THE OVARIAN NURSE CELL POLYTENE CHROMOSOMES OF *ANOPHELES QUADRIMACULATUS*, SPECIES A

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ABSTRACT. Nurse cells in the ovaries of adults of *Anopheles quadrimaculatus*, Species A, were used to prepare a polytene chromosome map. The chromosome quality is superior to that of salivary glands, and it is easier to use adults rather than larvae for cytological analysis of field populations. The most reliable homologies between the salivary and ovarian maps are located in the distal ends of the respective arms, and one homologous region is a prominent landmark in all of the members of the nearctic Maculipennis complex and related species. The left arm of chromosome 3 is uniquely dimorphic. The homokaryotype for $3L_1$ is synonymous with 3L of the published map of salivary gland polytenes. The 3L heterokaryotype is mostly asynaptic, except for two small homologous, synaptic areas, one of which is inverted. Each homokaryotype contains a unique, diffuse puff that is adjacent to the centromere.

INTRODUCTION

Past studies on the cytogenetics of *Anopheles quadrimaculatus* Say included a map of the salivary gland polytene chromosomes (Klassen et al. 1965), comparisons of homologies of polytene banding patterns among *An. quadrimaculatus* and other species of the Maculipennis complex (Kitzmiller et al. 1967), and descriptions of a complex arrangement of the left arm of chromosome 3 that was observed in both laboratory and field-collected mosquitoes (Frizzi and DeCarli 1954, Kitzmiller and French 1961, Mason and Brown 1963, Klassen et al. 1965, Kreutzer 1975). The presence of two sympatric species of *An. quadrimaculatus* was recently confirmed through the use of isozyme analysis, cytogenetic study, and hybridization crosses to analyze the genetic structure of field populations (unpublished data). Evidence from these investigations also demonstrate that the complex arrangement on 3L is the heterokaryotype of a unique chromosomal dimorphism for that arm.

In this paper we describe the polytene chromosomes in the nurse cells of the ovaries of *An. quadrimaculatus*, Species A. As previously demonstrated (Coluzzi 1968, Saifuddin et al. 1978), the polytene chromosomes in the nurse cells of anophelines are of excellent quality, and preparations are more reliable in comparison to salivary gland chromosomes. This map should prove to be a useful tool in elucidating the nature and cytological features of the sibling species complex, and it may also help in the delineation of the dimorphism for 3L.

MATERIALS AND METHODS

The primary source of *An. quadrimaculatus*, Species A, for this paper was the ORLANDO strain, which has been maintained in our laboratory for ca. 40 years, but field-collected material from Gainesville, FL and Lake Seminole, GA was also used. Females 3–5 days old were fed on a guinea pig which initiated ovarian development, and blood-fed females were held at 25°C for 27 hr and then the ovaries were dissected. Chromosomes were prepared by the method of Saifuddin et al. (1978), except for the slight modification of transferring the ovaries to 75% acetic acid instead of 45% which enhanced chromosome spreading. Staining and swelling of the chromosomes were frequently improved by floating the coverslip with 45% acetic acid and resquashing. The chromosome map was prepared by observing ovaries with phase contrast microscopy (900×) under oil immersion and with standard-magnification photographs (1562×).

RESULTS

The polytene chromosomes of the ovaries usually appear as three members, without chromocentric attachment. The centromeres of each chromosome are usually diffuse and the left and right arms are often separated at the centromere during slide preparation. The chromosomes are distinguished by different length and distinctive banding patterns (Fig. 1). The X chromosome is the shortest. Chromosome 2 is of intermediate length, and chromosome 3 is the longest. Similarities exist for the banding patterns of the autosomes within the subgenus *Anopheles*, and Klassen et al. (1965) labeled the autosomal arms of the salivary gland chromosomes of *An. quadrimaculatus* accordingly. Since the salivary and ovarian polytene chromosomes also have similar patterns, the zone designations (1–39) used by Klassen et al. (1965) were incorporated in the map of the ovarian polytene chromosomes (Fig. 2). Where similarities existed with the salivary map, the same zone and region notations were used, and where the banding patterns were different the zone and region designations were arbitrarily based on landmarks.

