Cytogenetic Observations on *Anopheles farauti* Laveran*

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Members of the *punctulatus* species complex of the genus *Anopheles* (subgenus *Cellia*) occur in the Moluccas, New Guinea, the Solomon Islands, the New Hebrides, and the northern part of Australia. At present, 4 species (*An. punctulatus*, *An. koliensis*. *An. farauti*, and *An. clowi*) are recognized within this complex (Belkin, 1962). *An. punctulatus*, *An. koliensis*, and *An. farauti* are most readily identified by the markings on the labium of adult females. The labium of *An. farauti* is dark-scaled except for a very narrow apical light ring.

Two colonies in which the females have a *farauti*-like labium have now been established in the insectaries at the Ross Institute. One of these colonies originated from eggs sent from Rabaul, New Britain, New Guinea, in 1965. The second colony was established from eggs of females caught near Innisfail, northern Queensland, Australia, in February and December 1969.

Cross-matings between these two colonies, using the induced-mating technique of Baker et al. (1962), have shown that the colonies are not conspecific; both the males and females of the F_1 generations are sterile (Bryan, 1970). A preliminary comparative study of the chromosomes of these two species and their hybrids has been made.

Materials and methods

Salivary-gland chromosomes were prepared from fourth-instar larvae using, with minor modifications, the lacto-acetic-orcein squash method described by Niccoletti (1960). Mitotic chromosomes from the pupal gonads were prepared following the method of French et al. (1962). No pretreatment was used.

A preliminary photographic map of the New Guinea species is shown in Fig. 1. Reference will be made to this map, arbitrarily taken as standard, when differences between the two species are described.

Results

The mitotic karyotype. In both species, the mitotic karyotype consists of one pair of rod-shaped heterosomes and two pairs of V-shaped autosomes (see Fig. 1). The acrocentric-like heterosomes (chromosome 1) are very similar to those described in the species A and B of the *An. gambiae* complex (Coluzzi & Sabatini, 1967), both in their shape and in the distribution of heterochromatin. The two autosomal pairs are clearly differentiated, one being nearly metacentric, the other submetacentric. Following the nomenclature first introduced in the *An. maculipennis* complex (Kitzmiller et al., 1967), we have designated the metacentric pair chromosome 2 and the submetracentric pair chromosome 3.

The salivary-gland chromosomes. The salivary-gland chromosomes are similar in both species and consist of five banded chromosomal arms (Fig. 1). One of these arms is much shorter than the others with the centromeric end characteristically swollen, presumably in contact with the heterochromatic zone. This arm is unpaired in the male complement and represents the euchromatic part of the X chromosome. The remaining four chromosomal arms may all remain associated at their centromeric ends but often divide into two groups as represented in Fig. 1.

No clear homologies have been discovered between the banding pattern of the *An. farauti* autosomes and that described in other species of *Anopheles*. This is not unexpected since *An. farauti* belongs to a primitive section of the subgenus *Cellia (Neomyzomyia)*, well differentiated from those species of the subgenus *Cellia* and the genus *Anopheles* so far studied from a cytogenetic point of view. However, parts of some of the arms resemble those of certain species of the genus *Anopheles* (particularly 2L, 2R, and 3R) and we have tentatively followed the same nomenclature (Kitzmiller et al., 1967). Accordingly, chromosome X contains zones 1–5; the 2R, zones 6–14; the 2L, zones 15–21; the 3R, zones 22–32; and the 3L, zones 33–39 (Fig. 1). The limits of the zones are arbitrary but have been chosen so that they occur at

* This work received financial support from the World Health Organization.

