THE SALIVARY CHROMOSOMES OF *ANOPHELES FREEBORNI*

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INTRODUCTION. A considerable body of data is accumulating pertaining to the genetics and cytogenetics of mosquitoes. In *Drosophila* and other Diptera, one approach to the meaningful correlation of genetic and cytological data became possible with the discovery, utilization and mapping of salivary gland chromosomes. Excellent salivary gland chromosomes are present in mosquitoes, and salivary chromosome maps have already been prepared for a few species. For a review of the pertinent literature see Kitzmiller 1953, 1963.

Among the species of the genus *Anopheles* in North America are four, *freeborni, occidentalis, aztecus* and *earlei,* which are considered to belong to the *maculipennis* complex. The European members of this complex of sibling species have been thoroughly studied by Frizzi, who showed in a series of papers (for citations, see Kitzmiller 1953, 1963), that the various species could be separated by aberrations, as seen in the salivary chromosomes.

This paper, which proposes a "standard" map of the salivary chromosomes of *Anopheles freeborni,* is the first of a series aimed at the elucidation of the evolutionary relationships among the Anophelines of North America, studied from the viewpoint of cytogenetics.

MATERIALS AND METHODS. The stock of *Anopheles freeborni* used in these studies was derived from the stock maintained at The Christ Hospital Institute for Medical Research, Cincinnati, Ohio. Eggs were supplied through the courtesy of Dr. Leon H. Schmidt. This stock was originally obtained at Marysville, California, and is maintained in several laboratories in the United States (Ward and Kitzmiller, 1963). The colony is maintained in routine fashion and presents no particular difficulties in rearing. The salivary chromosomes were prepared according to the methods described by French, Baker, and Kitzmiller, 1962. The maps themselves were prepared by photographing suitable chromosomes, printing at a standard magnification, carefully measuring total length and various widths, locating characteristic puffs, and drawing diagnostic banded areas. Working from such a "skeletal" map, individual areas were mapped by careful study of each region under oil immersion (1000X).

The salivary gland chromosomes appear as three pairs of synapsed polytene chromosomes with characteristic banding patterns. A short X-chromosome and two longer autosomes are present in each salivary nucleus. (Fig. 1). The X-chromosome averages about 70 microns in length, chromosome II about 250 microns, and chromosome III, about 270 microns. The X-chromosome appears to have a terminal centromere. Chromosome II has arms of approximately equal length, while in chromosome III, the left arm is considerably shorter than the right, these arms averaging about 110 and 160 microns respectively. Thus all three chromosomes are immediately recognizable by size and proportion; the X is the shortest; chromosome II has arms of almost equal length and chromosome III has arms of clearly unequal length.

There is no chromocenter of the *"Drosophila"* type. Even with moderate pressure, the chromosomes tend to spread singly. However, there is no question but that the chromosomes are loosely attached to one another at the regions which we are calling the centromeres. These regions stain very differently from the rest of the chromosome, and under phase, generally exhibit phase reversal. In different preparations the degree of attachment is more or less intimate, seemingly dependent upon the pressure during prepa-