The polytenized euchromatin of the X chromosome is ca. 71 microns (mu) long (unless otherwise indicated, all measurements of chromosomes, or portions thereof, indicate length) and occupies zones 1–5. Chromosome 2 (373 mu; zones 6–21) has arms of nearly equal length, with the right and left arms being ca. 201 and 172 microns, respectively. Chromosome 3 is very unique because of an unusual polymorphism in the left arm (Figs. 1 and 2). The right arm is quite long (277 mu; zones 22–32). The two dimorphic homokaryotypes of the left arm are readily identifiable, and $3L_1$ is 168 mu long and corresponds to chromosome 3L, zones 33–39, drawn by Klassen et al. (1965). The other homokaryotype, $3L_2$, is 189 mu and has not been represented diagrammatically in the literature; its zones (40–46) and regions were drawn arbitrarily. The most useful diagnostic features of each arm will precede the detailed descriptions.

X CHROMOSOME. The X chromosome is rather short in comparison to the other arms. The best diagnostic feature is an unusual puff that occurs in 3AB. There is a similar puff in salivary polytenes too, but it is located in 1B. Another useful feature is the constriction and dark bands in 4B. The centromeric end is often diffuse and appears to be attached to the nucleolus. The X chromosome is rarely associated with the other arms.

The terminal bands in 1A are usually diffuse; the next pair of bands are distinctive and the dark band preceding the puff in 1B is a reliable landmark. The puff of 1B has a diffuse band on each end. A constriction lies in 1C that is marked by a heavy dark band. Region 2A contains a series of small bands which sometimes mimic a single dark band and 2B has a single heavy band preceded by a doublet of thin bands. A lone dark band is in 2C. The large diffuse puff in 3AB is banded, but the exact nature of the pattern is not clear in all specimens. The two medium bands located proximally in 3B are usually clear as are the three medium bands of 3C. Region 4A consists of a dotted pair of bands followed by a medium band. A good pair of dark, diagnostic bands are located in the constricted area of 4B and often appear as a broad single band. Three very heavy bands follow in 4C. Region 5A is distinctively marked with a grainy band, a medium band and a dark band. The small puff in 5B is crossed by two dotted bands and followed by 4 medium-to-light bands. There are three heavy bands in 5C but the proximal part of this region is often diffuse.

CHROMOSOME 2. 2R. The right arm of chromosome 2 is slightly longer than 2L and is more recognizable for its lack of good diagnostic features, especially in zones 6–9. The most reliable landmark on the distal end of 2R is the pair of dark bands followed by a triplet of light bands in region 7B. In the middle of the arm in 10B there is a constriction that includes two heavy bands. Zone 13 contains two useful landmarks. A pair of very dark bands are in 13A and an elongated puff that is crossed by 3 heavy bands is in 13D. Zone 14 appears asynaptic in many field populations.

The dark terminal band of 6A is usually reliable, but it may be diffuse which can cause confusion with 2L. Regions 6A–D, which contain many light, broken bands, are in general void of consistent landmarks; the proximal medium band in 6D is usually clear. There are four medium bands in 6E followed by a medium band in 6F. The small puff in 6F normally appears grainy with a diffuse band proximally situated. There is a medium band in 7A. The pair of dark bands followed by a triplet of thin bands in 7B is a good landmark. Region 7C contains a heavy band and 8A has an elongated puff with 5 medium bands. The regions 8B–9C are often enlarged and diffuse with indistinct banding patterns. Region 8C contains 2 doublets of medium bands and a wide, diffuse band. When readable the two dark bands in 9A are useful and they are followed by a series of thin bands in 9B that usually appear broken. There are two medium and one light band in 9C. Beginning with 9E, the remaining zones have more distinct bands. In 9E in an elongated, clear puff there is a doublet of bands that often appears as a single; it is followed by a pair of medium-to-heavy bands. There is a pair of medium bands and a broad, diffuse band in 10A, and they are followed by a constriction with two very dark bands in 10B. The large clear puff of 10CD is distinguished by three doublets and one triplet, all of which may appear rather dark. The wide band in 10E usually looks dark and grainy. The two series of medium bands in the small puff of 11AB are good landmarks. Regions 11B–D contain four unusual, wide, grainy bands that are similar and consistent features. There is a dark band in 12A and a puff crossed by two medium bands in 12B. The constriction in 12C has a heavy and a medium band that is followed by a pair of broken bands. The two very dark bands in 13A are usually the most heavily stained of 2R. In 13B there is a small, elongated puff with a pair of medium bands, a solo medium band and then a doublet of medium bands. The 13C region contains two triplets, one of broken and one of thin bands, respectively. The elongated puff of 13D has three very dark, widely-spaced bands that are useful for distinguishing 2R from 2L. Region 14A has a single medium band and several light, broken bands; 14B contains two pairs of medium bands. The region adjacent to the centromere, 14C, is usually stretched and

