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A SIMPLE TECHNIQUE FOR THE PREPARATION OF POLYTENE CHROMOSOMES FROM *CULEX QUINQUEFASCIATUS*

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ABSTRACT. Preparation of polytene chromosomes in *Culex* species is very difficult and the available techniques are not always reproducible. Presuming these problems might be due to lack of an appropriate technique to deal with these long and fragile chromosomes invested with ectopic pairings and sticky ends, certain promising methods with several modifications have been tried. During the process, recently, a simple and reproducible method was developed for obtaining good polytene chromosomes from *Culex quinquefasciatus*. The detailed methodology with precautions and its advantages over other reported techniques are described.

Cytogenetic and molecular biology studies have been initiated in our laboratory on *Culex quinquefasciatus* Say, the principal vector for Bancroftian filariasis. Certain studies in this program, namely localization of heat-shock and heavy metal inducible loci, replication and transcriptional activity at those sites, and *in situ* hybridization of a few DNA probes on the polytene chromosomes have required well spread and readable polytene chromosomes.

A literature survey showed several reports on the preparation and cytological mapping of the polytene chromosomes in *Cx. quinquefasciatus* (Berger 1937, Kitzmiller 1956, Dennhoefer 1968, Sharma et al. 1969, Kanda 1970, Patnaik et al. 1989). An unequivocal agreement with regard to difficulties encountered in the preparation of these chromosomes has, however, been recorded by almost everybody who has worked with culicine species. On the other hand, *Anopheles* species yield good polytene chromosomes by a standard method (French et al. 1962). These differences have clearly been reflected in the dearth of cytological research in culicine species compared to anopheline species. Sharma et al. (1969) did not mention the difficulty in the preparation of these chromosomes and discussed the chromosome mapping in detail, but without providing chromosomal photographs. Kanda (1970) found it was very rare to see free ends of the chromosomes, and Tewfik and Barr (1974) felt that good preparations in this system, with 3 completely separated chromosomes, were difficult to obtain. Chaudhry (1981) described that these chromosomes were long, fragile, and spread with difficulty, and that it was impossible to make out the exact location of centromeres. Verma et al. (1987) and Patnaik et al. (1989) also reinforced technical difficulties in the preparation of polytene chromosomes in *Culex*.

Of all the publications I encountered in this area, the one by Rai (1967), who did not blame this system but noted the lack of an appropriate technique to deal with these chromosomes, impressed me the most. Consequently, all the available methods in mosquito and other dipteran systems were carried out to resolve the problem, but these culicine chromosomes were intransigent. Certain promising methods were tried several times, with many modifications. Recently, a simple and reproducible technique for obtaining well-spread and readable polytene chromosomes from the malpighian tubules of *Cx. quinquefasciatus* was developed.

Adult female *Cx. quinquefasciatus* mosquitoes collected from the environs of Calcutta, India, provided material for this investigation. The malpighian tubules were dissected out from bloodfed adults in a 7.3 pH phosphate buffer solution (Berendes et al. 1965). The tubules were transferred into a fresh drop of buffer solution and fixed in aceto-alcohol (1:3) for 50–70 sec based upon the thickness of the tubules. The fixative was drained out from the grooved slide and a drop of 2% lacto-aceto-orcein (2% orcein in equal volumes of acetic acid and lactic acid), diluted thrice with double distilled water, was added. The duration of the staining can range from 20 to 30 min. For chromosomal autoradiography 5 min of staining was found to be suitable. The stain was removed by adding a few drops of 45% acetic acid and the material transferred to a fresh drop of 50% acetic acid on a grease-free clean slide and covered with a clean coverglass and tapped carefully under a dissecting microscope. This is a very crucial step because one has to very carefully and patiently tap the nuclei in such a way that the spreading of the chromosomes can be monitored. Excess acetic acid was removed with a tissue paper and the preparations were observed under a low power light microscope (10× or 40× objective) to estimate the exact pressure required for final squashing. The squashed preparations were sealed with nail polish and observed under a phase-contrast photomicroscope.

