

SALIVARY GLAND CHROMOSOME MAP OF *ANOPHELES PSEUDOPUNCTIPENNIS FRANCISCANUS*¹

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ABSTRACT. A salivary gland chromosome map for *Anopheles pseudopunctipennis franciscanus* shows the same general pattern of previously described anophelines with a short telocentric X and two metacentric autosomes. The arms of chromosome 2 are almost equal in length, but the right arm of chromosome 3 is almost twice the length of the left. Comparisons of the chromosomes of

franciscanus with its nominative subspecies show a distinct difference between the X chromosomes involving at least one inversion and loss of chromosome material in *franciscanus*. Although similar in banding pattern, at least four inversions separate the autosomes of the two subspecies. No chromosomal aberrations were observed in the *franciscanus* material.

INTRODUCTION. *Anopheles pseudopunctipennis franciscanus* is the northernmost of the seven subspecies of the *Anopheles pseudopunctipennis pseudopunctipennis* complex (Stone *et al.*, 1959). The complex extends north and south through the Americas and occupies areas of great geographical diversity. This extensive distribution, with so many potential barriers to gene flow, led Baker *et al.* (1965) to suggest that the complex was a good candidate for a study in speciation and evolution.

The various roles of the several populations of *pseudopunctipennis* (*s.l.*) as vectors of malaria (Barber *et al.*, 1929; Barber, 1933, 1939; Boyd and Earle, 1939; Hoffmann, 1932; Shannon *et al.*, 1927; Shannon, 1930; Simmons and Aitken, 1942) leave open the question of genetic or ecological differences. *Anopheles p. franciscanus* is not thought to be a vector in nature (Herms, 1919, 1920) and even the reported case of laboratory infection of this species (Barber *et al.*, 1929) is in question since the mosquitoes used could have been *pseudopunctipennis* (*s.s.*) rather than *franciscanus*. These reports indicate that in addition to morphological differences there are possible physiological, or behavioral differences, which merit further study of the complex.

Recently mosquito cytogenetics has been

used to explore the relationships existing between species and within complexes. The growth and importance of these evolutionary studies are evident from the reviews of Kitzmiller (1953, 1963, 1967).

The first step in the evolutionary study of the *pseudopunctipennis* complex was taken by Baker *et al.* (1965) in their cytogenetic study of the salivary gland chromosomes of *pseudopunctipennis* (*s.s.*). The present paper adds the map of the salivary gland chromosomes of *franciscanus* as a further aid in elucidating the relationships of the complex. The taxonomic relationship between *franciscanus* and *pseudopunctipennis* (*s.s.*) will be examined in a later paper.

MATERIALS AND METHODS. The *franciscanus* used in this study were originally collected as larvae from residual streams in Capay Valley, Colusa County, California in 1969. They were colonized³ in the laboratory using methods outlined by Gerberg (1970) for anophelines except that the larvae were fed a diet of equal parts by weight of Fleischmann's yeast[®], Kellogg's[®] Concentrate and wheat germ ground to a fine powder (Kitzmiller, personal communication). This colony has been maintained in the laboratory without interruption for the past 2 years, but adults reared from field-collected larvae have been added at regular intervals.

The salivary gland chromosomes were prepared following the method described by French *et al.* (1962) except that the extracted glands were (1) dissected and

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fixed in 35 percent acetic acid and (2) stained in 0.5 percent lacto-aceto-orcein for 3 to 4 minutes.

The chromosome maps of these preparations were drawn in a series of steps. First, photographs of the individual preparations were printed with an imposed micrometer scale for later measurement. Second, outline maps of the individual chromosome arms, characteristic puffs and significant bands were drawn from the photographs. Based on these working drawings individual areas were mapped in detail from slides of over 300 glands using phase contrast microscopy at 1200 \times magnification.

The map of the salivary gland chromosomes of *franciscanus* (Fig. 6) has been lettered and numbered in the same arbitrary manner as the chromosome map of *pseudopunctipennis* (Baker *et al.*, 1965) to facilitate comparison.