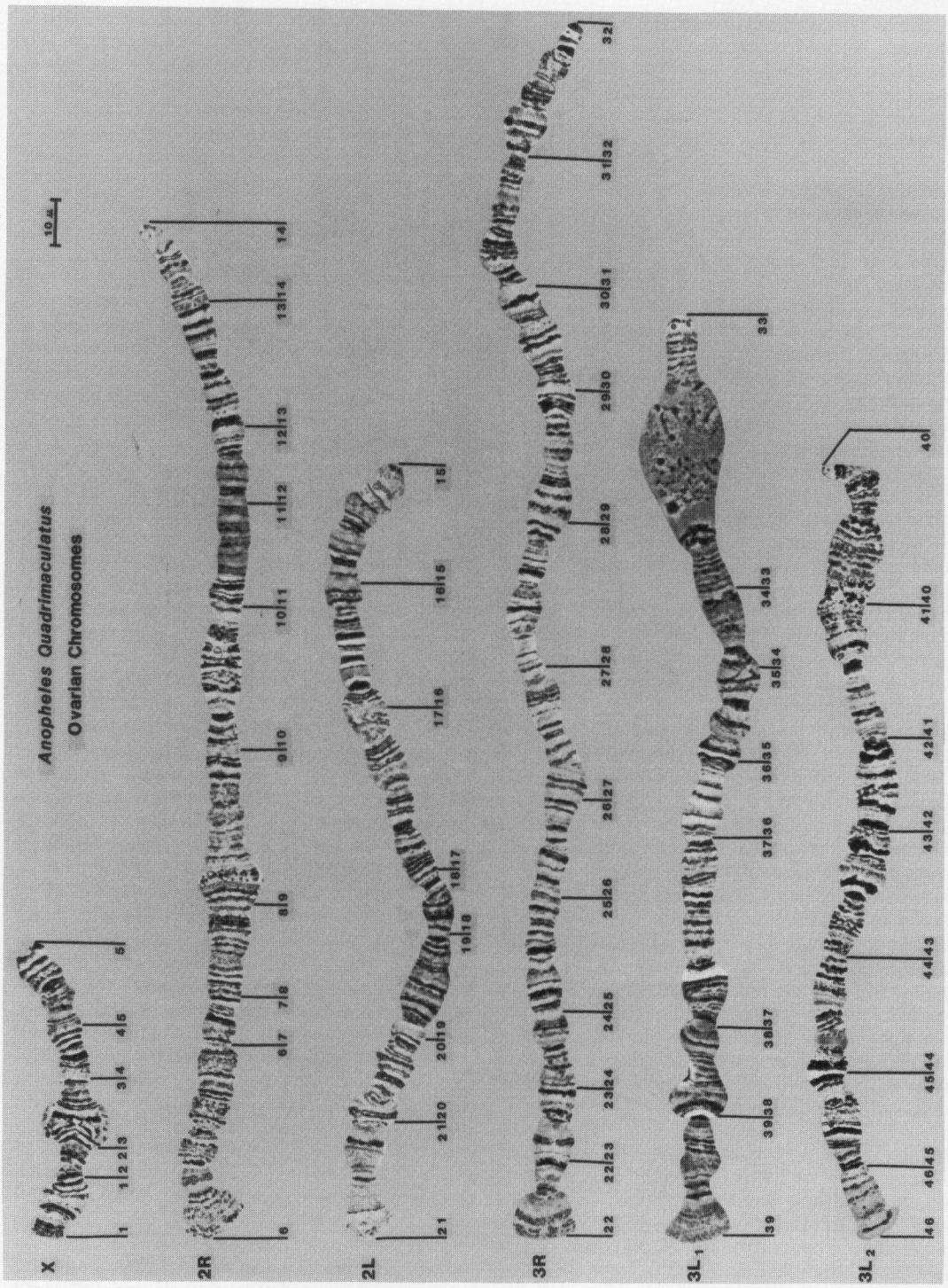

Fig. 1. Composite photographic map of polytene chromosomes from the ovarian nurse cells of *Anopheles quadrimaculatus*, Species A.

