THE SALIVARY CHROMOSOMES OF *ANOPHELES QUADRIMACULATUS* SAY

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Cytogenetic investigations involving the salivary gland chromosomes of *Anopheles quadrimaculatus* Say were begun by Frizzi and De Carli (1954) in a study comparing the chromosomal complements of the Nearctic and Palearctic species of the *maculipennis* complex of the genus *Anopheles*. They sketched regions of the chromosomes which differed in details of banding from that found in *Anopheles atroparvus*. Sketches, photographs and a preliminary map of the karyotype are shown in the doctoral thesis of French (1962). French noted the presence of intrachromosomal connectives, chromosome puffs but not of Balbiani rings, and that the chromosomes are sometimes attenuated. French also made the interesting observation that a complex arrangement in the left arm of chromosome 3, previously discovered by Frizzi and De Carli (1954), was characteristic of the majority of individuals in seven stocks of diverse geographic origin. An outline of this arangement is shown in a sketch of the karyotype by Mason and Brown (1963) who were able to show that DDT-resistance was unaccompanied by the appearance of new chromosomal inversions.

The present paper describes the salivary gland chromosomes of *quadrimaculatus* and attempts to summarize the chromosomal evidence for the relationship of this species to other members of the *maculipennis* complex in North America.

MATERIALS AND METHODS. The following strains were cultured and used:

Dothan: a strain collected in Dothan, Alabama in January 1964.

Bethesda: a strain obtained in 1962 from the Naval Medical Research Center, Bethesda, Md., where it had been in culture since 1945 as the "N" strain.

The best salivary gland chromosomes were prepared from large fourth instar larvae about 10 hours prior to pupation. Preparations were made by standard methods (French, *et al.*, 1962).

The maps were prepared by photographing the karyotype, printing at a standard enlargement and by sketching the form of the chromosome and the outstanding bands from the print. The details of each region were then mapped by careful examination under oil immersion (1000X) using phase.

RESULTS. The homologous chromosomes in the salivary glands are synapsed so that the karyotype appears to consist of five arms radiating from a common point of attachment which involves the centromere of each chromosome. No *Drosophila*-type chromocenter is present. Frequently the connection between the chromosomes is broken so that the chromosomes may superficially appear as three rope-like strands. (Figure 1)

The telocentric X-chromosome averages 72 microns in length and is about one-quarter of the length of the other chromosomes. The nucleolus is usually attached to the centromere region of the X-chromosome.

Chromosome 2 is metacentric; the right arm measures about 160 microns and the left arm measures about 147 microns. By contrast the right arm of chromosome 3 is much longer than the two known homozygous arrangements of the left arm, the respective lengths being about 234, 158 and 103 microns.

Clearly all three chromosomes are immediately recognizable by size and proportion: the X is the shortest, 2 has arms of similar length and 3 has arms of strikingly unequal length. In most smears chromosome 3 is recognized by the complex arrangement in the left arm.