DESCRIPTION OF THE CHROMOSOMES. The salivary gland chromosomes of *franciscanus* show the same general pattern of previously described anophelines with three pairs of synapsed polytene chromosomes appearing as five separate, or connected elements in each nucleus, a short X chromosome, chromosome 2 with right and left arms, and chromosome 3 with right and left arms. The telocentric X chromosome averages 50 μ . The autosomal arms average: 2R, 175 μ ; 2L, 140 μ ; 3R, 210 μ ; and 3L, 130 μ . The salivary chromosomes do not show a chromocenter as found in *Drosophila*, but are rather strongly connected in the region of their centromeres. This connection will break if sufficient pressure is applied to the preparation, the X chromosome separating with the least pressure. The nucleolus is normally associated with the common area of centromere attachment.

X CHROMOSOME. This chromosome is easily recognized by its length in salivary gland preparations. The free end, region 1A, was slightly flared with weak bands followed by a distinct puff in 1B-C. In 1B there were three heavy dark bands and then a clear area in 1C. Region 2A showed a densely banded puff followed

by a constriction in 2B. The puff in region 3 was lightly banded except for 3B which contained two dark bands. The most diagnostic feature was the large puff in region 4 with its heavy bands. In region 5 a small puff and constriction led to the broadly flared and weakly banded end of the chromosome (5B-C). In most preparations the terminal centromere showed attachment to the centromere region of either chromosome 2 or 3 or both when they were connected.

CHROMOSOME 2—RIGHT ARM. This arm is the second longest element in the chromosome complement. The free end was slightly flared (6A) and was difficult to see in most preparations. The large puff in 6B was easily seen and is diagnostic for this arm, but the three dark bands were not obvious in most preparations due to a folding back of the free end. This large puff was followed by a series of three smaller puffs in 6C, 7A and 7B and a clear area with few bands extending into 8A. The rest of 8A and 8B was densely banded while 8C and 9A were lightly banded. The dark band in 9B and multiple bands of 10B were characteristics of this portion of the arm. Region 10 was followed by a distinct banded puff in 11C which was visible in most preparations. Region 12 was distinguishable by the small puff containing five dark bands in 12A and the four dark bands of 12E. The small puff in 13D was the first of a series of puffs extending through 14B. This area of the chromosome arm, in particular region 14, was generally quite diffuse in preparations and easily distorted, though 13E was seen easily. The three terminal bands in 14C appear to be a part of the centromere region of this arm.

CHROMOSOME 2—LEFT ARM. The slight flare in the lightly banded free end of this arm (21A-B) separates it from 3L which is similar in length. Two puffs with a dark band are characteristic for region 20A. These puffs were followed by a light banded area in 20B with two large densely banded puffs in 20C-D. Region 19A was largely devoid of bands

while 19B contained three dark bands and a large clear area. A moderate puff in 19C led to a distinct constriction in 18A followed by multiple bands through 18C. The puff in 17A, with its two dark bands, was characteristic for this area of the arm, while 16A had a unique clear area with three distinct bands leading to a light banded 16C. The mass of bands at the juncture of 16C with 16D was diagnostic for this region of the arm. A small puff with two dark bands (15A) often appeared as the centromere end of this arm since 15B-C was generally distorted and unclear. However, the bands of the true centromere region in 15C can be determined.

CHROMOSOME 3—RIGHT ARM. This arm was the longest of all the salivary gland chromosome elements, a character which is shared with all members of the subgenus *Anopheles* studied to date. It shows the typical flared end with two dark bands (22A) followed by a diagnostic puff in 23A and two others. Regions 23B-C and 24A showed multiple bands which stained lightly. The small puff of 24C and 25A with its four dark bands was followed by a large puff in 25B-C. Region 26A-B was often distorted, but the puff in 26C-D and its three pairs of dark double bands were characteristic. This area is followed by a light staining portion leading to three prominent dark bands in a large puff in the distal (relative to the free end of the arm) portion of 27C. Another area which was often distorted was 28A-B preceding the clear section of 28C. Three light bands bounded on either side by a pair of dark bands are diagnostic for the puff in 29A. Region 30A-B was light staining except for a dark band at its distal end. The next region, 30C-D, showed three distinct bands at the proximal end of 30C. The four dark bands of 31B were characteristic. Region 32 was distinguished by the three dark bands in 32A and the three dark bands and single one in 32B. The centromere end of the arm was typified by a dark band in 32D.