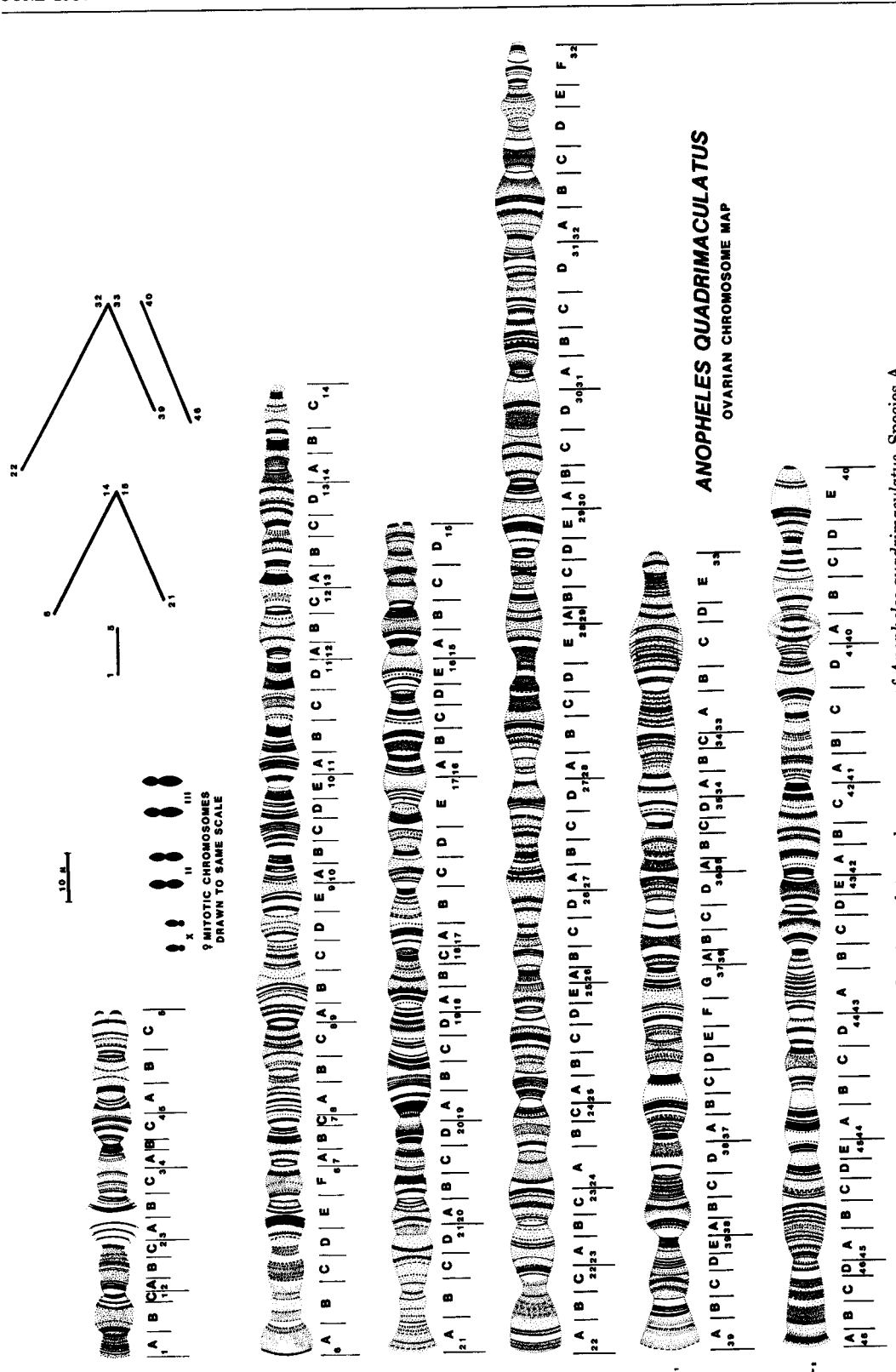

Fig. 2. Ovarian polytene chromosome map of *Anopheles quadrimaculatus*, Species A.

distorted. The distal, solo band is readable but the other bands may be too diffuse.

2L. The similarities between the salivary and ovarian chromosomes in the distal end of 2L in zone 21 are striking. The best feature of this zone is the dark band in the large 21D puff. The middle of the arm is well marked with 3 dark bands in 19D and a very heavily banded constriction in 18C. The proximal end of the chromosome is distinguished by two landmarks. The constriction in 16A is crossed by a very dark band and the puff that follows in 16B contains a broad, shaded band and a dark band. The constriction in 15A has a pair of dark bands that is followed by a clear area and another dark band. A common inversion occurs in zone 15 and this zone is asynaptic in many field populations.