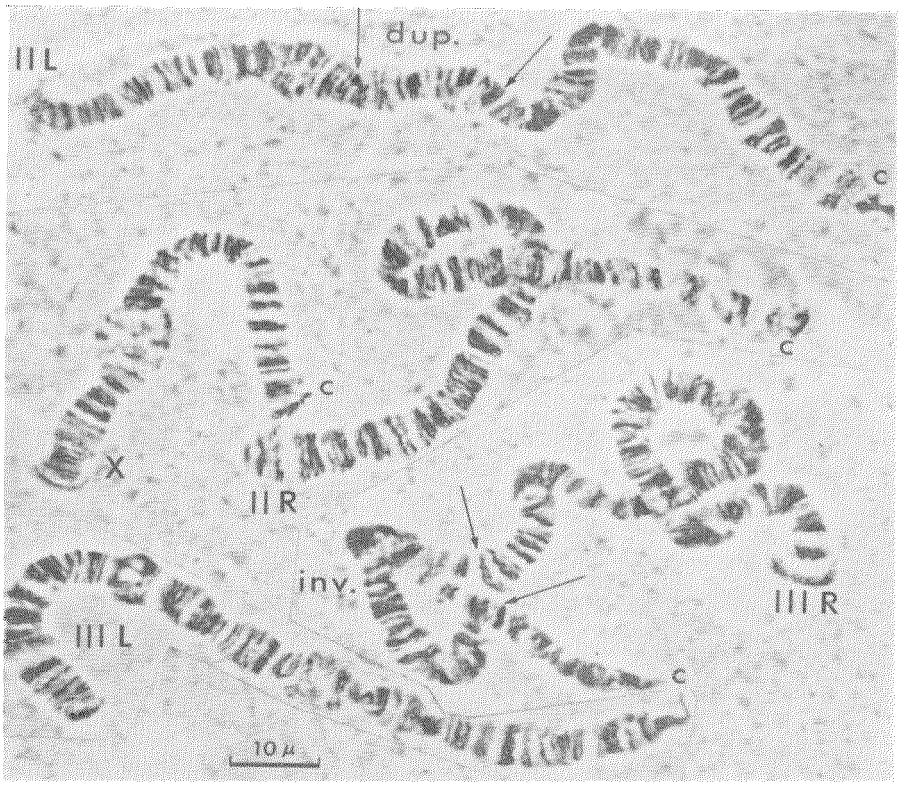

FIG. 1.—Salivary chromosomes of *Anopheles freeborni*. For details, see text, page 254.

ration. The terminal centromere of the X is usually attached to the centromere region of one of the autosomes, and sometimes all three centromere regions are seen to be joined.

A large nucleolus (Fig. 2c) adjoins the centromere regions. In well-spread preparations this nucleolus usually appears attached to the X-chromosome at the centromere end of the chromosome. The nucleolus is large, spherical, and averages about 50 microns in diameter. A dark staining body appears in the center of the nucleolus. In a few preparations, all three centromere regions appear to be attached to the nucleolus, although this may only be apparent, because of the proximity of the three centromeres. The nucleolus is most evident in preparations from 4th instar or earlier larvae; in later larvae, for example when the pupal trumpets have formed, the nucleolus usually disappears, leaving only a fine network of fibers connected to the chromosomes. Stainable chromosomal connectives which appear in smear prepartions are especially common in the region of the centromeres and nuceolus.

DESCRIPTION OF CHROMOSOMES. Following the usual arbitrary mapping

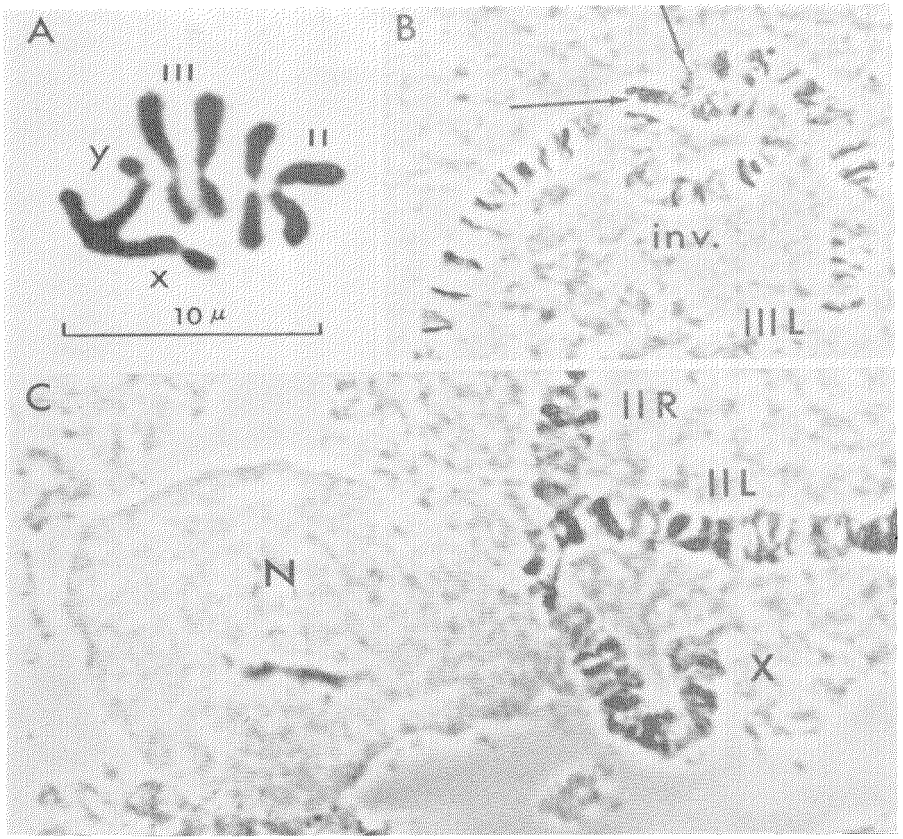

FIG. 2.—A. Metaphase chromosomes, testis. B. Inversion, III-L. C. Nucleolus. For details, see text, page 255.