CHROMOSOME 3—LEFT ARM. This arm

was generally the shortest of the autosomal elements in preparations although for graphic reasons it appears similar in length to 2L in the map (Fig. 6). It also showed similarities to the 3L of other described anophelines both in configuration and banding pattern. The dark bands in 38B and 38C were diagnostic along with the puff and dark bands in 37A. Region 37B also showed a puff with two dark bands followed by a light staining puff in 37C. The two dark bands in the constricted portion of 37D were characteristic and 36B showed a large puff with an unusual banding pattern. This puff was followed by a smaller puff in 35A leading to a large darkly banded puff in 35C. The rest of the arm was composed of a series of light staining puffs except for the dark band in 33B close to the light staining and diffuse centromere end.

ABERRATIONS. No aberrations were seen in any of the material examined including material collected from the type locality of *franciscanus* described by McCracken (1904) and other regions in northern California.

DISCUSSION. The salivary gland chromosomes of *franciscanus* are closer to those of *pseudopunctipennis* than those of *Anopheles hectoris* (Baker *et al.*, 1966). The three have a strong resemblance in banding pattern as a group and differ from other described anophelines, except in overall length and ends of the autosomes. As pointed out, the chromosomes of *franciscanus* are most similar to those of *pseudopunctipennis* and thus it is appropriate to compare the maps of these two subspecies. The banding patterns of the chromosome arms of the two are compared in Figs. 1, 2, 3, 4 and 5. Areas of apparent similarity and possible inversions (INV) are indicated by vertical lines.

The X chromosome of *franciscanus* (*f*), previously incompletely described by Baker and Kitzmiller (1963), shows a good comparison with *pseudopunctipennis* (*p*) in region 1, though a portion of 1A of *franciscanus* appears to be inverted from that of *pseudopunctipennis*. In addi-

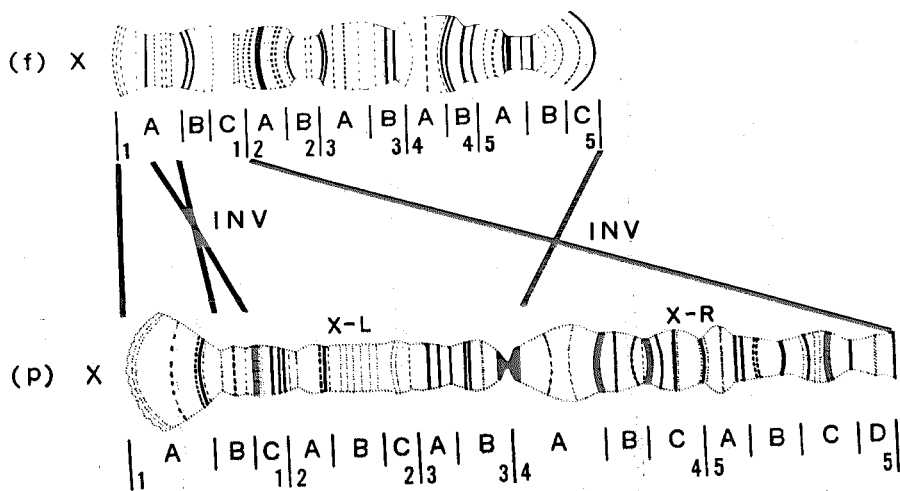


FIG. 1.—Comparison of X chromosomes of *A. p. franciscanus* (f) and *A. p. pseudopunctipennis* (p).

tion, the free end of the chromosome in *franciscanus* is not as flared as in *pseudopunctipennis*. The balance of the short telocentric X chromosome of *franciscanus* may be homologized with *pseudopunctipennis* by assuming a pericentric inversion of regions 4 through section 5D (p) with the loss of regions 2-3 (p). This situation is somewhat analogous to the condition described by Baker *et al.* (1966) for *Anopheles hectoris* although there is more similarity in the banding pattern of *franciscanus* with *pseudopunctipennis* than with *hectoris*. The telocentric condition of the X chromosome is a marked difference between the two subspecies and a condition shared with all other members of the subgenus *Anopheles*. *Anopheles p. pseudopunctipennis* is the only member of this subgenus to show a metacentric X chromosome to date.