The medium band of the 21B constriction and the dark band in the puff of 21D are the best landmarks of zone 21, otherwise, the numerous broken bands of this zone are not consistent. There is a doublet of medium bands in the puff of 20A and the proximal constriction in 20B contains a pair of heavy bands. The 20C region has a doublet of thin bands, then a heavy band, and then another thin doublet. The constriction of 20D contains a dark band; the distal portion of the small puff has a medium band and a grainy area. The large bilobed puff of zone 19 is usually a distinctive feature. The distal edge of the puff is marked by a lightly-banded grainy area. This is followed by two heavy bands that are separated by a wide, clear area. In 19C there is a dark band and also a pair of medium bands. Region 19D is easily recognized by the three heavy bands that cross this lobe of the puff. The puff of 18AB usually looks dark and grainy. It is crossed in 18A by a duo of medium bands and by a dark band that may appear diffuse. The heavy band of 18B is distinct and is followed by a medium band. The broad, dark band in the constriction in 18C is a good landmark; it is followed by a thin doublet. The elongated puff of 17AB has a medium band that is followed by a trio of medium bands. The doublet of dark, broken bands traverses the middle and is followed by a constriction with a triplet of medium bands. The 17C puff has a pair of thin bands and a pair of medium bands. The constriction in 17D shows a medium band followed directly by a thin doublet. The puff has a broken band with a medium band on either side. The broad, grainy band in 17E is unusual and is bordered on both sides by a thin band. Region 16A includes a trio of medium bands and a constriction with a very dark band. The puff of 16BC begins with a broad, gray band and a doublet of a heavy and light band, respectively. This is followed by a pair of light bands, one of which is broken, and a very dark band. The 16D region contains a constriction with a dark band that is flanked on both sides by a pair of light bands. The puff in 16E is crossed by a pair of medium bands that enclose a prominent, dotted band. There are two heavy bands in the 15A constriction that are followed by a clear area and then a dark band. The exact nature of the banding patterns in regions 15B–D is usually difficult to ascertain. There are several dark bands in 15B; 15C contains a medium-dark band and a series of lighter bands, and 15D has several pairs of light bands.

CHROMOSOME 3. 3R. Arm 3R is easily distinguished from the other arms by its length and club-shaped distal end. The banding sequences of this arm are important because of the inversion polymorphisms that exist in field populations. There is an important sequence of bands in 24C–25A that begins with a medium band, which is followed by a quadruplet of light bands. The next small puff has a trio of bands, then a dark band, then two light and two medium bands. Regions 26C–27A contain a series of six dark bands, two dark bands per region. The large puff in 29E is crossed by a conspicuous sequence of bands that is comprised of a quadruplet of medium bands, a clear area and another medium band. This landmark is followed by two prominent heavy bands in 30C. The superior feature of the centromeric end is the large puff of 32AB that is bisected by a pair of dark bands.

The bands of the "club" in regions 22AB are usually diffuse although the solo medium band at the base of the club in 22B is often discernible. The dark band near the constriction in 22C is the most reliable landmark of this zone. The puff of 23A is crossed by a pair of medium bands that may appear broken. The distal part of the 23C puff has a light, grainy band that is followed by a prominent, dark band. A clear area precedes a medium band in 24A, and further down the puff is a grainy band and then a medium band. The constriction in 24B contains a heavy band. The regions of 24C–25A begin with a medium band followed by a quadruplet of light bands. A clear space in the constriction precedes the three light bands and one dark band in 25A, and this is followed by a quadruplet of two light and two medium bands, respectively. A light area precedes the medium band in 25B and the constriction contains two light bands. The puff in 25C begins with a medium band and is bisected by a prominent, dark band that is followed by a thin band. Region 25D, which often is clear, contains a medium band that precedes a constriction which includes a doublet of thin bands. The small puff in 25E has a medium to heavy band and a series of dotted bands. The constriction in 26A contains several dotted and broken bands as well as a heavy band. 26B has a triplet of

