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THE CHROMOSOMES OF *ANOPHELES CULICIFACIES*

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ABSTRACT. A polytene chromosome map has been prepared from the ovarian nurse cells of female adults in *Anopheles culicifacies*. These chromosomes are distinct and well-banded when prepared approximately 23–25 hr after a blood meal. No naturally occurring aberrations were observed in the polytene chromosomes from a laboratory strain, but differences in the mitotic X-chromosomes were found.

Genetic and cytogenetic studies have been initiated in our laboratory with *Anopheles culicifacies* Giles, one of the principal vectors of malaria in the Indo-Pakistan subcontinent. One sex-linked eye mutant, *rose eye*, showing an X-Y sex determination has been reported (Sakai et al. 1977), and a number of additional morphological and isozyme variants are under investigation. The discovery of these variants along with chromosomal studies of the mitotic and polytene chromosomes have made possible the induction and isolation of inversions and translocations which may be useful in the control of this species.

This paper presents a polytene chromosome map of *An. culicifacies* prepared from adult ovarian nurse cells. As has been demonstrated by Coluzzi (1968) for the Gambiae complex, these cells contain distinct, well-banded polytene chromosomes. With the techniques used in our laboratory these cells provide better polytene chromosome preparations than those of the larval salivary glands. The karyotype of *An. culicifacies* has been previously described by Aslamkhan and Baker (1969). Recent additional studies described below have shown variation in the relative length of the X-chromosomes in comparison to the autosomes.

MATERIALS AND METHODS

The ovarian polytene chromosome map was prepared from the Sattoki laboratory strain colonized in 1975 (Ainsley 1976). This colony, which is maintained by a circular mating scheme (Sakai et al., 1977), is very vigorous and may represent a "standard" reference strain for future

genetic studies. This strain has also been used in the production of inversions and translocations. An additional advantage is that mating routinely occurs in 1 gal "ice cream" cartons. To initiate ovarian development, 3–4 days old females were offered a mouse blood meal. The period of time after feeding which resulted in the best squash preparations appears to be related to the ambient temperature of the blood-fed female. Optimum preparations were obtained from females held at 28°C and dissected 25 hr after feeding, 24 hr at 30°C, and 23 hours at 32°C. Slides were prepared according to the methods described by French et al. (1962), with the following modifications. The ovaries were dissected in a drop of diluted modified Carnoy's (9 parts absolute ethyl alcohol: 19 parts of glacial acetic acid: 52 parts water) and were transferred to a small drop of 45% acetic acid for 30–60 seconds on a silicone-treated coverslip (22 mm^2) after which one drop of 0.5% lacto-aceto-orcein was added. Since the ovaries are so large at the time of dissection only 1 ovary was used per coverslip. Thus, normally 2 slides were made from each female. The polytene map was prepared by studying the slides under phase contrast at 1250–2500X oil immersion, and drawing with the aid of photographs printed at a standard magnification.

The mitotic chromosomes from the ovaries were prepared from females less than 15 hours post emergence as described by Baker et al. (1977). The chromosomes from testes were prepared as described by French et al. (1962).

DESCRIPTION OF THE CHROMOSOMES

MITOTIC CHROMOSOMES. The karyotype of *An. culicifacies* was first described (Aslamkhan and Baker 1969) from ovary and testes as having 1 pair of short subtelocentric sex chromosomes and 2 pairs of longer autosomes, 1 submetacentric and 1 metacentric. Recent examinations confirm this description although some figures have variations in the long arm of the X-chromosome. Normally, the smallest pair appears as subtelocentric (acrocentric) chromosomes approximately 4.3 μm in length. In females the 2 homologs are usually identical (Fig. 1A) while in males the smaller arm is usually heteromorphic (Fig. 1D): the homolog with the smaller short arm has been designated as the Y-chromosome and the one with the larger short arm the X-chromosome. The 2 larger pairs of chromosomes (generally considered autosomal as no dimorphism has been noted in either sex) have been designated chromosomes 2 and 3. The larger pair, chromosome 2, is submetacentric and average 6.7 μm, while the other pair, chromosome 3, is metacentric and is approximately 6.2 μm in length. However, in some preparations the X-chromosomes in females are as long as the 2 autosomes (Fig. 1C) whereas in other females 1 of the X-chromosomes is as long as the autosomes while the other is shorter (Fig. 1B). This variation in the length appears to be due to the presence of extra chromosomal material on the long arm of the X-chromosome and not to an artifact as all the X-chromosomes from a particular female exhibit similar karyotypes. This variation in the long arm of the X-chromosome has also been re-

