

set up. A young baboon (*Papio ursinus* (Kerr)) or vervet monkey (*Cercopithecus aethiops* (Cuvier)) is anaesthetized by intramuscular injection with "Sernylan" (phencyclidine hydrochloride) and roped to the bait platform which consists of a piece of 50 mm wire mesh. This platform can either be supported by poles of electric conduit tubing 1 m above ground level or is suspended on a rope at a height of 10 m or more in the tree understorey. Two rubber-bladed suction fans housed in galvanized iron cylinders, painted black outside, are suspended from the bait platform as shown so that the openings of the cylinders through their 5 mm wire mesh filters are about 70 mm away. Eight-watt "autofan" motors operated by a 12 volt car battery are used in the suction units. Insects are sucked downwards into 2 organically collecting cages as they come near the bait to feed.

In the eastern Transvaal this collecting method was employed during the 2 hr after sunset, the bait animals usually remaining asleep over this period, although occasionally a 2nd injection of anaesthetic became necessary. Large numbers of female and male mosquitoes

of the *Ae. furcifer/taylori* group were collected at 2 levels, particularly by traps in the understorey. Identification of the males from their genitalia showed that they were mainly *Ae. furcifer*. Unbaited control traps set 1 m from the ground gave almost negative catches. Fair numbers of Phlebotomines and *Culicoides* species were also sampled in the traps baited with vervet monkeys.

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#### References

- McIntosh, B. M., P. G. Jupp and J. De Sousa. 1972. Mosquitoes feeding at two horizontal levels in gallery forest in Natal, South Africa with reference to possible vectors of chikungunya virus. *J. Entomol. Soc. Sth. Afr.* 35: 81-90.
- McIntosh, B. M., P. G. Jupp and I. Dos Santos. 1977. Rural epidemic of Chikungunya in South Africa with involvement of *Aedes (Diceromyia) furcifer* (Edwards) and Baboons. *S. Afr. J. Sci.* 73: 267-269.

### A PRACTICAL METHOD FOR SEXING PUPAE OF *ANOPHELES* *ATROPARVUS*

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The described method here for sexing pupae was found very useful during cage population studies with *Anopheles atroparvus*, van Thiel 1927. For this study each week a number of unmated females and males of the same age was required. Data collected in the course of this experiment are presented in order to give some quantitative information concerning efficiency and usefulness of this method.

Larvae of *An. atroparvus* were reared in trays. Once pupae developed they were collected in a small beaker by means of a pipette or a small

strainer of the size of a teaspoon. The sex of each individual pupa was subsequently determined by examining the shape of the hypopygium (Moorfield 1951), using a binocular dissecting microscope with a magnification of 50 X.

To facilitate examination each pupa is sucked into a narrow glass tube, fixed to an adapted sucking tube. Glass tubes with an inner diameter varying between 1.5 and 2 mm were used. The size of the inner diameter is so chosen that the pupa is fixed in a position with a stretched abdomen. The glass tube is turned

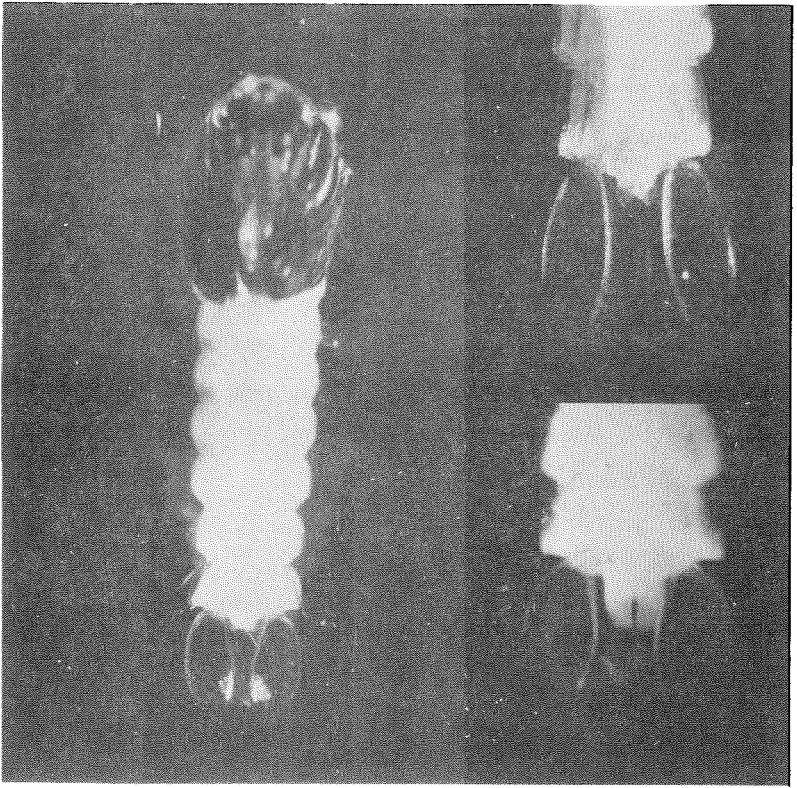


Fig. 1. Pupa of *A. atroparvus* stretched in a capillary tube.