light to medium bands that is a good landmark. Regions 26C–27A each contain a pair of heavy bands so this sequence of six dark bands is easily recognized. Region 27B includes three diffuse bands. Region 27C contains a triplet of thin bands, a medium band and a thin, diffuse band. The puff in 27D has a trio of medium bands that may appear diffuse. Region 28A contains three light to medium bands. A puff encompasses 28BC; the distal half includes several thin and broken bands, and the center is bisected by a prominent, dark band flanked by two thin bands. A wide, grainy area that contains several thin bands is located in 28C. A very dark band, a light band and a wide grainy area are within 28D. Region 28E, which contains the distal half of a prominent puff, is noteworthy for its pair of heavy bands that encompass a clear area. The proximal side of the puff, in 29A, contains a heavy band followed by a grainy band. (This puff is often enlarged which diffuses the banding.) The 29B region has a triplet of medium bands. The constricted area that occupies 29CD is marked with two pairs of dark bands, the first of which usually appears diffuse. The puff in 29E is easily recognized by the quadruplet of medium bands that is followed, after a clear space, by another medium band. The sequence of seven bands that border the constriction of 30AB is also a good landmark. The elongated puff of 30C is crossed by two heavy bands. The grainy area in 30D includes several dotted and light bands and it is followed by a dark band that is a consistent feature. In 31A the constriction has a pair of dark bands. The elongated puff of 31B contains a light band, a heavy, diffuse band, and a trio of light bands. Region 31C includes a doublet of medium bands, a heavy band followed closely by a light band, and then another doublet of a light and a medium band, respectively. Region 31D contains a dark band flanked on both sides by a light band; this is followed by a light, diffuse band and a pair of light bands. The prominent puff of 32AB begins with a dark band at the distal base. The center of the puff is bisected by a broad, grainy band, a wide, clear area and a doublet of dark bands. Another wide, grainy band covers much of the proximal side of the puff. The 32C region contains a small, dark puff that is bridged by three medium bands. A medium band, a pair of thin bands, and several broken bands follow in 32D. Two doublets of light bands occupy 32E and three heavy bands can usually be seen in 32F, although this region is frequently stretched.

3L. There are several similarities between the salivary gland map and the ovarian map ($3L_1$) worth noting: 1) the series of double bands in 37BC; 2) the two pairs of dark bands in 38AB (salivary) correlates to the same in 39E–38A (ovarian); 3) a pair of heavy bands appear in 39B of both maps.

The first two areas of correlation are noteworthy because they also are the two areas of homology between $3L_1$ and $3L_2$ of the ovarian map. The initial homologous area is comprised of a series of bands in 37A–D and 44A–45D of $3L_1$ and $3L_2$, respectively. The second area consists of the constriction and dark band at 39E and 43B of $3L_1$ and $3L_2$, respectively. Although other banding sequences on the two arms may show a likeness, the ones cited above are the only two that synapse in the ovarian polytenes of the heterokaryotype.

$3L_1$. The two homologous areas previously mentioned are reliable landmarks. The two heavy bands in 36D–35A that are separated by a clear section are usually distinctive. The large, clear puff of 35D–34A, with its five dark bands, is a good area of recognition. The very large, diffuse puff in 33C is the most obvious landmark of this arm.

The terminal, broken band in 39A is frequently not discernible and the remainder of this region is grainy and diffuse. The two dark bands in 39B are easily recognized and distinguish $3L_1$ from $3L_2$. The 39D region contains a trio of medium bands and a light band. The small constriction with the very dark band in 39E and the large puff that is crossed on the distal end by a medium and heavy band in 38A represent a striking "dot and crescent" landmark. The center of the puff, in 38B, is bisected by a diffuse band. Region 38C contains a sequence of eight bands. Region 38D encompasses a very wide, clear area that is bisected by a heavy band; this is followed by a wide, grainy band. The distinctive 37A region includes a medium band, a trio of light bands, a medium-heavy band, a grainy band, and another light band. Regions 37BC are unique because of the wide, clear section that is encompassed between the two pairs of dark bands. There is a dotted band in 37D that is flanked on both sides by a medium band. A wide, grainy band, two broken bands, and a pair of light bands follow in 37E. A heavy band and a pair of light bands occupy region 37F. Region 37G contains two thin doublets, which may appear fused or broken, and a constriction that contains a heavy band. The 36A region has a light pair of bands and a medium band. The wide, grainy band in 36B has a solid band on its distal edge. Region 36C is a clear region that is crossed by a dark band and a doublet of a medium and a light band, respectively. Regions 36D–35A have a good landmark in the puff that is crossed by two dark bands, which are separated by a wide, clear area. The adjacent puff is traversed by a light-to-medium doublet. The constriction in 35C has a thin band

followed closely by a very dark band. The clear puff that occupies 35D–34A is crossed by two dark bands on the distal side, between which lies a broken band. Another broken band that crosses the proximal portion of the puff precedes a heavy band; a duo of medium bands lie at the base. Regions 34BC contain several thin bands and several medium bands that are often diffuse, and culminate in a doublet of medium bands. The eight bands in 33A are: two medium bands; a light, diffuse band; a pair of narrow, grainy bands; two medium bands; and another narrow, grainy band.