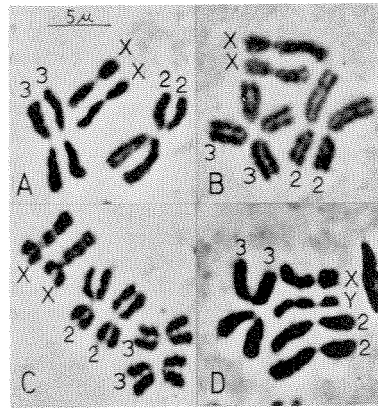

Fig. 1. Mitotic chromosomes. A, B, C from ovary and D from testes. For further details see text.

ported in *Anopheles punctipennis* (Baker and Kitzmiller 1964). Preliminary comparative cytogenetic studies in our laboratory of chromosomal aberrations support the correlation of the above number designations given for the mitotic chromosomes and those of the polytene chromosomes as described below.

POLYTENE CHROMOSOMES. The polytene chromosomes from ovarian nurse cells in *An. culicifacies* consist of 5 elements with a characteristic banding pattern (Fig. 2 and the map): a very short X-chromosome apparently with only 1 arm and the right and left arms of chromosomes 2 and 3. Since the banding patterns at the free ends and the total lengths of the arms of *An. culicifacies* are similar to those of the previously described 16 species of the subgenus *Cellia* (Colluzi and Kitzmiller 1976, Kitzmiller 1976, Sharma and Chaudhry 1976), especially those of *An. pulcherrimus* (Baker et al. 1968) and *An. stephensi* (Sharma et al. 1969), we have designated X, 2R and 2L, 3R and 3L accordingly. However, because the overall banding patterns are different (although many areas of all arms show strong similarities with the maps of the other species) we have arbitrarily numbered and lettered the zones and regions for *An. culicifacies*. Extended comparisons between *An. culicifacies* and the other species were not attempted as most previously reported chromosome maps are from the larval salivary glands and not from ovaries. Detailed comparisons between species will probably have to await various hybridization experiments, or the preparation of a map of *An. culicifacies* from salivary glands.

The X-chromosome in *An. culicifacies* measures about 79 micra in length and has been divided into zones 1–6. The position of the centromere in the X-chromosome is difficult to ascertain as the centromere end is flared and diffusely stained with no consistently identifiable structural features. Moreover, this end of the chromosome is usually asynaptic and appears stretched out even in the best preparations, while the autosomes at their extreme centromere end have a single dark band present. Thus, although the mitotic X-chromosome has two arms, in the polytene chromosomes 1 arm does not appear to be present. Since the banded arm is considerably shorter than any of the autosomal arms, it is possible that only the short arm of the X-chromosome has a banded appearance in the polytene nucleus. Chromosome 2 (444 μm in length) has 2 unequal arms. 2R, the longest arm in the complement (287 μm) contains zones 7–19, and 2L, the shortest autosomal arm (157 μm). Chromosome 3 (432 μm) has arms approximately equal length. 3R (209 μm) has zones 29–37, and 3L (223 μm) zones 38–46.

Thus, all 3 chromosomes are immediately recognizable by their relative sizes and the positions of their centromeres. In the polytene nurse cells there is no distinct chromocenter. The X-chromosome generally appears isolated from the remaining 4 arms, while the right and left arms of chromosomes 2 and 3 may be attached by their respective common centromere ends. Thus, in this species in ovarian nurse cell preparations it is possible to determine unambiguously which arms share centromeres 2 and 3.