Chromosome 2R (Fig. 2) of the two subspecies is almost identical in both configuration and banding pattern. Regions 6-7 (f) show the only major difference while the other differences may be attributed to staining and the loss or gain of some minor bands in *franciscanus*.

In chromosome 2L (Fig. 3) the major areas of difference are two inversions. The first is in region 20D-19B (f) including a portion of 20C (f) compared with 20D-19B (p). The region 20D (p) shows a subsequent included reinversion to be equivalent with 19A-19B (f). The second inversion is in region 17B-16B (f) as compared with 17B-17D (p) with an included reinversion of 17D (p) to be equivalent with 17B (f). Region 15A (f) is also difficult to homologize with *pseudopunctipennis*.

The free ends of chromosome 3R (Fig. 4) are almost identical in the two subspecies. The rest of the arms are similar though two inversions appear present in *franciscanus*: 26A-C (f) inverted from 26A-C (p) and 28A-C (f) inverted from 28C (p). Regions 31 and 32 in *franciscanus* are difficult to homologize with the same areas in *pseudopunctipennis*.

The configuration and most of the banding pattern of the arm of chromosome 3L (Fig. 5) of *franciscanus* are essentially identical to *pseudopunctipennis* apart from some difference in staining and orientation of bands.

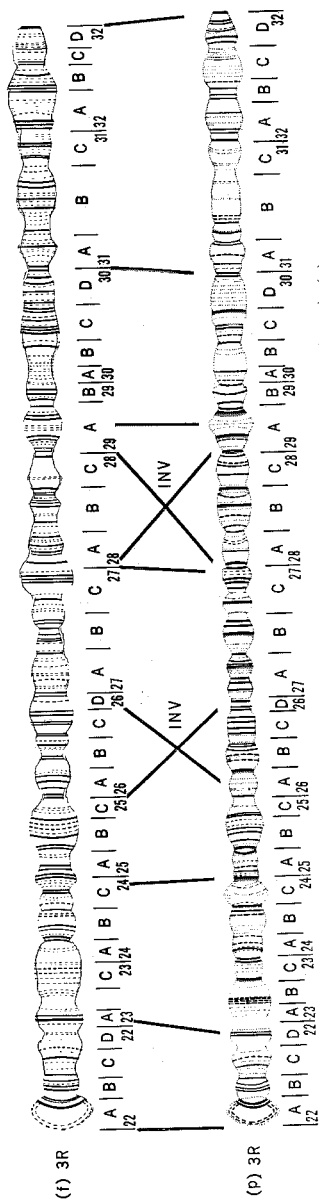


FIG. 4.—Comparison of 3R of *A. p. franciscanus* (f) and *A. p. pseudopunctipennis* (P).

