

A SIMPLE TECHNIQUE FOR PREPARATION OF SOMATIC CHROMOSOMES OF APHIDS

A simple squash technique for preparation of somatic chromosomes of aphids is described. The embryos are dissected in 0.67 N. NaCl and are treated with 0.9% Tri Sodium Citrate Solution and fixed in 1 : 3 Acetic acid & methanol. Squash is made in 50% Acetic acid and the slides are stained with 2% Giemsa.

The techniques for preparation of aphid chromosomes were tried since 1905 (Stevens 1905, 1909; Morgan 1909). However the detailed account of aphid chromosomes was given by Shinji (1931) for the first time, using Ringer's solution and Carnoy's (Acetoalcohol and Chloroform) mixture as fixative. The stains used in his studies were Hematoxylin, Orange G, and Acid fuchsin. All these workers based their studies on sectioned material. The squash method was first tried by Smith (1947) using Osmic acid as fixative and Acetocarmine as stain. Dionne and Spicer (1957) advocated the use of Acetoalcohol and chloroform as fixative and staining with Gomori's Hematoxylin after hydrolysis in Hydrochloric acid. Harper and Mac Donald (1965), Sun and Robinson (1966) used Feulgen stain simultaneously. Blackman (1976) introduced Giemsa as a stain, however, his earlier studies (1971-1974) were based on rapid Feulgen squash techniques. More recently Kurl and Narang (1978) have described an air dry technique for cytological preparations which seems to be similar to our method in general except in minor details.

The present technique described here has been in vogue in the Cytotaxonomy laboratory of the Zoological Survey of India, which is simpler in every respect. Slides prepared by this technique have shown good results with satisfactory chromosome spreads revealing their detail morphology.

The embryos are taken out in 0.67 N. NaCl. After two or three minutes these embryos are transferred to hypotonic solution of 0.9% Tri Sodium Citrate for 60-120 minutes, which varies from species to species. The hypotonic treatment enables the cells to swell, thus spreading the chromosomes. Embryos in the early developmental stages have shown better results. A mixture of 1 : 3 acetic acid and methanol is used as a fixative in which the embryos are transferred after the hypotonic treatment. Material is treated with this fixative for a minimum period of one hour and satisfactory results were obtained even up to three months when stored in a domestic refrigerator at 4°C. Subsequently the embryos are transferred in 50% acetic acid for 3-5 minutes depending upon the hardness of the fixed material. The material is then squashed in the usual manner. The slides are put in a vertical slide box which has at its base, a blotting paper dipped in 50% acetic acid and stored in a refrigerator at 4°C for over night. In the next step, the slides are brought to the room temperature and immersed in the fixative (1 : 3 acetic acid & methanol) for an hour. The cover slips are

pried off in the fixative and both slides and cover slips are air dried at room temperature. Staining is done in freshly prepared 2% Giemsa* for 15-20 minutes (pH is maintained between 6.8-6.9) and dipped in distilled water. After staining, the slides are dried in dust proof chamber at the room temper-

DIONNE, L. A. and SPICER, P. B. 1957, *Can. J. Zool.*, **35** : 711.

KURL, S. P. and NARANG, R. C., 1978, *Curr. Sci.*, **47** (21) : 837-838.

MAC DONALD, M. D. and HARPER, A. M. 1965, *Can. J. Genet. Cytol.*, **7** : 18.

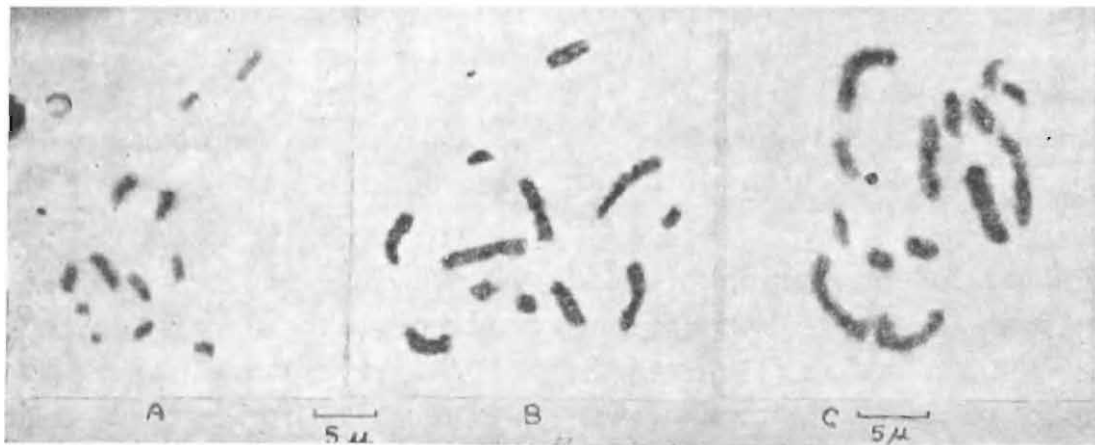


Fig. 1. A & B—*Myzus persicae* Sulz. ($2n=12$)
C—*Megoura lespedezae* (Essig & Kuwana) ($2n=14$)

ature and mounted in the D. P. X. after a dip in Xylene. Slides and coverslips bearing the material are mounted separately. The stained material remains in good condition for more than 40 days.

REFERENCES

BLACKMAN, R. L. 1971, *Experientia*, **27** : 704.

BLACKMAN, R. L. 1974, *Ibid*, **30** : 1136.

BLACKMAN, R. L. 1976, *Chromosoma*, **56** : 393.

MORGAN, T. H. 1909, *J. exp. Zool.*, **7** : 229-350.

ROBINSON, A. G. AND CHEN, Y. H. 1969, *Entomologist*, 101-110.

SUN, R. Y. AND ROBINSON, A. G. 1966, *Can. J. Zool.*, **44** (4) : 649.

SHINJI, O. 1931, *J. morph.*, **51** : 373-421.

SMITH, L. 1947. *Stain technol.*, **22** : 17.

STEVENS, N. M. 1905, *J. exp. Zool.*, **2** : 313-333.

STEVENS, N. M. 1909, *J. exp. Zool.*, **6** : 32.

Zoological Survey of India,
Calcutta

P. P. KULKARNI
R. K. KACKER

*Stock solution : Add 25 ml of Glycerol, 25ml of methanol with 800 mg. of Giemsa powder and keep at 60°C for over night, filter and store in refrigerator.

Working solution : 1.5 ml. of methanol, 1.5 ml. of 0.2 m Disodium hydrogen phosphate, 2.5 ml. of stock solution of Giemsa.

Dissolve the above in 50 cc of distilled water.