Fig. 2. Enlarged male hypopygium.

Fig. 3. Enlarged female hypopygium.

under the microscope, till the hypopygium is clearly seen. The movements of the pupa are very limited and as the differences between the female sex and the male hypopygium are so clear, the sex can instantly be distinguished (figs. 1-3). Also the spermatheca is easily recognized in the female pupa, giving another point for differentiation. After the sex has been determined, the capillary tube is emptied into separate beakers for males and females.

It is evident that the inner diameter of the glass tube is of utmost importance. The difference in size between individual pupae can be considerable. Nevertheless one single tube is usually sufficient for any sample of pupae of the same species and originating from the same batch of larvae, reared under the same conditions. For practical purposes it may be advisable to have 2 or 3 different sizes of glass

tubes ready for use. We found capilette® capillaries with an inner diameter of approximately 1.5 mm very useful.

**RESULTS.** The time required for sexing various samples of pupae by one person can be noted from the table. The careful reader will notice that on a few occasions the sexes in the selected sample came close to a 1:1 ratio. This is by no means coincidental. Males are on average smaller than females, the extremes of this range are readily visible. Thus it is possible to approach the desired result by picking the pupae for sexing selectively and reducing time.

As shown in the table, 902 pupae were sexed in 209 minutes, which is almost 260 pupae/hr. When the sexing is carried out by 2 persons a considerable increase in numbers sexed per hr can be reached. For our study 2262 pupae were sexed by this method, consisting of 1161

Table 1. Time required for sexing female and male pupae of *An. atroparvus*.

Sample number	Sample size	Time (in minutes) required to sex sample		
		Females	Males	
1	56	35	21	10
2	28	23	5	9
3	50	25	25	11½
4	112	46	72	25
5	50	25	25	13
6	50	25	25	14
7	111	47	64	27
8	53	26	27	11
9	49	28	21	12½
10	66	40	26	13
11	46	8	38	9
12	60	25	35	15
13	67	37	30	18
14	63	38	25	12
15	35	22	13	9
Total	902	450	452	209

females and 1101 males of which 71 (6.1%) and 128 (11.6%) respectively failed to emerge. Not too much significance should be ascribed to the difference in failure to emerge between female and male pupae as differences between 2 samples of the same sex were often much more pronounced.

Sharma et al. (1972) reported 3.6% mortality among pupae of *Culex fatigans* sexed by means of a grid system. It is unlikely that the higher mortality in the reported series is caused by the sexing procedure only, as many factors during the rearing of larvae have a great influence on pupal mortality. In any case the average loss of almost 9% was acceptable for our cage population study. The greatest merit of this method is, that it provides a simple and practical means to separate the sexes in the pupal stage with

complete certainty; pupae that fail to emerge can readily be replaced.

The distinction between male and female pupae, based on the hypopygium has been described by Moorfield (1951) for 3 *Aedes* species, 3 *Culex* species, *An. punctipennis* and *Psorophora ferox*, thus it may be possible that this method has a general application in mosquito species when reliable sexing is required, provided that glass tubes of adapted size are available.

#### References

- Moorfield, H. H. 1951. Sexual dimorphism in mosquito pupae. *Mosquito News* 11 (3) 175-177.  
 Sharma, V. P. et al. 1972. A device for the rapid separation of male and female mosquito pupae. *Bull. Wld. Hlth. Org.* 47 429-432.

## ERRATUM

In the note "Observations on the time of attraction of some Pakistan mosquitoes to light traps," by Suleman et al., Vol. 37(3):531-533, page 531, col. 2, paragraph 2, line 6 reads "1977) and swarming (Reisen 1976, Reisen et al. 1977) and (Aslamkhan 1976) rhythms at Sattoki." This should read "1977) and swarming (Reisen and Aslamkhan 1976, Reisen et al. 1977) rhythms at Sattoki."