DIAGNOSTIC FEATURES

X CHROMOSOME. This chromosome can be recognized easily by its short length. The free end is characterized by a fan-shaped tip with 2 medium bands at the end of 1A. A characteristic sequence of 5 dark bands in a 2:1:2 pattern is always present in 1B which sometimes appears as 1 thick dark area. 1C is a diffuse area with 3 medium and 6 very light bands including a small puff. Zone 2 contains a diagnostic puff marked in 2A by a dark and a medium dotted band, followed by a wide clear area. At the center of the puff in 2A and 2B are 5 somewhat darker bands with a thin band separating them from the 5 distinctive dark bands at the end of the puff in 2B. Regions 3A, 3B and 3C are a

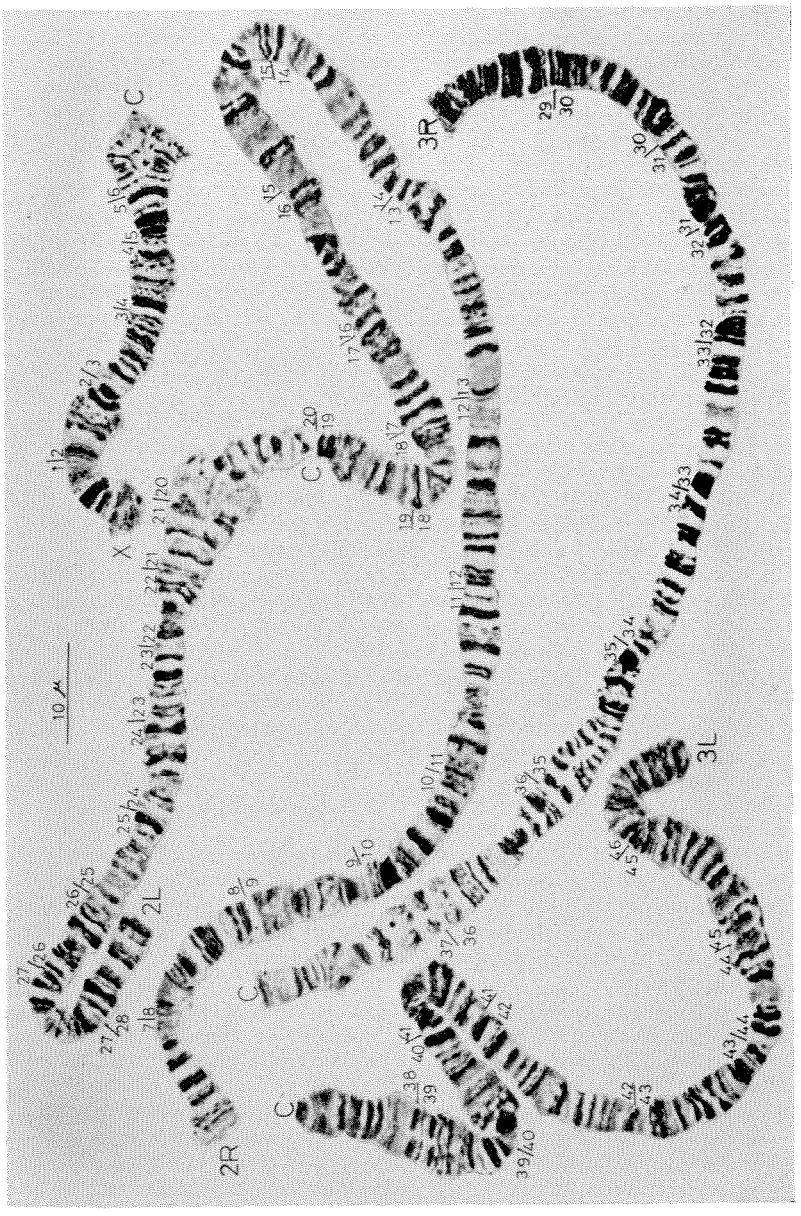

Fig. 2. Polytene chromosomes from adult ovary.

series of small puffs. 4A and B are two puffs with A marked with one distinct heavy band and a series of faint bands. 4B begins with two heavy bands, a series of broken bands in the center of the puff and ending with a heavy band and a faint band. 5A represents one of the best landmarks for the chromosome. It begins with a medium band in a constriction followed by eight dark and one faint band (barely visible) in a 2:2:1(faint band):2:2 pattern.