The area within regions 33B–D is quite unusual and is comparable to the large, diffuse puff in region 40A of $3L_2$. The exact nature of the banding pattern in the puff is unclear, however, the distal side appears to have a pair of medium bands followed by a heavy band. The center may contain several pairs of light bands, but, these are always very diffuse. The proximal side includes two heavy bands. The final region, 33E, contains eight bands and is usually stretched toward the centromere. The last band, which is fairly dark, is usually diffuse.

$3L_2$. The heavy band in the terminal region helps to differentiate the two homokaryotypes. The next two diagnostic areas are the regions that are homologous with $3L_1$ (45D–44A; 43B). The series of heavy bands in the elongated puff of zone 42 are quite characteristic. The two dark bands in 41C are prominent landmarks. The diffuse 40A puff is a unique feature, and it also may appear as a large, well-defined, gray puff or a heterochromatic constriction.

A clear, distal band that is closely followed by a dark band distinguishes the terminal region of this arm. The remainder of the elongated puff in zone 46 is crossed by three wide, grainy bands, and the latter two each terminate with light bands. The elongated puff in zone 45 is usually difficult to diagnose. The constriction in 45A has a dark band which is followed by several light and medium bands; these culminate in the center of the puff with a large, dark band. Region 45C contains a clear space, a pair of light bands and two diffuse, broken bands. The 45D region has a diffuse, medium band followed by a trio of medium bands. Regions 45E–44A contain a wide, clear section that is bordered on both sides by a pair of heavy bands. The latter pair of heavy bands is followed by a light band, a grainy band, and a medium band. The distal portion of 44B is unique because it usually appears gray and featureless; it is followed by two light bands and a dark band. The small puff in 44C has a wide, grainy band on its proximal side, and there are two dark bands and a light band in 44D. The elongated puff in 43AB contains many bands. The first two are diffuse and medium, respectively, and they are followed by a doublet of light bands, three medium bands, and a light band. A medium band and a thin doublet follow, and the puff culminates in a very narrow constriction that is crossed by a large, dark band. This constricted band and the heavy band on the distal side of the puff in 43C represent the "dot and crescent" landmark of this arm. The 43C puff is bisected by a wide, grainy band and another grainy band is located on the proximal side. The constriction in 43D has a dark band, after which is another puff that begins with a clear area and a dark band. The center of the puff in 43E appears grainy. The elongated puff in zone 42 is bridged by six very dark bands which makes this an excellent landmark. Also of note in this puff is the medium, grainy band in 42B that precedes the dark band. Several faint bands precede the doublet of the light band and dark band, respectively, in 41A. Region 41B contains several dotted bands and a light band and 41C has a very heavily banded constriction that is followed by a thin doublet and another heavy band. There are approximately seven medium and light bands in 41D that approach the large, diffuse puff of 40A. This puff, which corresponds to the one in zone 33, possibly contains 2–3 dark bands. There is a pair of light bands, a dark band and a wide, grainy band in 40B. Region 40C contains a thin, a medium and two heavy bands. The constriction in 40D has a triplet of light bands, and this is followed by a grainy band and a thin doublet. The last region, 40E, is usually diffuse, although it appears to begin with two heavy bands.

DISCUSSION

With the exception of the X chromosome, the salivary gland polytene chromosomes are somewhat shorter than the ovarian chromosomes (Table 1). Klassen et al. (1965) stated that the length of the two 3L homokaryotypes were 158 and 103 microns, respectively, and they used the longest of arms for their diagrammatic map. Their standard 3L is analogous to our $3L_1$, but the explanation for the discrepancy between the

Table 1. A comparison of the lengths of the respective arms of salivary gland and ovarian polytene chromosomes of *Anopheles quadrimaculatus*, Species A.

Arm	Salivary gland	Ovary
X	72*	71
2 R	160	201
2 L	147	172
3 R	234	277
3 L1	158	168
3 L2	103	189

* Measurements in microns.

length of their salivary gland $3L_2$ and the ovarian $3L_2$ is unclear.

The dissimilarities in the banding patterns, staining intensities of bands and location of puffs between the salivary and ovarian polytenes are numerous, so we recommend that due consideration should be given before making comparisons between the ovarian chromosomes and the salivary chromosomes of other Maculipennis species. However, there are some likenesses between the two maps that are not just coincidental. Homologies in the salivary gland chromosomes of *An. quadrimaculatus* and the other species of the nearctic group of the Maculipennis complex have been compared and discussed at length by Klassen et al. (1965) and Kitzmiller et al. (1967). They concluded that the best homologies are on the terminal ends of the respective chromosome arms. There are many similarities between the salivary and ovarian chromosomes of *An. quadrimaculatus*, and the most analogous regions also lie in the respective ends.

