THE SALIVARY GLAND CHROMOSOMES OF ANOPHELES ARGYRITARSIS COMPARED WITH THOSE OF CERTAIN OTHER SPECIES IN THE SUBGENUS NYSSORHYNCHUS

R. D. KREUTZER,¹ J. B. KITZMILLER ² AND M. G. RABBANI ²

ABSTRACT. The chromosome number of Anopheles argyritarsis R.-D. is six, one pair of sex chromosomes and two pairs of autosomes. There is a high level of banding homology in the autosomes of the salivary gland chromosomes between A. argyritarsis and A. darlingi. If sections of 2L and 3R are inverted the sequences in these arms are identical; in addition half of the other two autosomal arms are identically banded. There is significantly less homology between A. argyritarsis and A. aquasalis and A. munozovari; however there are identically banded regions among these species at the free and centromere ends of the autosomes. Data indicate that an inversion recovered from 3L of argyritarsis populations is confined to the south and central portion of the distribution.

INTRODUCTION

Within the subgenus Nyssorhynchus the members of the argyritarsis series, Anopheles albitaris, argyritarsis, brasiliensis and darlingi, show a great deal of morphological similarity. Cytogenetic studies of other morphologically similar anophelines (Kreutzer et al. 1970; Kreutzer and Kitzmiller 1971) show that species with morphological similarities have similar banding patterns in their salivary gland chromosomes. An investigation has been undertaken to determine the amount of homology among the members of the series argyritarsis. The cytogenetic relationships of darlingi with other species of the subgenus Nyssorhynchus have been reported (Kreutzer et al. 1972). This report concerns similarities in the banding patterns of argyritarsis and certain other species in the subgenus Nyssorhynchus.

MATERIALS AND METHODS

The specimens used to prepare the chromosome map of argyritarsis were collected near Colon, Panama; at various localities in the states of Guanabara and Rio de Janeiro, and near Brasilia, D.F., Brasil; near Villavicencio, Meta and near Tibú, Norte de Santander, Colombia. Slides were made following the method, slightly altered, described by French et al. (1962). The “dry-ice” method was used to make the slides permanent. Photographs were taken using a 40X objective and an 8X ocular. Detailed observations of the banding patterns were made at 1000X using a Zeiss phase contrast system. The chromosome complement is shown in figure 1, and the proposed salivary gland chromosome map is shown in figure 2. Figures 3 and 5 are section by section comparisons of the argyritarsis and darlingi complements.

DESCRIPTION OF THE CHROMOSOMES

The chromosome number in argyritarsis is 6, as in all other species of the genus Anopheles studied to date; 2 pairs of autosomes and 1 pair of sex chromosomes (Guedes et al. 1957). The males are heterogametic. The X chromosome is telocentric and averages 73 micra, the right arm of submetacentric chromosome two averages 212 micra, the left arm 158 micra; the right arm of metacentric chromosome three 140 micra and the left arm also 140 micra. These chromosome lengths are approximately the same as those of darlingi. The numbering system for the arms is the same as in the darlingi system: X-chromosome, zones 1 through 5; 2R, zones 6 through 15; 2L, 16 through

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Fig. 1. Salivary gland chromosome complement of Anopheles argyritarsis.
25; 3R, zones 26 through 35; 3L, 36 through 45.

**X-Chromosome.** The telocentric X-chromosome is the shortest and most easily identified element of the complement. The free end is usually flared and contains a thick dark double band in region 1B. Another thick dark band followed by three, possibly four broken bands is found in 2A. At the end of 2B is a series of two or three broken, light bands which mark the breakpoint of an inversion which probably extends to the light-broken bands at the end of 4B; this inversion has been found in the Tibú preparations. The darkly staining bands at the beginning of 5A are good landmarks, and can be used to begin study of this section of the chromosome. In region 5B a pair of dark bands is followed by a pair of lighter bands. Region 5C begins with a lightly staining band, has a thin dark band in the center, and ends at the centromere.

**Chromosome 2, Right Arm.** The free and centromere ends of this and the other autosomal arms have sections which are not only similar to *darlingi* (figure 3) but also to other members of the subgenus. The free end, 6A–7B, is drawn in Figure 2 as in the *darlingi* map, as are all sections of the complement which have homologous banding patterns. This is done to facilitate comparisons with other members of the series and subgenus and with anophelines classified in other subgenera. Any band intensity differences in these regions are noted in the description of the chromosomes.

There is a series of dark bands at the beginning of 8B followed by 3 dark bands which often appear as a single thick band. In 9B there is a single thick dark band followed by a pair of light bands and 2 series of 3 bands each in 9C. The 2 dark bands in 10A each followed by 2 lighter bands and the dark band in 10B flanked in front by 1 and in back by 3 light bands are good landmarks. The wide dark band in 11B preceded and followed by a series of light bands is another landmark area in the center of the arm. The dark bands near the beginning of 12B occasionally appear as 1 wide dark band. There are 4 or 5 wide dark bands flanked by a pair of light bands in 13A.