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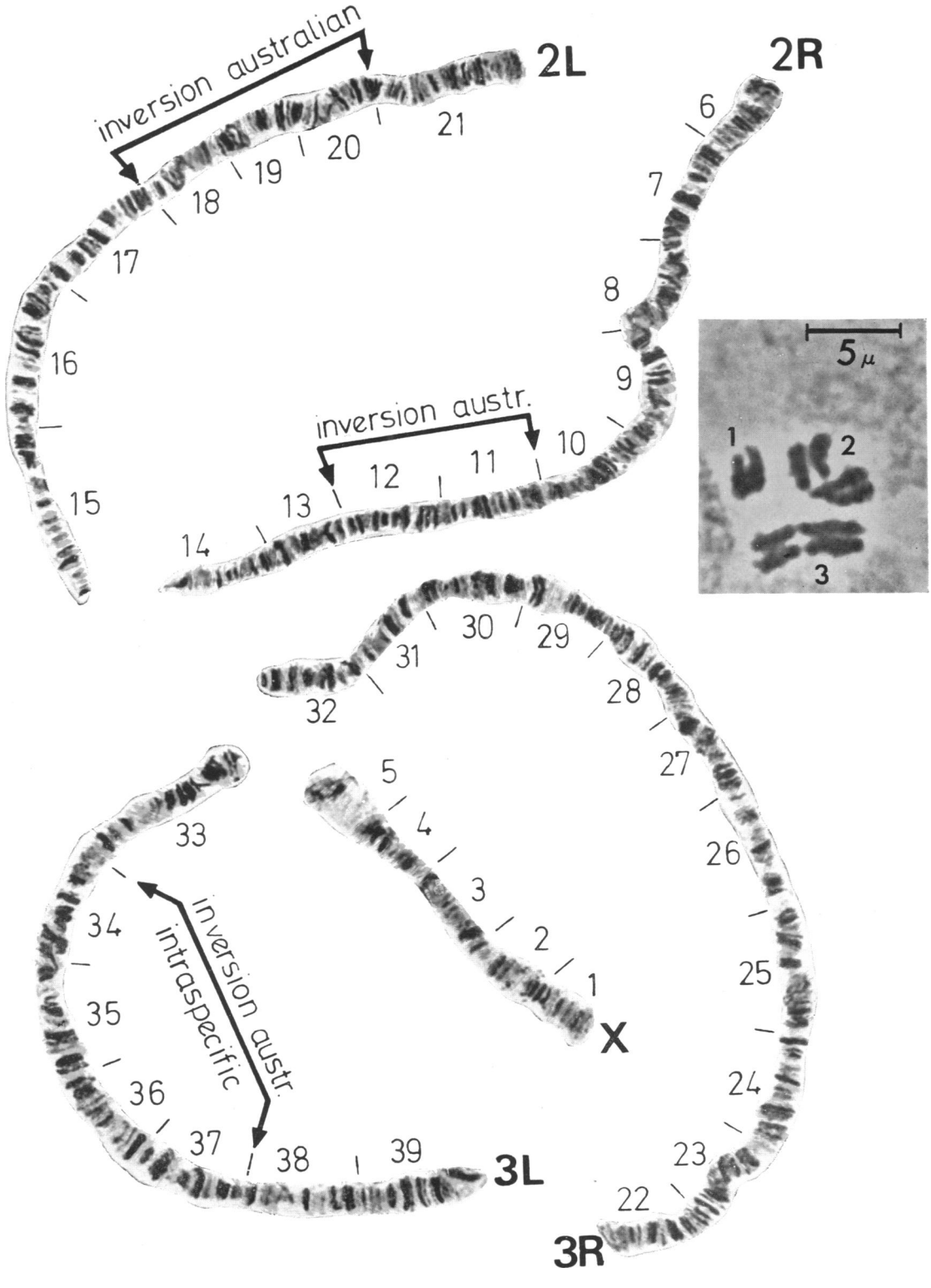

Fig. 1. Chromosomes of *An. farauti* No. 1 Laveran from Rabaul, New Guinea and, inset, the mitotic karyotype.