Region 1A of the salivary X resembles its ovarian counterpart, but this if often distorted by the large puff in 1B. Zone 6 on 2R demonstrates the fewest similarities of the different arms because of the terminal flair in 6A (that appears much like 3R but is not represented diagrammatically) and the dark band in 6B of the salivary chromosome which is not present on the ovarian 2R. On the other hand, the terminal ends of 2L are similar for the salivary and ovarian chromosomes. The terminal end of 3R is flared in the salivary chromosome whereas it is club-shaped in the ovarian; however, both have dark bands in region 22C so the homology is apparent. The distal band of zone 39, $3L_1$, of the salivary map is frequently indiscernible, and the bands in 38AB correspond to the "dot and crescent" in $3L_1$. The bands in 39B are similar for both maps, and the puff of 37BC and the series of bands in 37G–36A of the salivary map closely resemble the respective regions of the ovarian map.

The constriction and puff of 37AB of the salivary map, which appears in our salivary preparations more like the constriction and puff of 39E–38A and 43BC of the ovarian map, is the most common landmark among the Maculipennis (nearctic) species. The corresponding map regions for these species are: *Anopheles freeborni* Aitken, *Anopheles aztecus* Hoffman, and *Anopheles occidentalis* Dyar and Knab, 36E–35A; *Anopheles earlei* Vargas, 38D–37A. This same polytene configuration can be found in other, less related North American species such as *Anopheles punctipennis* Say and *Anopheles perplexens* Ludlow (36E–35A), and *Anopheles atropos* Dyar and Knab and *Anopheles walkeri* Theobald (36C–35A). Kitzmiller et al. (1967) referred to this reoccurring feature as the "dot and crescent" or "birdseye."

Klassen et al. (1965) described a "loop" on $3L_1$ in zone 33, which is near the entromere, but they refrained from calling it an inversion. Kreutzer (1975) reported two paracentric inversions on 3L in field collections from southeast Florida. Although we frequently observed the "loop" in salivary polytenes, we have not seen a loop or an inversion on a 3L homokaryotype in the ovarian polytenes in either laboratory or field material (unpublished data). However, there could be an analogy between the loop in salivary polytenes, which has an association with the chromocenter, and the diffuse puff in ovarian polytenes, which appears to have a connection with the nucleolus. We have observed different forms, either a diffuse puff (Fig. 1), a well defined puff, or a heterochromatic constriction, of region 40A in ovarian preparations of $3L_2$.

References to the dimorphic arms of 3L have been cited several times during the past 30 years (Frizzi and DeCarli 1954, Kitzmiller and French 1961, Mason and Brown 1963, Klassen et al. 1965, Kreutzer 1975), but unfortunately, there has been no determination to study this unique polymorphism in *An. quadrimaculatus*. Ironically, 3L has always been considered the most conservative of the arms in the Maculipennis complex. The literature references indicate that one of the homokaryotypes is much more prevalent than the other; however, we have found approximately equal numbers of each 3L karyotype in laboratory and field populations. As reported previously, we did observe a predominance of heterokaryotypes which indicated the populations were not in a Hardy-Weinberg equilibrium (unpublished data). As usual in the Maculipennis complex, arm 3R contains the most inversion polymorphism. We observed three commonplace paracentric inversions on 3R (Species A) that were observed in several field populations; homozygotes for each inversion were also seen. We also saw a small paracentric inversion at the base of 2L in both laboratory and field material which corresponds to the asynaptic region 15C of the salivary map.

Cytological studies of field populations revealed two different karyotypes in adults, both of which were identified as *An. quadrimaculatus* with the taxonomic keys available for North American anophelines. Notable differences between the karyotypes were observed for the X, 2L and 3R; the variations involved both fixed and floating inversions. Hybrid crosses produced individuals with the type of polytene asynapsis and the degree of sterility that is consistent with that of crosses between sibling species (unpublished data).

The nature and quality of the ovarian polytene chromosomes in *An. quadrimaculatus* make them the preferred material for cytogenetic analysis. Also, since it is easier to collect wild anopheline adults than larvae, it facilitates the study of field populations by cytological methods. Other nearctic anophelines may also be suitable for study with this technique, and preliminary examinations of the ovarian polytenes of *An. freeborni* and *An. punctipennis* tend to confirm this. The existence of a sibling species complex and a rare chromosomal dimorphism in *An. quadrimaculatus* may hopefully provide inducement for further genetic investigations on this important mosquito, and the ovarian map should prove useful in such studies.

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