**Chromosome Two, Left Arm.** Except for inverted regions, the left arm of chromosome two of *argyritarsis* is quite similar to that of *darlingi*, (figure 3). There are some band intensity differences throughout the arm. The study of the free end of the arm can best be initiated in zone 23A with the series of 4 bands, the first pair lighter than the second. The darkly staining series of 3 bands at the end of 22A is a second diagnostic character for the free end of the arm. The 2 dark staining bands in 20B are a good landmark for the center of the arm. The 3 dark bands near the end of 19B of *argyritarsis* are very close, but in *darlingi* these bands are usually widely spaced. The puff beginning in the center of 18B extends into 17B in *argyritarsis*, and the 2 pairs of bands at the front of 17A appear as 2 thick, dark bands. The first pair of double darkly staining bands in 16B is usually involved in a wide Balbiani type puff.

**Chromosome Three, Right Arm.** The major differences in 3R of *darlingi* and of *argyritarsis* are in the center section of the arm, and can be explained by 2 paracentric inversions, (figure 5). The areas which are the breakpoints for both inversions contain broken light bands. In 31C there is a series of 3 darkly staining bands, the last one flanked by 3 lighter bands, and the 4 together often appear as one thick, dark band. The pair of bands at the front of 34B is light, the pair at the 34B–34C division is darker, and the pair of bands at the end of 34C is dark.

**Chromosome Three, Left Arm.** The left arm of chromosome three has certain homologies with *darlingi*, especially at the free and centromere ends (figure 5). The thin broken bands in 42B, the group of 3 broken bands in 41A and the 2 wide dark bands at the end of 41A make this area a good one to begin the study of the arm. The series of 2 dark thin bands in
Fig. 3. Chromosome two of *argyritarsis* compared with that of *darlingi*. Regions which contain only one numbering unit are identical and in the standard *argyritarsis* sequence in both species. In regions which contain two numbering units the upper number refers to the *argyritarsis* standard region, and the lower number refers to and numbers the region of the *argyritarsis* chromosome which has been relocated in the *darlingi* chromosome by one of the hypothetical inversions explained in the text.
40B followed by light bands, a pair of dark bands at the end of 39A, the first thin and the second wide, are diagnostic for the center section of the arm. A wide Balbiani type puff is often found in 36A. The bands in this region are usually broken, and their intensities vary from slide to slide. Region 36B contains a pair of darkly staining bands, the second in some preparations appears double.

Inversions. Only 2 inversions have been recovered from natural populations of argyritarsis. One in the X chromosome involves region 3A-4B, and has been recovered only heterozygous and only in the Tibú population. The second is in 3L, 40A-39B. This inversion has been recovered both heterozygous and homozygous inverted (table 1 and figure 7). The

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limited data infer that the inversion is confined to the south and central portion of the distribution. Furthermore, the reduced number of karyotypes recovered heterozygous or homozygous inverted indicates that the inversion is either new or does not impart much advantage to its carriers. The breakpoints of the inversion are located in regions where the bands are light and broken. Areas of this same type, lightly-staining with broken bands, have been found to be inversion breakpoints in 2L and 3R.

DISCUSSION

The morphological similarities between argyritarsis and darlingi are reflected in the similarity in the banding patterns of their salivary gland chromosomes; morphologically similar species are expected to and do have more banding pattern similarity than species which have little morphological similarity (Kitzmiller et al. 1967). Except for the X-chromosome, which is distinct in all species of anopheles thus far studied, and the center sections of 2R and 3L, the chromosomal banding patterns of these 2 species differ only in the sequences of the bands. The free and centromere ends of the arms of both autosomes are identical.

Region 6A-7B at the free end and 13A-15C at the centromere end of 2R are the same in both species. The center of this arm is distinctive in argyritarsis and is difficult to homologize with darlingi. The left arm of chromosome two has been involved in 3 separate paracentric inversions. The probable genesis of the argyritarsis arrangement from that of the darlingi 2L (or vice-versa) is diagrammed in figure 3. The first inversion involves region 22B(part)-19B(darlingi), the second inverts 25B-24B(part) and the last inversion, 25C(part)-23B, reverts and displaces region 25C(part)-25B. These sections have been renumbered in argyritarsis. There are additional band intensity differences in this arm, but the inverted regions of argyritarsis can be easily identified and homologized with darlingi. The right arm of chromosome three is very similar to that of darlingi. The differences in sequence are derived and diagrammed in figure 4. Except for minor intensity differences the argyritarsis sequence can be produced by inverting region 29B(part)-32A(darlingi) and then re-inverting region 31A-32A. In 3L regions 45A-43C and 37A-36B are almost the same in both species, and region 42A-43B(part) is similar in both species; however, region 43A-43C has been in-
Fig. 4. Probable derivation of argyritarsis 2L from that of darlingi. Arrows indicate inversion breakpoints.
Fig. 5. Chromosome three of *argyiarias* compared with that of *daringi*. Refer to figure 3 for explanation of the numbering.
Fig. 6. Probable derivation of *argyritarsis* 3R from that of *darlingi*. Arrows indicate inversion breakpoints.
Fig. 7. Naturally occurring arrangements of 3L in *argyritarsis*. “A” standard sequence. “B” inversion heterozygote for region 40A–39B. “C” inversion homozygote.
verted in *argyritarsis*. The center section of this arm, like the center of 2R, is difficult to homologize with *darlingi*. Region 37A—36B has been drawn as it is in the *darlingi* map. The similarities between these two species are compared in figure 5. The regions with identical and inverted sequences are numbered using the *argyritarsis* system.