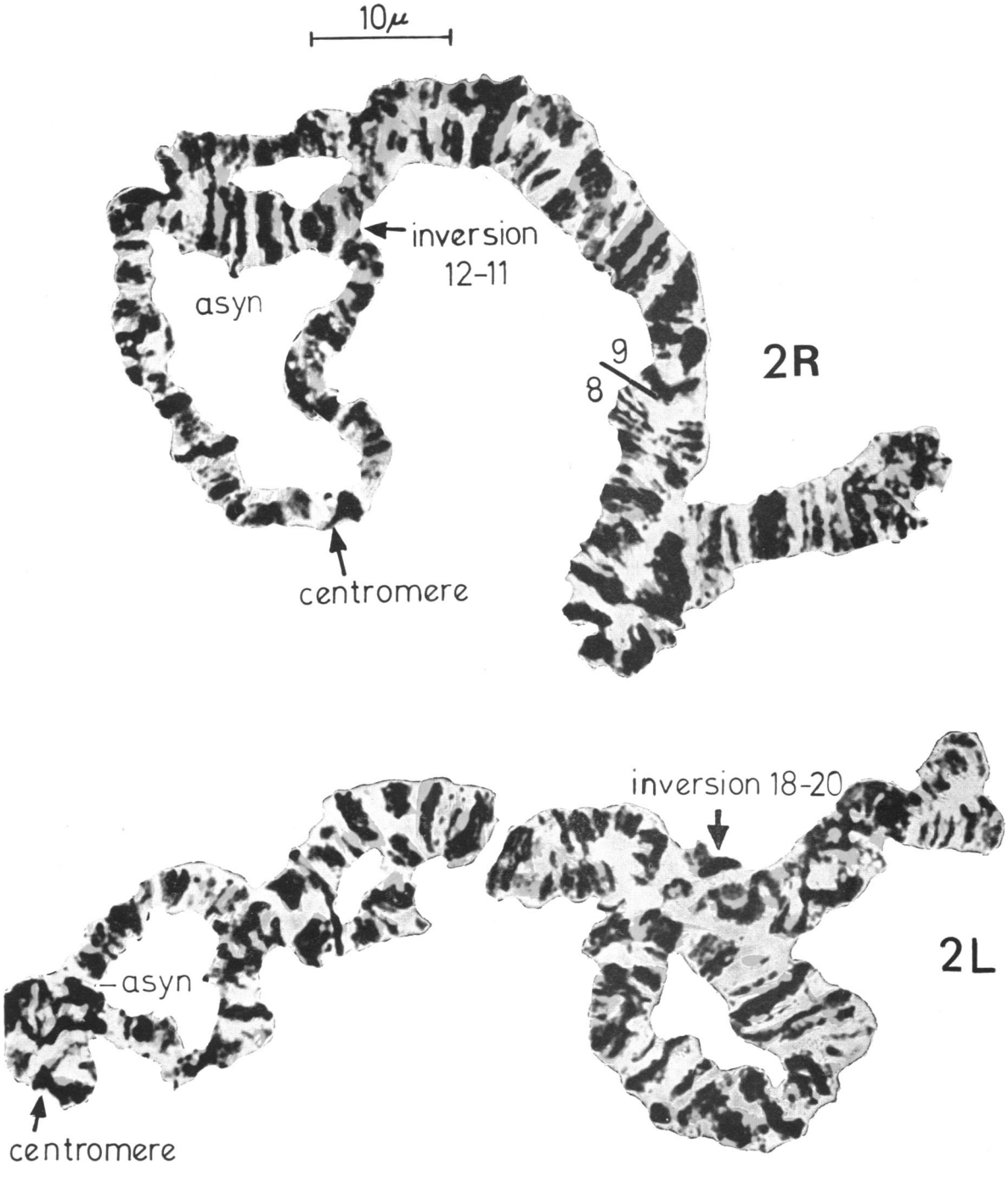

Fig. 2. Salivary-gland chromosomes of hybrid larvae from the cross New Guinea male (*An. farauti* No. 1) and Australian female (*An. farauti* No. 2).

clearly recognizable bands or areas. No further subdivisions have been made.