techniques, the chromosome complement has been broken into five units, one the X-chromosome and the other four the four arms of the autosomes. See Plate I for these and other details mentioned in the accompanying text. We have generally followed Frizzi's (1947) scheme, starting with the X, then the right arm and left arm of II, finally the right arm and left arm of III. Regions in each chromosome have been designated by numbers, in a completely arbitrary fashion, although we have attempted to choose the regions so that they begin and end at clearly recognizable bands or areas. Within each numbered region, smaller zones have been designated by letters, again in an arbitrary fashion.

Since it was clear from the start that certain regions of each of the salivary chromosomes in *A. freeborni* were clearly homologous with certain regions in comparable chromosomes in *A. atroparvus*, we have designated "right" and "left" arms of chromosomes II and III to correspond with similar arms in Frizzi's map

of *A. atroparvus*. We have not attempted any correspondence in numbered or lettered areas between the two species.

Following the standard *Drosophila* usage, we have designated the arms of the autosomes as II-R, II-L, III-R and III-L rather than the D(dextral) and S(sinistral) used by Frizzi. In our maps of *A. freeborni* the numbered zones are as follows: X-chromosome, zones 1 to 5, beginning at the free end of the chromosome, and with zone 5 ending at the centromere; II-R, zones 6–14, ending at the centromere; II-L, zones 15–21, with zone 15 beginning at the centromere and zone 21 at the free end of the chromosome; III-R, zones 22–32 beginning at the free end and ending at the centromere; III-L, zones 33–39, beginning at the centromere and ending at the free end.

As discussed below, certain individuals show heterozygous inversions in chromosome III. Again, in a completely arbitrary fashion, we have selected as the "standard" chromosome arrangement for *A. freeborni* one of the two homozygotes of chromosome III. The other homozygote, the heterozygotes, and the location of inversions will be discussed below.

X-CHROMOSOME. Diagnostic features. The "X" may be readily recognized by size alone, being about one-fourth the length of either autosome. It is often attached to one or both of the autosomes, and to the nucleolus. The crescent-shaped, flared end (1A) and two series of three heavily staining bands (1A, 1B) mark the left end of the chromosome. A large puff with weakly staining bands is typical of 2C and 2D. Three heavy bands occur in 3C. A series of widely-spaced heavily-staining bands in 4C and 5A and 5B mark the right end of the chromosome.

Detailed description. Although individual bands vary somewhat in staining intensity from slide to slide, they are remarkably constant. Generally, we have attempted to designate four grades of staining intensities in bands, ranging from a heavy dark band to a thin, almost invisible one.

The left end of the X is characteristically flared with a heavy terminal band. This flared region is often broken by pressure, but usually may be recognized. Two series of three bands each are typical of regions 1A and 1B. A light area, usually puffed and containing light bands occurs in 1C. Two heavy bands in 1D and a 1–3 series in 2A mark the area distal to the main puff. This large puff begins with two sharp bands in 2B, ends with a heavy double in 2D. The puff itself is noticeably wider than the rest of the chromosome, appears diffuse, and contains a series of bands which usually stain weakly. Proximal to the puff, area 3A contains a pair of thin but dark bands and 3B a series of three bands which usually stain with a medium intensity. The three heavy bands in 3C are diagnostic. Zones 4A and 4B are characteristically lightly-staining and puffed. Area 4C contains two heavy bands usually single, each surrounded by an area which stains more darkly than the rest of the background of the chromosome. Area 4C ends with a heavy double band. A single heavy band marks the end of 5A, and a double heavy band occurs near the end of 5B. Two light, thin double bands occur near the centromere in 5C.

CHROMOSOME II. Right arm. Diagnostic features. Chromosome II may readily be recognized as the autosome with arms which are approximately equal in length. The right arm may be identified by one or a combination of the following areas. The end of the right arm is typically expanded into a broad bulb (6A, B) followed by two narrower puffs (6C, 7A, B, C). The bands in region 6 are generally diffuse; the most prominent are a single dark band in 6A and two thin singles in 6B. Two dark thin bands in 7B and a 1—3 series in 7C mark this puff. These characteristics are quite uniform for this end of the chromosome and will usually serve to identify it. Region 10 is also diagnostic, with a series of 4 dark heavy bands in 10A, a puff, and three heavy bands, in 10C. At the centromere end of the right arm, region 14 is usually dif-

fuse, lightly staining, often asynaptic, with one heavy band in 14B, flanked on either side by a diffusely staining area. A thick beaded band, more lightly stained, often appears in 14C.

