines based on morphological characters is laborious at the adult stage and very difficult at the larval stage.

Puri (1960) studied various morphological characters for species identification of Indian anophelines in the larval stage; however sexual dimorphism has not been attempted. Some methods have been described by various workers to sexually segregate male and female mosquitoes at the larval stage, with varying degrees of success at the larval and pupal stages, using morphological characters, size differentiation, mechanical methods, and genetic sexing systems (Jones 1957, Fay and Morlan 1959, Krishnamurthy et al. 1962, Dame et al. 1974, Focks 1980, Malcolm and Mali 1986, Alphey 2002, Catteruccia et al. 2005, CDC 2007, Emamia et al. 2007).

In the present study, attempts have been made to identify the characters for sexual differentiation in male and female mosquitoes at the larval stage with the naked eye and its success rate for sexual dimorphism in *An. stephensi, An. culicifacies*, and *An. subpictus*.

For the study ten 3rd and 4th instars of *An. stephensi, An. culicifacies*, and *An. subpictus* with and without 2 spots visible on the 6th abdominal segment were kept in 200 ml of dechlorinated water in separate Petri dishes for development at 27 ± 1°C and 70% ± 5% relative humidity. For both the instars of each species 5 replicates were kept under identical conditions. Larvae were provided with yeast tablets as food ad libitum. After pupation, pupae were transferred to 100-ml beakers placed in mosquito cages for adult emergence. The emerged adults were anesthetized and identified to sex.

Table 1 reveals the number of 3rd and 4th instars of the 3 species, with and without 2 spots visible on the 6th abdominal segment, which subsequently developed into adult males and females, respectively. It is apparent from the data that larvae with abdominal spots developed into adult males and those without the spots into adult female. The spots visible on the 6th abdominal segment in the 3rd and 4th instars were not apparent in the 1st and 2nd instars of the 3 mosquitoes.

The diagnostic spots visible through the transparent cuticle possibly correspond to the developing male gonads on both sides of the 6th abdominal segment in the 3rd and 4th instars of *An. stephensi, An. culicifacies*, and *An. subpictus*. These spots are visible to the naked eye. For convenience, the term "abdominal spots" is given for this diagnostic character. The presence of this diagnostic character has also been confirmed in field-collected larvae of these species. Under the microscope, both the structures appear dark brown in color and spindle shaped with a narrow terminal tail-like structure protruding into the anterior half of the 7th abdominal segment (Fig. 1). The mean length and width of spots in the 3 species vary from 0.128 to 0.156 mm and 0.043 to 0.057 mm in the 3rd instar and 0.170 to 0.213 mm and 0.106 to 0.099 mm in the 4th instar, respectively. The tail length of the spots

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Table 1. Sex differentiation of 3rd and 4th instars of *Anopheles stephensi, Anopheles culicifacies,* and *Anopheles subpictus* with and without 2 spots on 6th abdominal segment

Species of mosquito	Instar	Larvae with spots on 6th abdominal segment		Larvae without spots on 6th abdominal segment	
		No. of larvae	No. of male mosquito emerged/dead larvae	No. of larvae	No. of female mosquito emerged/dead larvae
An. stephensi	3rd	50	45/5	50	46/4
	4th	50	50/0	50	49/1
An. culicifacies	3rd	50	46/4	50	44/6
	4th	50	48/2	50	47/3
An. subpictus	3rd	50	45/5	50	48/2
	4th	50	48/2	50	48/2

ranges from 0.03 to 0.057 mm and 0.071 to 0.114 mm in the 3rd and 4th instars, respectively.

Percentage development to adult stage of 3rd and 4th instars with and without spots indicated a 100% success rate at using this character to differentiate male and female larvae. With this identification mark, larvae of the 3 species can be separated at 3rd or 4th instars with the naked eye. Sex-specific studies can be conducted at the larval or adult stage, but the latter is comparatively more cumbersome. In this way, adult males or females could be selectively developed after segregation at the larval stage. Further in population dynamics studies in field conditions, this character can be used for possible prediction of adult male and female ratio after estimation of larval density in natural habitats.