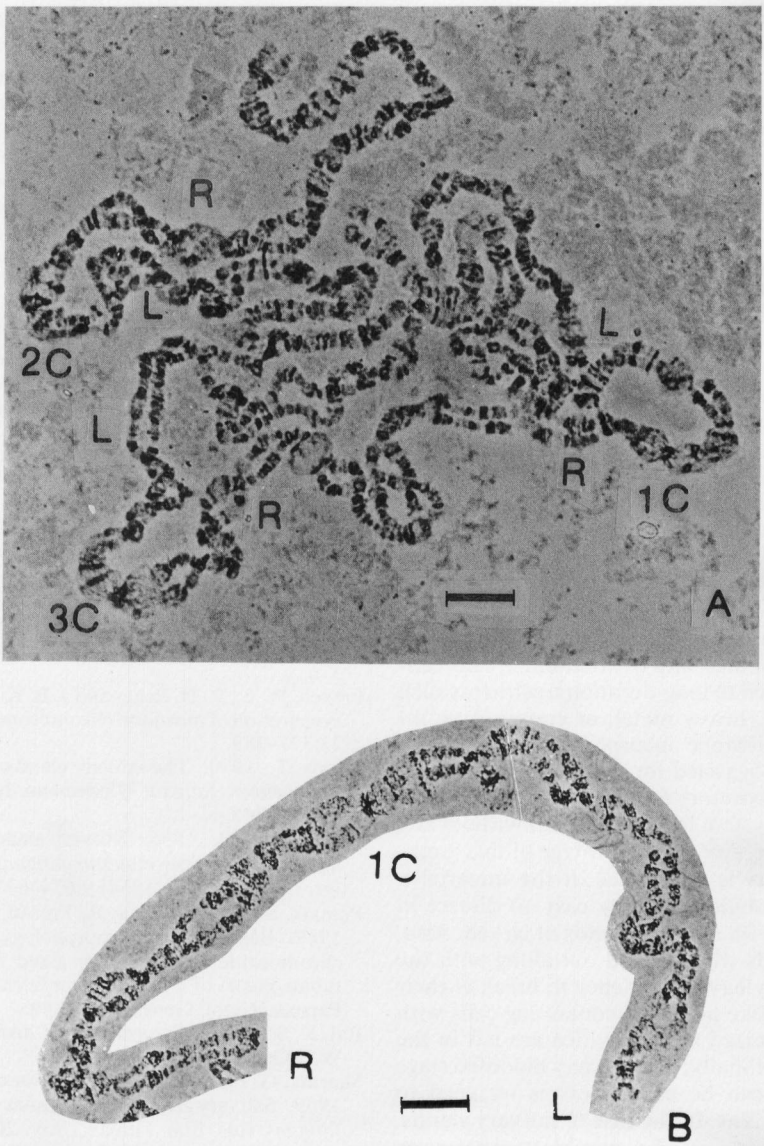

Fig. 1. Polytene chromosome preparations from the malpighian tubules of *Culex quinquefasciatus*. A. Complete chromosomal complement. B. Isolated first chromosome with free ends. (1C, 2C, and 3C are centromeric regions of respective chromosomes; R and L are right and left arms of chromosomes.) The bar represents 10 μm.

The following precautions should be heeded: 1. Filtered stain is required. 2. Part of the digestive tract attached to the tubules should be removed before squashing. 3. Only 2–3 tubules per slide should be used. 4. Coverglass breadth should be the same as that of the slide. 5. While tapping with a needle on the coverglass, the fingers of the left hand (index finger and thumb) should keep the coverglass in the same position. Any displacement will roll the soft chromosomes into dark masses. 6. Careful observation of threadlike chromosomes and patient tapping is required for well-spread and isolated chromosome preparations. 7. The temporary preparations should be sealed with nail polish immediately to prevent air bubbles from entering beneath the coverglass. 8. The final squash should be very gentle, otherwise the chromosomes will be over-stretched.

The polytene chromosome preparations made from *Cx. quinquefasciatus* using the above methodology are presented in Fig. 1, which shows a chromosomal complement consisting of 3 pairs

of synapsed chromosomes (A) and a free first chromosome (B) invested with a number of asynaptic regions. These routine preparations, with demarcated bands, interbands, puffs, centromeres, and free ends, show a high degree of superiority in quality over those published to date. This is solely attributed to the crucial steps involved in the methodology, namely, short acetoalcohol fixation and longer acetic acid treatment followed by tapping of the malpighian tubules. While postfixation in acetic acid dissociated the fragile attachment between the chromosomal ends, careful tapping under a binocular microscope facilitated good spreading. The technique was also good for ^{3}H-thymidine and uridine autoradiography after heat-shock treatment and *in situ* hybridization studies.

Here a note on certain technical advantages of this method over others is worth mentioning. Regarding the dissection or incubation media, Kanda (1970) used 0.1% sodium oxalate and Tewfik and Barr (1974) used 0.1% sodium citrate. Because both are not physiological buffered solutions, live materials such as salivary glands, malpighian tubules, and ovarian nurse cells cannot be subjected to long-duration treatments such as heat-shock, heavy metal, or certain drug induction and isotope incorporation. The phosphate buffer suggested for this method has been used in our laboratory for various treatment purposes in this system for the past year without any inconvenience. Another advantage of this method over others is the choice of the material—malpighian tubules are very easy to dissect in comparison with salivary glands of larvae. Also, salivary glands when pulled out along with the head generally have a tendency to break at their lower ⅓ bulblike portions (containing cells with highly polytenized nuclei), which are left in the thorax. Additionally, because any bloodfed stage of the adult can be used, there is no need to culture the strains. In the case of salivary glands, culturing the larvae at a suitable temperature, relative humidity, and age is a must. With this technique, culicine species may hopefully be exploited at par with *Anopheles* or any other established group for various cytogenetic and molecular investigations.

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