Detailed description. The characteristic free end of II-R has been described above. A small puff with two weak bands in the center and a strong band at the end marks 8A. A series of heavy bands, the first three heaviest are seen in 8B and 8C. Region 9 stands out usually as a series of dark bands, usually in two puffs. Four dark bands, the last one darkest, occur in 9A, a pair of heavy bands in 9B, and a series of 3 dark bands in 9C. A heavy band in 9E followed by two lighter bands mark the end of region 9. The strikingly characteristic heavy banding of 10A, B, C has been described above. The rest of region 10, D, E and F, is variable, but is usually present as shown on the map, as an expanded region marked with four double bands followed by 3 single dark ones. Regions 11A and B are unusual in that they contain series of very thin, closely approximated bands separated by a clear space. If the stain is too heavy, these close bands often appear as two heavy areas. The wide puff in 11C and 11D is marked by a 2–1–2 sequence of heavy bands. The double bands in 12A almost always appear dotted, followed by a series of heavy bands in 12B and 12C. Five fairly dark, thin bands appear in 13A followed by two, thin, dark bands in a puff in 13B. The middle of the 13B puff is marked by a heavy double. Region 13C contains a single heavy band followed by three single, more lightly-staining bands. Region 13D often contains four heavy bands, although these are sometimes more diffuse. As described above, region 14 is often stretched, diffuse and lightly staining.

CHROMOSOME II. Left arm. Diagnostic features. The free end of the arm is readily recognizable. The tip is usually slightly flared with only one prominent band, a thin dark band in 21C. Although there are several other bands in region 21, they usually are thin and lightly staining, so that the entire region gives the impression of being lightly stained. Following in region 20 and in 19A is a series of heavy bands. This combination of a light tip with one heavy band, plus a heavy series, will identify this free end.

Another very characteristic region is a wide puff in 19B, C, and D, with a series of thin, medium intensity bands. This wide puff almost always can be recognized by three thin but heavy bands in 19C, of which the first is the heaviest. This puff always ends in a heavy double in 19D. A series of five evenly spaced heavy doubles in 17C followed by a lightly staining puff in 17D are good markers. The centromere end of this arm is easily recognized by a series of 4 dark areas in 15B and C, followed by a heavy broad double in 15D, near the centromere. All of these dark areas in Zone 15 are composed of several bands as shown on the map, but in most preparations these bands appear condensed so that one sees typically a 4–2-centromere series.

Detailed description. The flared tip (21A) contains a 3–1–2 series of medium, thin bands, followed by a pair of lighter bands in 21B. Following the diagnostic dark band in 21C are two pairs of lighter bands in 21C and D. Region 20 appears dark, due to the several heavy bands contained therein.

Region 20 is usually a puff, with a heavy double in 20A, ending in a heavy double in 20C, with a symmetrical series of bands between, as shown. The small puff in 19A usually contains three fairly prominent bands, the last of which is darkest. A pair of heavy bands marks 19B, and the characteristic puff in 19C and D has been described above. Region 18 is another puff, marked by two heavy bands in 18A, two heavy bands in 18B, and two lighter doubles in 18C. Region 18 is interesting in that in some slides it appears duplicated (see below). Region 17A usually contains 4 dark bands. 17B is normally a lighter, more diffuse puff, followed by a series of light bands, then the 5 prominent doubles

in 17C. Region 16 contains a series of heavy bands, which are, however, variable in different preparations. Dark bands in a 1–2–3 sequence are seen in 16E and 15A followed by a characteristic light space, then the 4–2-centromere series described above.

