

## A FIELD TECHNIQUE FOR SQUASH PREPARATION OF ANOPHELINE CHROMOSOMES<sup>1</sup>

S. L. SHRESTHA

Nepal Malaria Eradication Organization,  
Kathmandu, Nepal

Stimulated by the cytogenetic studies of the *Maculipennis* Complex and the *Gambiae* Complex, chromosomal investigations of these mosquitoes have become commonplace; both salivary and ovarian polytene chromosomes have been used. Most of the reports have come from well-equipped laboratories, or from field operations which are extensions of these laboratories (French et al. 1962, White, et al. 1975, Kanda 1979). Very few studies have utilized field preparations, especially from developing countries. In our studies in Nepal for example, slide quality was inferior for one or more of the following reasons: electricity is rarely available in the field; temporary slides, even when sealed with wax or nail polish, tend to dry out under our conditions; dry ice and liquid nitrogen are not available. Faced with these difficulties we have evolved a system, mostly by trial and error, which permits field preparation of slides, about 50% of which are readable and useful upon return to the laboratory.

1. Half gravid females were collected during early morning hours.

2. After the tip of the abdomen was severed at the 9th segment, the ovaries were gently teased from the abdomen and placed in a drop of prefixative (5% modified Carnoy's, see below) on a nonsiliconized slide.

3. The body of the female was pinned and labelled, with appropriate collection and identification data.

4. Ovaries which were of Christophers'

stage III were transferred to a drop of modified Carnoy's solution (equal volumes of glacial acetic acid and 95% ethyl alcohol). Under tropical conditions this fixative is good for one week.

5. Add one drop of stock stain (2% lacto-aceto-orcein). Mix gently.

6. Touch a clean glass slide to the preparation and invert, so that the coverslip faces up. Stain for 2 minutes.

7. Spread chromosomes by tapping gently. The amount of pressure and tapping will vary with the specimen and the species.

8. Slides may be made permanent in the field by a modification of the old *Drosophila* method. (Steps 9-11).

9. Store slides for 24 hours in a coplin jar or staining dish with a small amount of 95% ethyl alcohol in the bottom. Seal well, with vaseline or similar material so that the slide is surrounded by saturated alcohol vapor.

10. After 24 hours, fill the coplin jar with absolute alcohol. Some coverslips will drop off the slides; others may be flicked off with a razor blade.

11. Add a drop of euparal or other mounting medium and cover with a nonsiliconized coverslip.

Slides prepared in this manner are permanent. In our experience about 50% are readable and about 20% are good for detailed study.

### References Cited

- French, W. L., R. H. Baker and J. B. Kitzmiller. 1962. Preparation of mosquito chromosomes. *Mosq. News* 22:377-383.
- Kanda, T. 1979. Improved techniques for the preparation of polytene chromosome for some anopheline mosquitoes. *Mosq. News* 39:568-574.
- White, G. B., A. R. Zahar, and M. Coluzzi. 1975. Review of cytogenetic studies on anopheline vectors of malaria. Unpublished document WHO/MAL/75.849.

<sup>1</sup> It is a pleasure to acknowledge support from UNDP/WORLD BANK/WHO.