A medium and a light band is present at the beginning of 5B along with 2 diagnostic heavy bands flanked by thin bands. A series of 5 sharp bands and 4 thin light dotted bands are present in 6A and 6B. 6A also marks the beginning of the characteristic asynaptic centromere end. 6C represents a widely flared indistinctive diffuse area presumably the centromere region.

CHROMOSOME 2. *2R.* This arm is the longest element of the complement and is easily recognized by its scolecoid free end. At the tip of this arm (7A) is a sharp narrow broken band followed by 2 light bands and 1 heavy band. At the broadest part of the "head," a group of 4 bands of varying intensity are present. At the "neck" 1 light, 1 dark and 2 light broken bands mark the end of 7A. The 3 pairs of heavy bands in 7B are a consistent and diagnostic feature of this arm. Another area easily recognized is the heavy doublet flanked by light bands in 8D followed by the 3 medium wavy bands at the center of the elongated puff in 9A. Another elongated puff occurs in 9B and 9C which always contains 1 dark band composed of large triangular-shaped dots. Zones 10A-D and 11A have many light, thin dotted bands but the 2 sharp bands each found in 10B, 10C, 10D and 11A are characteristic for the area. The exceptional 6 dark heavy bands in a 2:2:2 pattern are a constant feature of 11C and 11D. 12B also contains 6 heavy bands in a 2:2:1:1 sequence of which the last is usually a thicker wavy band. This band is followed by a diffuse area with a number of faint bands in 12C which develops into a puff containing 4 dark bands, with the first 2 dotted. The 3 small puffs in 13C, 13D and 14A with many dark bands may be used as a reference for the middle portion of this arm. The puff in 13C has 5 dark bands in a 3:2 sequence followed by a clear area. The puff in 13D stands out beginning with a medium and a dark band with a wide space in the center of the puff followed by 2 medium and 2 dark bands. The puff in 14A has 1 doublet on either end with 2 medium and 1 thick band in the middle. Near the centromere end the 3 dark bands in 17D and the 5 dark bands in the puff of 18A of which the last 2 are doublets in the center stand out. The last puff in this arm, 19C, contains a series of medium bands of which 2 or 3 are usually wavy. The centromere end is characterized by 2 very heavy bands close together in 19D.

2L. This is the shortest autosomal arm with many recognizable areas. At the free end, 28A, a heavy band is present just at the tip followed by a light dotted, 2 heavy, a light, a heavy and a medium dotted band. 28B begins at the center of the first puff and ends in a constriction with 3 dark bands. A dark thick doublet following 5 light bands is present in 28C. The 4 heavy bands at the beginning of the puff at region 28D-27A are a diagnostic feature. Except for 1 sharp dotted band and a heavy band, the rest of 27B is composed of at least 10 light bands. The 3 heavy bands in the form of a triplet following 2 other dark bands in 1:1:3 pattern are diagnostically present in 25C. The 5 sharp bands in a 3:1:1 pattern are always present as drawn in 24B. These 5 bands may at times appear asynapic as is frequently found for the bands in regions 24C and 23A. The latter two regions also usually appear as an elongated puff. Another asynaptic area begins in 22B, continues through the big puff in 20A and ends at the dark bands in 20B. The most distinctive feature of this arm is the big "spongy" puff in 20A which is preceded by 2 heavy dark bands. This area may well be used as an initial reference point from which to study this arm as this puff is

usually the largest in the complement. This puff has no distinct outline and contains hazily stained patches ending in a medium band. This puff is frequently asynaptic. The centromere end is marked by a small puff in 20D with 2 dark, 2 light and 2 dark bands. The extreme end is marked by a sharp band.