CHROMOSOME III. Right arm. Diagnostic features. Chromosome III is recognizable immediately by means of the differential arm length. The right arm is considerably longer than the left (160:110 microns) and in stretched preparations may appear even longer. Several diagnostic areas occur in this arm. The free end is typically fan-shaped, expanded, with two heavy dark bands (22A). A series of heavy double bands, evenly spaced in Region 22 and 23A, combined with the flared tip, are sufficient to recognize the end of the chromosome. The most prominent of these bands are a pair of thin dark bands at the end of 22A, a single dark band in the middle of 22B and a pair of dark bands at the end of 22B, two thin dark bands in 22C, three dark bands in 22D, and two very heavy bands in 23A. Another excellent area for identification of this arm is a series of dark bands in area 26. In practice, we use this area, plus the tip, as positive identification for this arm. Two areas stand out at once: a series of five closely-spaced heavy bands in 26D and a series of 4 or 5 heavy bands at the end of 26E. Between these two series, in the center of the puff, are three thin, more lightly-staining bands. This puff is an excellent point at which to start to identify the adjoining regions of the chromosome. Proceeding toward the end of the arm, a heavy band in 26C, two heavy bands at the end of 26B and another series of 5 heavy bands in 26A are excellent landmarks. In the other direction, two small puffs in 27B and 27C are diagnostic. Heavy bands at each end of 27B mark this puff; the 3 thin bands in puff 27C, followed by a dark band at the end of 27C, clearly identify this puff. Also very useful is the series of six dark, widely spaced bands in 28B and 28C. Of these the fifth (28C) is invariably the heaviest and darkest. This series is also very useful in that it marks one end of a common inversion. The centromere end of the arm is unmistakable, with an excellent series of bands. Starting at the centromere, and proceeding toward the free end of the arm, the sequence is as follows: two dark "dotted" bands, followed by a series of 4 lighter bands of which the two most distal are darkest; then a "dark-light—light-dark" sequence in 32B; a pair of very heavy bands at the end of 32A; then a series of four dark bands in 31A, B and C.

Detailed description. The diagnostic tip (Region 22) has been described above. Region 23 begins with two heavy bands, then a lighter one, and ends in a series of three thin, fairly dark bands. A series of 4 dark bands, followed by a pair of lighter ones, then three dark ones, characterize regions 23B, C and D. Most prominent in region 24 is a pair of dark doubles in 24B and C, although these do not consistently stain heavily. Another pair of dark doubles mark 25A and B. The puff in 25C is consistent, with the three light, thin bands as shown on the map. Regions 26, 27 and 28 have been described above. Regions 29 and 30 are likely to be least clear of any on this arm. They are the areas contained with a common inversion, and even in homozygotes often appear diffuse, stretched, twisted and indistinct. The puff in 29B and C, bounded by dark doubles is usually consistent, with two dark bands in its center. The three heavy bands in 30C and the series of heavy bands in 30D and E are sometimes run together, and may appear as two dark areas with little detail visible.

CHROMOSOME III. Left arm. Diagnostic features. This arm is impossible to miss. It is the shortest arm of the autosomes. A striking feature is the "dot and crescent" and "birdseye" in 35A. These bands, always darkly staining, appear as a dot, plus a curved band closely approximated to it. Detailed study shows the "crescent" or "parenthesis" to be com-

posed of a double band, plus another dark band as shown on the map in 35 A; at low power, or in somewhat condensed chromosomes this area typically appears as one dot and one crescent.

The tip of the arm is easily recognized. Typically it is squarish, not flared and with three dark, widely spaced bands in 39A and B. In 39C is a series of 4 heavy bands followed by 5 thin light ones. The puff in 39D is consistent, usually light with one heavy band. The three dark bands in 39E, two heavy ones in 38A and three heavy ones in 38B are typical. The puff in 37B is one of the most consistent features of the arm. It is bounded at each end by curved bands, is lightly staining, and contains indistinct banding. The three heavy bands following it in 37C are also typical.

Detailed description. The tip of the arm has been described. Region 38C is marked by a 2–3 series of heavy bands and 38D by a 1–1–1 series of dark bands, with the last two actually doubles. The diagnostic puff (37B) and the three dark bands in 37C are followed by a light puff with dotted bands in 37D and E, ending with a heavy double band. Region 36 consists of two broad puffs with heavy bands. Consistently the most heavy bands are at the ends of the puffs as shown. Following the dot and crescent in region 35A is a puff which ends in a series of dark bands (35C), followed by a sequence of five dark bands in 35D. Region 34 is usually wide with a series of dark bands in 34A and 34B and ending in a closely spaced series of dark bands in 34C. Region 33 also contains dark bands, usually widely spaced, as shown on the map.

ABERRATIONS. Approximately 300 slides of salivary chromosomes have been prepared and read from this stock of *Anopheles freeborni*. Thus far no aberrations of any kind have been found in the X-chromosome. In chromosome II we have found one duplication but no inversion. In chromosome III an inversion commonly occurs in the right arm; a rare inversion, found only once thus far, in the left arm.