Chromosomal differences. The chromosomal differences between the New Guinea and the Australian species were studied by direct comparison and by examination of the pairing configuration in the F_1 hybrids. The salivary-gland chromosomes of these two species differ by two paracentric inversions. One occurs on 2R and involves zones 11 and 12; the other, on 2L, involves zones 18, 19, and 20. These two inversions always appear in the heterozygous state in the hybrids where typical synapsed inversion loops are observed (Fig. 2). Although no clear differences in the banding pattern are detectable, asynaptic areas occur in all the arms in the hybrid preparations, suggesting the presence of genetic differences that do not affect the expression of the bands.

Chromosomal polymorphism. No polymorphism was seen in the New Guinea species, but this may have resulted from a loss of polymorphism caused by the rigid selection pressures exerted during 5 years of laboratory rearing. One intraspecific inversion in the heterozygous state has been seen in the Australian species, involving zones 34–37 of the 3L chromosome. This inversion was seen in larvae of the third laboratory generation.

Ovarian nurse-cell chromosomes. No organized, banded polytene chromosomes were observed in the ovarian nurse cells. In other species of the subgenus *Cellia* excellent giant chromosomes have been found (Coluzzi, 1968).

Discussion

This cytogenetic study confirms the previous findings obtained by crossing experiments showing that the *An.* "*farauti*" colonies from New Guinea and Australia are not conspecific but belong to two sibling species (Bryan, 1970). Provisionally, the New Guinea species is designated *An. farauti* No. 1 and the Australian species *An. farauti* No. 2.

The most obvious chromosomal differences between these two species are two paracentric inversions, both on chromosome 2; one inversion occurs on the left arm and the other on the right arm. These inversions appear to be fixed in the homozygous state and are likely to constitute useful cytotaxonomic characters. However, the material examined is insufficient to exclude their intraspecific occurrence.

No chromosomal rearrangements were observed in the salivary X chromosome. This is in contrast to the situation in most of the sibling species of the *An. maculipennis* complex (Kitzmiller et al., 1967) and the fresh-water species of the *An. gambiae* complex (Coluzzi & Sabatini, 1967), where the chromosomal differences are mostly localized in the X chromosome.

Areas of asynapsis were also observed in the salivary-gland chromosomes of the hybrids, involving all the chromosomal arms. Asynapsis occurs in areas where there are apparently identical banding patterns in the parental chromosomes. This has also been recorded in crosses between members of the *An. maculipennis* complex (Kitzmiller et al. 1967).

ACKNOWLEDGEMENTS

The authors record their thanks to Mr A. Sweeney, formerly of the Malaria Service, Territory of Papua and New Guinea, who supplied the eggs for the New Guinea colony and to Mr and Mrs M. P. Hines for valuable assistance in collecting the eggs for the establishment of the Australian colony. They also thank Dr G. Davidson of the Ross Institute for his help and encouragement, and the technical staff of the Monticelli Experimental Station, Italy, and the Ross Institute, London, for their indispensable help.

REFERENCES

Baker, R. H., French, W. L., Kitzmiller, J. B. (1962) *Mosquito News*, **22**, 16

Belkin, J. N. (1962) *Mosquitos of the South Pacific*, University of California Press, vol. 1

Bryan, J. H. (1970) *Trans. roy. Soc. trop. Med. Hyg.*, **64**, 28

Coluzzi, M. (1968) *Parassitologia*, **10**, 179-184

Coluzzi, M. & Sabatini, A. (1967) *Parassitologia*, **9**, 73-88

French, W. L., Baker, R. H. & Kitzmiller J. B. (1962) *Mosquito News*, **22**, 377-383

Kitzmiller, J. B., Frizzi, G., Baker, R. H., (1967) *Evolution and speciation with the* maculipennis *complex of the genus* Anopheles. In: Wright, J. W. & Pal. R., ed., *Genetics of insect vectors of disease*, Amsterdam, Elsevier

Nicoletti, B. (1960) *Drosoph. Inf. Serv.*, **33**, 181