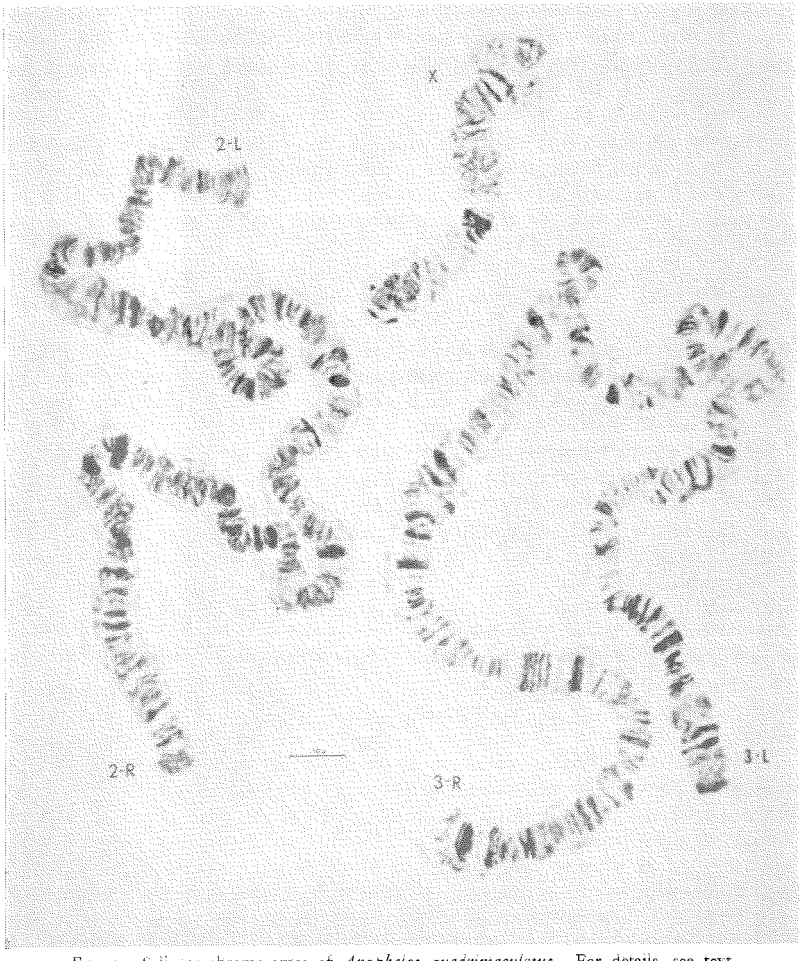

FIG. 1.—Salivary chromosomes of *Anopheles quadrimaculatus*. For details, see text.

A large nucleolus adjoins the centromere regions when these are intact, but it remains connected to the X if the chromosomes become dissociated. The nucleolus measures about 40 microns in diameter, and a darkly staining diffuse web-like structure may be seen within its center.

DESCRIPTION OF THE CHROMOSOME. For descriptive purposes the chromosome complement has been divided into five subdivisions after the scheme of Frizzi (1947) which begins with the X, then the right arm of 2, the left arm of 2 and finally the right and left arms of 3. Zones in each chromosome are established so that each zone usually begins and ends at a clearly recognizable band or series of bands. The zones are numbered and further subdivided into segments to which letters are assigned. Since the arms of these chromosomes are similar in many details to the arms of previously described species (Baker and Kitzmiller 1964, 1965a, 1965b; Kitzmiller and Baker 1963, 1965) we have adopted similar numerical zones in all chromosome arms.

In our map of *Anopheles quadrimaculatus* (Figure 2) the numbered regions are as follows: X-chromosome, zones 1 to 5, beginning at the free end of the chromosome and with region 5 ending at the centromere; 2R, zones 6 to 14, the latter at the centromere; 2L, zones 15 to 21 with zone 15 beginning at the centromere and zone 21 at the free end of the chromosome; 3R, zones 22 to 32 beginning at the free end and ending at the centromere; 3L, zones 33 to 39 beginning at the centromere and ending at the free end. The choice of an arrangement to designate as the "standard" arrangement in 3L was somewhat arbitrary. A high percentage of all individuals of all populations and strains examined thus far is heterozygous for a complex series of aberrations in 3L. We have recovered two different homozygous arrangements and suspect that still others exist. The 3L arrangement shown herein is the one most commonly recovered as a homozygote. In the following descriptions the term distal is used to designate the free end of a chromosome arm, while proximal is used to designate the region of the centromere.

X-chromosome. The X-chromosome is readily identified by size, being one-fourth the length of either autosome. It is usually attached to the nucleolus and sometimes to the autosomes at the centromere region. Further, the X-chromosome differs from the X-chromosome of any other mosquito that has been cytologically studied by the presence of a large subterminal puff in zone 1B (Baker and Kitzmiller, 1963). The two deeply staining bands in the center of zone 3C are an excellent "landmark" on the map; these bands often appear as a broad single band.

The terminal bands in zone 1A are often difficult to see, and only rarely is the terminal band as dark as in the figure. The three proximal bands in 1A are consistently present. The bands in the subterminal puff 1B are sometimes difficult to resolve. Three heavy bands in 1C lie adjacent to the puff. The three heavy bands in 2C are always readily found; they are followed by a clear segment and then by two widely spaced dark bands in 3A and four heavy bands in 3B. The two "landmark" bands in 3C are followed by two slightly less intense bands, a clear segment and a variable puff in 4A. The form and banding pattern of 4C is quite constant.

