

This was a typical male "crescent" and it was not until several more films were examined that scanty trophozoites were detected. Whether or not this subject would have infected mosquitos we do not know, but we consider this to be doubtful.^e

The observation that gametocytes of *P. falciparum* seldom, if ever, appear in the peripheral blood until about the eighth day of an attack is of considerable importance to the epidemiologist. During the Second World War, one of us (P. G. S.) was sent to Italy with instructions to infect a batch of mosquitos with *P. falciparum* by feeding them on volunteers in a hospital near Naples. The blood of many patients with fever was examined without a single film being found with numerous gametocytes. It was then

^e Grant, D. B., Perinpanayagam, M. S., Shute, P. G. & Zeitlin, R. A. (1960) *Lancet*, 2, 469.

decided to track down some patients who had been discharged from hospital up to a week previously and who had received a course of quinine therapy. This entailed visits to military units and convalescent camps and the results obtained were both interesting and enlightening. The blood of most of these recently discharged patients was found to contain numerous gametocytes, and mosquitos fed on them became very heavily infected. It was obvious that these symptom-free patients were being returned to their units at the very time when they were most highly infectious to mosquitos and so constituted an important link in maintaining the man-mosquito cycle. For at least one month, and possibly for a much longer period, these symptomless carriers were infectious to mosquitos and were probably much more dangerous than indigenous infections in which the attack has ended in spontaneous recovery.

Cytological Aspects of the Taxonomy of Anophelines (Subgenus *Nyssorhynchus*)

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In the taxonomy of the anophelines, the researches of Frizzi^{a, b} opened up a new path as regards the analysis of relationships between species belonging to the European and North American *maculipennis* complex. Comparison of the salivary bands made it possible to recognize a common structural plan and to analyse the differences between the species constituting the complex.

This method, which commenced with the chromosome analysis of *A. aquasalis*, by Frizzi & Ricciardi,^c is at present being applied in this laboratory, in relation to Brazilian anophelines, to the study of the whole subgenus.^{d, e, f} This note reports

^a Frizzi, G. (1947) *Sci. genet. (Torino)*, 3, 80.

^b Frizzi, G. (1954) *Affinità genetiche fra Anopheles delle regioni paleartiche e neoartiche rilevate attraverso lo studio dei cromosomi salivari*. In: *Proceedings of the Ninth International Congress of Genetics (Caryologia (Torino), Suppl.)*, p. 671.

^c Frizzi, G. & Ricciardi, I. (1954) *Symp. genet.*, 2, 172.

^d Guedes, A. S., Amorim, E. M. & Schreiber, G. (1957) *Rev. bras. Malar.*, 9, 247.

^e Schreiber, G. & Memória, J. M. P. (1957) *Rev. bras. Malar.*, 9, 101.

^f Schreiber, G. & Guedes, A. S. (1958) *Cytological and ecological researches on Brazilian Anophelids*. In: *Proceedings of the XVth International Congress of Zoology*, London, Section IX, paper 20.

two cytological phenomena: firstly, the frequency of chromosome mutations (heterozygous inversions, asynaptic zones and translocations) among the different species of the subgenus, related to the formation of "variety complexes"; secondly, variation of the X chromosome, in which there is progressive loss of one of the arms, probably due to heterochromatin formation.

The subgenus *Nyssorhynchus* includes two "series": the *albimanus* series, with the species *aquasalis*, *albimanus* (not Brazilian), *strodei*, *noroestensis*, etc.; and the *argyritarsis* series, with the species *darlingi*, *argyritarsis*, *albitarsis*, etc.

The *albimanus* series includes some species which are well characterized and geographically distinct, as well as other species which are more difficult to characterize and consequently constitute an "*albimanus* complex" (species complex) similar to the *maculipennis* complex. We have not yet sufficient data on the salivary chromosome pattern to enable us to make any distinctions within this complex. The pattern of the right arm of the X, the only one so far studied, shows a certain homogeneity of the bands as between species, while the quantitative

variations in the left arm of the X might possibly serve better for the characterization of the species in this complex.

In the *albimanus* series and in the *argyritarsis* series there are two species which each form a "variety complex". Study of the salivary chromosomes in these species reveals a very clear fact—namely, that the two species which form "complexes" show a much higher chromosome mutation frequency.

Among the species of the subgenus, *aquasalis* and *argyritarsis* show no chromosome mutations (unless there are homozygous inversions, which were not investigated). *Darlingi* has two heterozygous inversions in one of the autosomes, and some heterozygous inversions were found in *noroestensis*. We have no original data on *albimanus*, but a figure by Frizzi & Ricciardi shows the chromosomes of these species as being completely free from inversions. Contrarily, the two species *strodei* and *albitarsis* (each one forming "variety complexes") show chromosomes full of mutations, particularly heterozygous inversions, asynaptic zones and, rarely, translocations. In the case of *strodei*, where research was carried out using a large number of larvae,^e we were able to find a variability per nucleus of 3-11 anomalous zones. In the few cases examined so far of *albitarsis*, we found 4-6 anomalous zones per nucleus. Consequently, we may conclude that the two species in which taxonomists find complexes have chromosomes showing high polymorphism and a considerable degree of heterozygosis. The "*strodei* complex" varies especially as regards the structure of the chorion of the eggs, and it may be mentioned that Frizzi found a good correlation between variation in the eggs and frequency of inversion in Italian anophelines. The variation of the "*albitarsis* complex" is connected with the chromatic characteristics of the adults.

It is of interest—as an initial contribution to the cytological classification of this subgenus—that the species forming "complexes" show much higher chromosome mutation frequencies than those which are uniform taxonomically.

Another field of cyto-taxonomic research in this subgenus is opening with the comparative study of

the X chromosome in the neuroblasts and the salivary glands.^g The species of the subgenus can be arranged in a series in which one arm of the X chromosome is gradually lost; this loss in the different species does not occur simultaneously in the two types of somatic cells studied. Thus *aquasalis* has a metacentric X both in the neuroblasts and in the salivary cells. The map of this chromosome was published by Frizzi & Ricciardi. *Noroestensis* shows a metacentric X in the neuroblasts but an acrocentric X in the salivary cells. The single arm of the salivary X in this species corresponds to the right arm in Frizzi & Ricciardi's map for *aquasalis*. In the other species, the X is acrocentric both in the neuroblasts and in the salivary cells, and in these species the arm present has a pattern corresponding to the right arm in *aquasalis*. In the *albitarsis* nucleolus we found a much larger quantity of heterochromatin linked to the X centromere than in the other species. We also observed heterochromatic zones in *aquasalis*, more extensive in the left arm than in the right—an observation which does not appear in the paper by Frizzi & Ricciardi.

The progressive loss of the left arm of the X bears apparently no relation to the taxonomic position of the species in the subgenus. It appears to be a phenomenon which occurs independently of the two "series". Frizzi describes similar phenomena in *atroparvus*, *freeborni* and *claviger* species in which the mitotic X is sub-telocentric, while the salivary chromosome loses the larger arm as a result of heterochromatin formation. We must await further research in this direction before drawing any systematic conclusion, although these phenomena indicate the probable existence of a cytological basis for species formation within the subgenus.

It would seem to be worth while to make a more detailed study of these cytological variations, which consist in the elimination of various parts of the chromosomes in different somatic tissues during embryonal development, as well as a comparison of them in the different species.

^g Schreiber, G. & Guedes, A. S. (1959) *Ciência e Cultura*, 11, No. 3, p. 128.