CHROMOSOME 3. *3R.* The free end of this arm begins in 29A as a very wide area with 4 light bands followed by 1 thin dark chain-like band and 2 thin dotted bands. The 2 pairs of broken bands at the constriction at the end of 29B are quite distinct. A group of 6 light and medium bands in 29C is diagnostic, and it stands out since the areas preceding and following them are bandless. The puff in 30D is very prominent beginning with 2 thin sharp bands of which the first is broken. In the center of the puff there is a characteristic very thick heavy dark "fire" band flanked by a sharp broken band. The series of dark bands in 32D and 33A as a whole are very diagnostic for this arm. In 32D there are 6 heavy bands and a very light one of which the fourth is a thin doublet. The series in 33A begins with 2 faint bands then a heavy band followed by 6 faint and medium bands ending with 2 heavy scalloped bands in the middle of the puff. The central areas in 33B and 33C appear similar with only 3 very indistinct bands whereas the constriction at their juncture is marked by a 2:1:2 sequence of dark:light:dark bands. A small but diagnostic puff in 35A with 7 sharp bands in a 3:1:1:2 sequence is always present followed by another small puff. These 2 puffs then develop into one of the best landmarks of this arm denoting the presence of a "zebra" area in 35C consisting of 3 very thick and 2 somewhat thinner dark bands in an alternating sequence which is frequently asynaptic. At the centromere end there is an interesting puff, 37C, with a dark patch on the periphery continuing in a semi-circular transverse band which is clear only in good preparations. The puff in 37D begins with 2 heavy bands separated by a light band and ends with a series of faint bands. The centromere end is made by a dark band.

3L. The free arm of this chromosome is easily identified by the absence of heavily stained bands in the distal part except for the one in 46B. The sequence of light and thin bands in zone 46 are fairly constant, but there is differential puffing within the regions. Region 46C always ends in a constriction with a single heavy band. The best series of landmarks of this arm begins at 44B with a distinctive puff containing 3 pairs of heavy bands of which the second and third are "fire" bands. This puff is followed by a unique small bandless puff, 44C, which is delimited at both constrictions by a thin, faint band. This puff is followed by a medium band and 2 "sausage-shaped," very dark doublets, in 44C and 44D. These dark doublets are the most intensely stained bands in this area of the arm, but this area also has a tendency to be stretched and frequently the chromosome arm is "broken" into 2 parts. Another prominent puff occurs in 42B starting with 3 intensely stained bands of which the third is a "fire" band, followed by many dark and light thin bands. This region ends in a very narrow area with a medium band. The large distinctive puff in 40C is normally the largest puff of 3L. In the center of this puff are 2 "fire" bands which sometimes appear to have lateral connecting "cross" bands as drawn on the map. The regions starting from 39B till to the end of 38A are frequently asynaptic. This area and all of regions 38B and C contain mostly light thin dotted bands. The centromere end of this arm ends in a dark band.

DISCUSSION

While radiation-induced aberrations have been isolated in *An. culicifacies,* no naturally occurring inversions or translocations have been found in the Sattoki colony except for the differences described earlier in the arm lengths of the mitotic X chromosomes. Females from the Sattoki laboratory colony which were originally colonized during the summer of 1975 were crossed to male progeny

reared from field material collected in the winter of 1976. The chromosome preparations from the F_1 also showed no aberrations. We have examined the ovarian polytene chromosomes from another strain colonized from Kot Baghicha (Ainsley 1976) and found no aberrations. Additional populations from different geographical areas need to be examined.

The differences in the arm lengths of the long arm of the mitotic X-chromosomes also need further study. So far we have not detected any differences in the length of the ovarian polytene X-chromosomes, although as described above the centromere end of this chromosome is usually stretched, diffusely stained, and asynaptic. Moreover, if the long arm of the mitotic X-chromosome is not present as a banded element, this difference would probably not be detected in the polytene chromosomes.

The salivary gland chromosomes from *An. culicifacies* are now under investigation. As expected, these chromosomes when compared to those from the ovarian nurse cells show many similarities as well as differences in the positions of the puffs and the staining intensity of the bands.

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