Chromosome 2—Right arm. The right arm of chromosome 2 may be recognized by one or a combination of the following areas. The free end is usually expanded and within it several diffuse bands may be seen. In some preparations the segment of the chromosome from the end to the heavy band in 6B is condensed to about one-quarter of the length indicated on the map. The two puffs in 6C and 6D may be used to distinguish this arm from the left arm of the chromosome; care should be taken not to mistake the band in 6B for the 21C band similarly positioned in the left arm of the chromosome. The asynaptic puff in 8C followed by an arc-shaped band are constant features. Perhaps the best landmarks are regions 9A and 9B which consist of two heavy bands, three light bands, then another light band followed by four closely spaced heavy bands. Zones 6E, 6F show much variability in the intensity of the bands and the degree of puffing. The light bands between the proximal two heavy bands in 6F are not always visible. The most distal heavy band in 9E may often be resolved into two bands. In some preparations the bands in 12A appear as heavy bands, however the puff in 12B may be regarded as a constant and very useful landmark for the proximal end of the chromosome. In some preparations a connective joins the heavy band in 14B to the centromere.

Chromosome 2—Left arm. The distal end of chromosome 2L is characterized by the presence of a single dark band which may be seen in 21C. This band is followed by a very characteristic puff in 21D and 20A and a characteristic series

of crescent-shaped bands in 20B. Zones 20C, 20D and 19A are variable in form and in resolvable detail. Perhaps the most constant feature of the entire arm is zone 19C, in which two heavy bands are followed by a light band, a blank segment and a series of light bands adjacent to the proximal heavy band. The five dark bands in 17A are an excellent landmark for the middle of the arm. Zone 17C is often asynaptic and difficult to resolve. The two dark bands in 16A always stain very heavily and they are readily seen in all preparations. The heavy bands in 16C usually show the pattern of two medium intensity bands flanked by heavy bands. Zones 16D, 16E, 15A show a tendency to stretch so that the space relationships between the bands may become distorted. Zone 15C was found to be asynaptic in all preparations.

Chromosome 3—Right arm. The right arm of the third chromosome may be most readily recognized by its unusual length, by the flared distal end followed by two dark bands in 22A and by the series of bands in zones 24C, 24D, 25A, 25B.

The extent of puffing in 23A and 23D was found to be variable. In all preparations the proximal segment of 23C was seen to be asynaptic. The expression of 24A proved to be constant while 24B often was found to be puffed. The segment 24C through 25B showed a tendency to twist in most preparations, so that careful focusing may be required to follow this strikingly constant series of five dark bands, four light bands, two dark bands, two light bands and five dark bands. Zone 26C was found to show extremely wide variation in the extent of puffing so that when maximally distended the puff is nearly four times as wide as the adjacent regions of the chromosome. The puff in 27A is consistently present. In some preparations 27D is puffed. The region 28C through 30A was found to show considerable variation in the intensity of the bands and in the interband distances; the latter being due to distortion during preparation. However in some preparations 28C through to 29B appeared to be exactly duplicated in 29B through 30A. The dark band in 31A always stained intensely and served as a landmark for the proximal portion of the chromosome. The three heavy bands in 32B may be used to recognize the centromere end of the chromosome as may the 1-3-1 heavy series in 32C and D.

Chromosome 3—Left arm. The free end is very similar to the typical end found in all other Nearctic species. The squarish end and the banding pattern in 39A and B are similar, as is the banding sequence in 39D through 38B. Conspicuously absent are the three heavy bands in 39C which are characteristic landmarks of the end of the chromosome in all other Nearctic species. Perhaps the best landmark of the free end of the arm is a series of four heavily staining areas in 38D through 37C. This series characteristically appears as two heavy areas, then two heavy bands which are usually much wider than the average width of the chromosome, then two heavy bands in 37C. The typical sequence may be used as an excellent marker for identifying arrangements in which this end of the chromosome is inverted. The series of dark bands in 37G and 36A are consistent, as is the long spindle-shaped series of dark bands in 36D and E. A single heavy band in the middle of 36E is almost always sinuose. The expanded puff in region 35 characteristically appears empty except for the heavy bands at its beginning. The five heavy bands in 34C followed by a clear area in 33A usually are sufficient landmarks for this region. In most specimens of most strains the areas 33C, D and E are in the form of a loop with definite connections between the heavy band at the end of 33B and the one at the end of 33E.