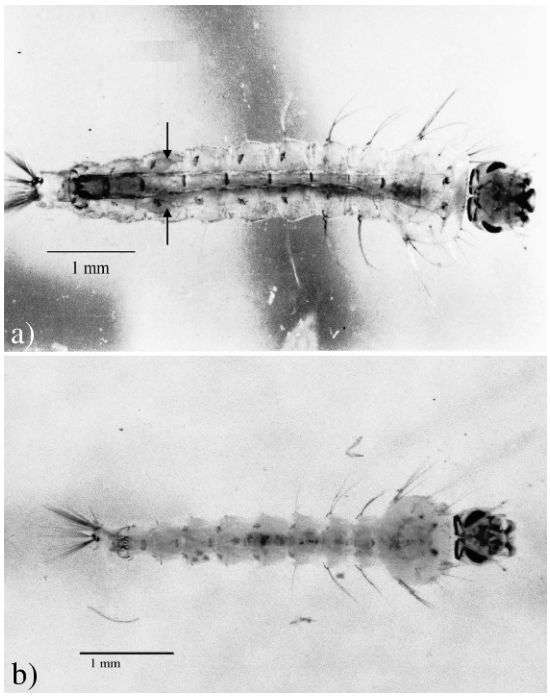

Fig. 1. Photomicrograph of *Anopheles stephensi* 4th instar, (a) male larva with abdominal spots indicated with arrows, (b) female larva without abdominal spots.

Some mechanical methods have been developed to separate anophelines sexually at the pupal stage (Focks 1980). However, the major problem encountered was incomplete separation of males from females on the basis of size, since in anophelines pupae, the sexes do not differ so markedly as in culicine species (Curtis et al. 1976). Moreover, inconsistent culture conditions may also influence the size of the developing stage. Further, in field conditions, size-based methods have limitations on their feasibility with regard to inconsistent nutrients and other ecological conditions in natural habitats. A genetic sexing system has been developed by some workers for separation of male and female anophelines based on artificial linking with translocation of insecticide resistance or other conditional lethal genes to the chromosome in some pests and vector species (Baker et al. 1978, La Chance 1979, McDonald and Asman 1982, Lines and Curtis 1985). Malcolm and Mali (1986) described a method for sexing in *An. stephensi* based on Y-linked translocation induced by X-ray, which leads to the formation of visible mutant black larvae (Bl). Catteruccia et al. (2005) reported the expression of green fluorescent protein (EGFP) in larvae of *An. stephensi* on the basis of transgenesis for sex separation. These methods of the genetic sexing are complicated, need highly specialized techniques, and are difficult to operate in the field. The Centers for Disease Control and Prevention (2007) has reported a method for sexing of *An. gambiae* larvae in late 4th instar on the basis of a preantennal lobe. However, the method is difficult for sexing in *An. stephensi* because of difficulty in visualizing the imaginal disc. Emamia et al. (2007) also described the morphological method for sexing the larvae of *An. stephensi* and *An. culicifacies* with the difference in the length and width of a tube-like organ in the 9th abdominal segment using light microscopy. However, this character cannot be used in lab or field for sex separation of larva with the naked eye.

The authors consider the use of the diagnostic spots visible on the 6th abdominal segment to be a simple, reliable, safe, and accurate method for sexing males and females of the 3 species. This may be the first report on sexual differentiation of male and female larvae of *An. stephensi, An. culicifacies*, and *An. subpictus*, different from other markers reported by Malcolm and Mali (1986) and Emamia et al. (2007). The use of this simple, convenient, and accurate method for larval sexing of the 3 anophelines with the naked eye may be useful in studies on dealing with mosquito taxonomy, toxicology, genetics, physiology, and control.

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