Inversion III—R. This is a paracentric inversion occurring within the right arm. It begins at region 29B and continues through region 31A. We have not calculated the frequency in a random sample from our strain, but in the slides examined, it occurs with a frequency of about 50 percent. This inversion is shown in Figure 1. Both homozygotes are present in the population in the laboratory, as well as the heterozygotes. The relative frequencies of the homozygotes and the heterozygotes have not been investigated.

Inversion III—L. This paracentric inversion begins in region 36A and extends to the centromere (Fig. 2B). It has been recovered only once in two years. One homozygote only has been seen in this stock. Nothing is known about the relative frequencies of the heterozygotes and homozygotes.

Duplication II—L. This is an interesting duplication of regions 18A and 18B in the left arm of chromosome II. It involves a series of 9 bands in an obvious puff (Fig. 1, Fig. 3). The bands are in

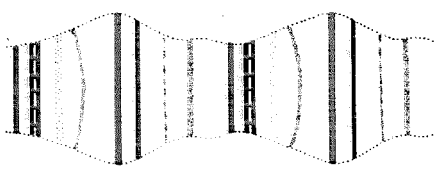

FIG. 3.—Duplication, Regions 18A, 18B.

the same sequence in the duplication. It has been seen in fewer than 10 slides, but when present is unmistakable.

DISCUSSION. Frizzi and DeCarli (1954) compare the salivary chromosomes of *A. freeborni* with those of *A. atroparvus*. They note the general agreement of the salivary complement in the two species, and make several detailed observations concerning areas which seem to be homologous. From their drawings certain areas of *freeborni* as pictured, are very similar to our maps; other areas are less securely homologous. The right end of the X compares favorably, as do in general the

ends of the autosomes. III—L is very close to their drawing and III—R compares well along most of the length. It is more difficult to compare our maps of II with their drawings.

It is of interest that the two inversions described by Frizzi and DeCarli agree almost exactly in location with ours. We are convinced that we have the same inversions as described by them. Their stock of *freeborni* was obtained from Rozeboom; this is in all probability the same stock which we have (Ward and Kitzmiller, 1963). One difference seems to be in the frequency of the inversion in III—L. We have found this only once; evidently it was quite frequent in their stock.

In addition to the homologies of certain areas between *freeborni* and *atroparvus* mentioned above, there are certain clear homologies with other species. III—L is, as far as we can tell, identical or very similar in *atroparvus, freeborni,* and *punctipennis.* III—L in *earlei, crucians, aztecus,* and *occidentalis* are also very similar to *freeborni.* Preliminary observations clearly indicate the similarities.

The mitotic and meiotic chromosomes of *A. freeborni* consist of one pair of sub-telocentric heterosomes, and two pairs of metacentric autosomes (Fig. 2A). One pair of autosomes has arms of approximately equal length; we consider this to correspond with salivary chromosome II which also has approximately equal arms. The other pair of autosomes clearly has one arm shorter than the other; this corresponds well with salivary chromosome III. The X-chromosome in the mitotic configuration is as long as either autosome. Yet in the salivary preparations, the X is very much shorter, only about one-quarter the length of the autosomes. It is possible that the salivary X corresponds only to the shorter arm of the mitotic X, and that the longer portion, presumably heterochromatic, of the mitotic X, does not appear in salivary preparations.

SUMMARY. Salivary chromosome maps are presented for *Anopheles freeborni.* All chromosomes are mapped as homozygotes, and it is proposed that this be used as the "standard" map for this species. Supported in part by grant E 3486 USPHS.

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CORRECTION

In the paper entitled "The Anatomy of a Naturally Occurring Sterile Adult Female *Aedes aegypti* (Linnaeus)" (Vol. 23, No. 2, June, 1963, page 165) by Jack Colvard Jones, line 16 in Column 1 continues in Column 2, lines 1 through 11. Also, line 24 in Column 1 continues in Column 2, lines 12 through 26. The author would also like to add that Spielman (Spielman, A., 1957: "The inheritance of autogeny in the *Culex pipiens* complex of mosquitoes." Amer. J. Hyg. 65:404–425) was apparently the first to report cases of ovarian atrophy, "apparently without developed follicles," in a mosquito *(Culex pipiens).* He found 51 cases of bilateral ovarian atrophy out of 6,000 females (see his paper, page 409).—J. C. J.