MITOTIC AND MEIOTIC CHROMOSOMES. During mitotic divisions two large pairs of chromosomes were observed by Frizzi (1953) to be mediocentric, while one dot-like pair was judged acrocentric; the members of this latter pair were seen to

be of equal length in females, while one member of the pair was seen to be of reduced length in males. Subsequently Kitzmiller and French (1961) described the smallest pair as subtelocentric, while Rai and Craig (1961) described them as metacentric. During meiotic divisions the smallest pair was seen to be the first to separate at Anaphase I (Kitzmiller and Frizzi, 1954). In the ovaries both members of this pair were seen by Kitzmiller and French (1961) to be 5 microns long, while one member of the pair was seen to be only one-half of this length in the testis. In resting sexual cells Frizzi and De Carli (1954) could observe only a very small heteropycnotic region. We consider that the smallest pair of mitotic (and meiotic) chromosomes corresponds to the shortest element in the salivary set. The much reduced length of the mitotic X in *quadrimaculatus* as compared with the much longer mitotic X in all other species within the complex, is indirect evidence that only the dot-like portion (in *freeborni, punctipennis,* etc.) is represented in the salivary complement.

DISCUSSION. The chromosomal affinities of *quadrimaculatus* clearly seem to be with the *freeborni-aztecus-occidentalis* group. The X-chromosome is difficult to homologize, but zones 3B through 4C are possibly homologous with *freeborni*. At the left end of the chromosome, a simple inversion involving zones 1C through 3A of the *freeborni* map would produce a chromosome not too different from *quadrimaculatus*.

Chromosome 2 shows some interesting relationships. The free end of 2R is difficult to homologize with *freeborni* but is very similar to *aztecus,* from 6A through 8C. The banding is not exact, but is very similar. Regions 9A through 10B of the *quadrimaculatus* map appear to be a simple paracentric inversion of 10B–9A of *freeborni*. Zones 10C through 12C are almost perfectly homologous with *freeborni*. Zone 13A, with weakly staining bands is of uncertain homology but 13B, 13C and 13D have exactly the same banding pattern in both species. The variable centromere end of the chromosome is not exactly similar, but is very close. The region 9A through 10B as stated above represents an inversion of similar segments in *freeborni*. This is entirely contained within a larger inversion between *freeborni* and *occidentalis,* and therefore should appear in the *occidentalis* chromosome in the same order as it does in *quadrimaculatus*. Comparison of the *quadrimaculatus* and *occidentalis* maps bears this out.

The right arm of chromosome 2 therefore, seems to be very close to that of *freeborni,* except for the free end, which apparently has been derived from *aztecus*.

The left arm of chromosome 2 does not differ from that of *freeborni* in any major aspect. As far as can be determined the bands and areas homologize almost perfectly. A possible exception is in regions 15C and 15D near the centromere, which in *quadrimaculatus* usually appears asynaptic and twisted, but even here the bands are very similar. There do not appear to be any major inversions or other aberrations. The only differences noted are in intensities of some bands and variable stretching.

At first, the left arm of chromosome 3 appears to be different from the consistently conservative pattern found in the other species but closer study shows that this impression is false. The free end, through 38A is identical with the typical *freeborni* arm, except that the heavy bands in 39C are missing. With only slight differences in band intensities, the homologies are good through 37D. The centromere end of the chromosome 35A through 33B is almost identical with the *freeborni* pattern. Additional material, 33C through 33E, is found in *quadrimaculatus*. This material is the segment contained within the "loop" of this arm. A short segment 33F, lies between the loop and the centromere. It is of considerable interest that *atroparvus, occidentalis* and *aztecus* also have the extra material at the centromere end of 3L. This scheme leaves 37E

through 36E of the *quadrimaculatus* map not represented in the chromosomal maps of other species. Region 36 of *quadrimaculatus,* with its two large puffs corresponds almost exactly with region 36 of *freeborni.* Region 37G of *quadrimaculatus* is very similar to 37E of *freeborni,* which would leave only a few bands different (37E and F, *quadrimaculatus*). This is very close similarity indeed.