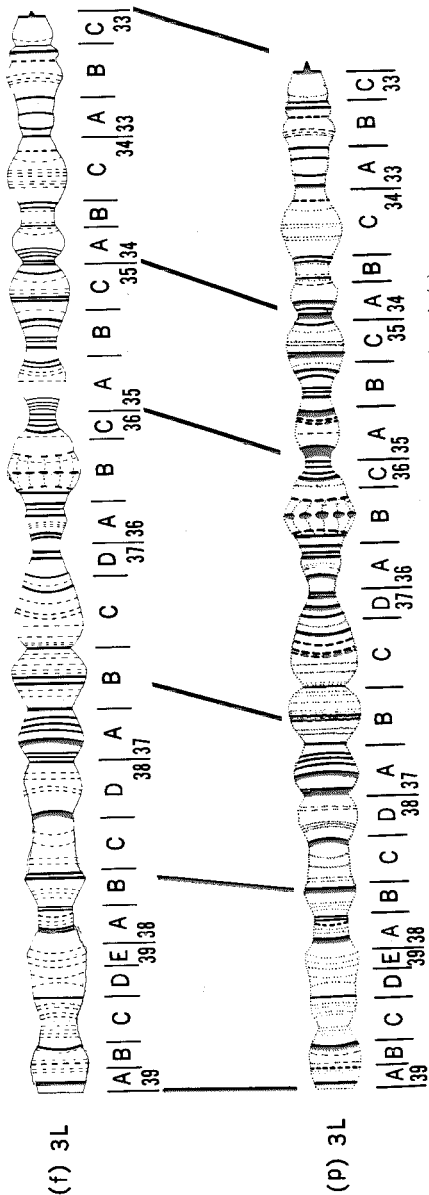


FIG. 5.—Comparison of 3L of *A. p. franciscanus* (f) and *A. p. pseudopunctipennis* (P).

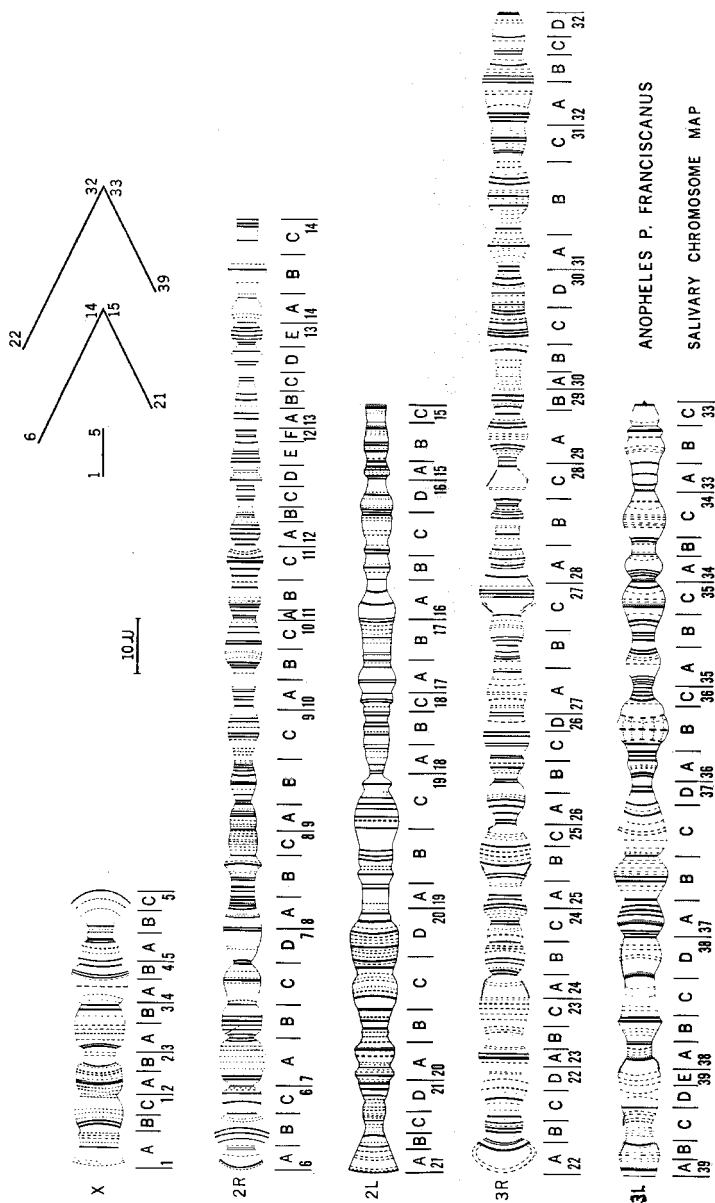


FIG. 6.—Salivary gland chromosome map of *Anopheles p. franciscanus*.

SUMMARY. A proposed salivary gland chromosome map for *Anopheles pseudopunctipennis franciscanus* is presented. The proposed map is compared with the nominative subspecies and the major areas of difference enumerated.

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