As is usually the case, the homologies of 3R are difficult, but there are enough similarities to identify this chromosome arm clearly with the *freeborni-occidentalis-aztecus* series. In *occidentalis* and *aztecus* 3R is almost identical from 22A through 26E. In *quadrimaculatus* the similarity extends from 22A through 24B, then a long inversion, 24C–26C represents the 23C–26D segment in *occidentalis* and *aztecus.* The homologies are not perfect, but close. The centromere end, 32E to 32A (*quadrimaculatus*) is very close to 32D–30C (*aztecus*) and 32C–31A (*occidentalis*). Other regions also show some similarities. There is clearly more material in the *quadrimaculatus* and *occidentalis* chromosomes than in *freeborni* or *aztecus,* and the *quadrimaculatus-occidentalis* similarities are also closer when the bands are compared.

In summation, the chromosomal evidence clearly points to a close relationship among *freeborni, occidentalis, aztecus* and *quadrimaculatus.* The remaining species, *earlei* and *punctipennis,* show close similarities but of course share much in common with *freeborni.* The chromosomal evidence therefore bears out the closeness of the *freeborni-occidentalis-aztecus* relationship as suggested by the crossing studies, but indicates a closer relationship for *quadrimaculatus* than do the crosses (Kitzmiller *et al.* 1965).

SUMMARY. A map of the salivary gland chromosomes of *Anopheles quadrimaculatus* is presented and it is suggested that this map be used as the standard for future work. Comparison of the banding patterns in *quadrimaculatus* clearly indicates that it is closely related to the *freeborni-occidentalis-aztecus* group of the Nearctic *maculipennis* complex of species.

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EFFECTIVENESS OF VARIOUS DOSAGES OF DICHLORVOS AGAINST *AEDES AEGYPTI* IN CISTERNS, ST. THOMAS, V. I.[1]

G. D. BROOKS, H. F. SCHOOF AND E. A. SMITH

Of the myriad of man-made breeding sources for *Aedes aegypti*, the water storage cistern presents one of the most difficult in which to exercise control of breeding. The necessity of preventing contamination of water by foreign substances, the often limited access to areas to be treated, and the diversified types of cisterns frequently restrict the use of standard insecticidal techniques. Under these highly restrictive conditions, a logical approach is that of the sustained release of insecticidal vapor within the cistern. This method can operate in a wide variety of cistern configurations, can treat the inaccessible areas of the cistern, and avoid the necessity of the direct addition of a foreign substance to the water. Work on the residual fumigant technique for the control of *Culex* in confined spaces, *i.e.*, catch basins, (Maddock *et al.*, 1963 and Brooks *et al.*, 1963) indicated possible usage of this approach against adult *Ae. aegypti*. The objective of this study was to establish dosage levels of this residual fumigant that could be used to control *Ae. aegypti* in cisterns in the Virgin Islands.

MATERIALS AND METHODS. Eighteen cisterns infested with *Ae. aegypti* were selected for treatment in Charlotte Amalie, St. Thomas. The cisterns chosen were of the exposed type located exterior to the residence (Figure 1). All cisterns were

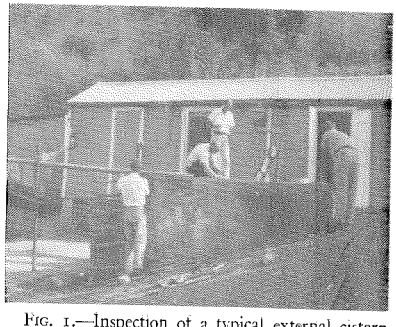

FIG. 1.—Inspection of a typical external cistern on the island of St. Thomas, U. S. Virgin Islands.

rectangular in shape ranging in size from 3′ 0″ x 6′ 6″ to 9′ x 11′ 3″. They were constructed of concrete with either a wood or metal access hatch in the top. Capacities of individual cisterns varied from 127 cubic feet to over 1,128 cubic feet. Water depth varied from 6 inches to more than 7 feet, while the distance between the water line and the roof of the cistern ranged from 3 inches to 10 feet. All cisterns contained rain water collected by roof gutter systems attached to adjacent buildings.

The dispensers utilized in this study were of the 20 percent dichlorvos in resin-

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