As does *darlingi* the free and centromere ends of chromosome two of *argyritarsis* show a great deal of similarity with the corresponding regions in *aquasalis*. The free (6A—7B) and centromere (14A—15C) ends of 2R and the centromere end (16A—16C) of 2L have been drawn, as they were in *darlingi*, the same as in the *aquasalis* map, and any band intensity differences have been noted in the description of the chromosomes. The free end of 2R, 6A—7B, shows some similarity to *Anopheles nuneztovari*, (Kitzmiller, et al. 1973) and at the centromere end, region 15, is identical in both *argyritarsis* and *nuneztovari*. In 2L only the centromere end, 16A—16C, shows any similarity to *nuneztovari*. In addition, the free end, 26A—27A and the centromere end, 32B—35C, of 3R and the free, 45A—43B, and centromere, 38C—36B, ends of 3L show at least a gross similarity to the same regions in *aquasalis*. The banding pattern of the X chromosome and the center sections of the arms do not homologize either with *aquasalis* or *nuneztovari*.

The breakpoints of the hypothetical inversions needed to produce the *argyritarsis* sequence from that of *darlingi* are in some cases the breakpoints of inversions found in natural populations of *darlingi*. Although *darlingi* is a highly polymorphic species, (10 different aberration arrangements have been recovered), the *argyritarsis* populations studied have in comparison relatively conservative karyotypes. Two aberrations have been recovered from natural populations. The inversion in the X chromosome involves region 3A—4B. As has been noted the X-chromosomes of both species are distinct and it is difficult to find homology within them; however, both contain an inversion, which has been recovered only in the heterozygous condition, in the center of the arm. This is also true of the 3L inversion. Although the banding patterns in the center of 3L of *argyritarsis* and *darlingi* show little similarity, the region of the *argyritarsis* inversion is about the same as an inversion recovered both heterozygous and homozygous from *darlingi* populations.

Although the data in Table 1 are limited, they indicate that the 3L inversion frequency is greater in the south and lesser in the north. In 2R, 6A—7B and 13A—15C are areas which are very similar in both species. In *darlingi* both 7B and 13A are breakpoints for naturally occurring inversions. The breakpoint in 2L at the end of 23B of *argyritarsis* corresponds to the end of 23B of *darlingi*, and is a common breakpoint with an inversion in *darlingi*. Another hypothesized inversion in this arm has a breakpoint at the beginning of 18A; region 18A—18B is usually asynaptic in *darlingi*. The inverted section at the free end of 3L, 43A—43C (*argyritarsis*), is often found homozygous in this same inverted sequence, naturally occurring, in *darlingi*. These similarities further emphasize the chromosomal relationships between the 2 species.

The amount of chromosomal relationship between *argyritarsis* and the other 3 species can be estimated assuming *argyritarsis* as 100% and the other values as percent similarity in the species indicated.

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The greatest amount of homology is with *darlingi*, however, there are significant similarities with *aquasalis* and lesser, distant similarities with *nuneztovari*. The
relationships might be diagrammed as follows:

\[ \text{argyritaris} \rightarrow \text{aquasalis} \rightarrow \text{nuneztovari} \rightarrow \text{darlingi} \]

As in other studies the breakpoints of observed or suggested inversions are usually in broken, lightly staining bands. As evidenced by their staining intensities, there is a reduced concentration of DNA in darkly staining bands. This reduction might account for the weakness of the area, and its susceptibility to breakage.

SUMMARY

The salivary chromosome map of *Anopheles argyritaris* is presented and proposed as the standard for this species. The complement consists of five paired elements: a telocentric X-chromosome, right and left arms of submetacentric chromosome two, right and left arms of metacentric chromosome three. The banding pattern is compared with that of *Anopheles darlingi*. The differences in the left arm of chromosome two between these species may be explained as the result of three paracentric inversions, and the differences in 3R the result of two paracentric inversions. The free and centromere ends of 2R and 3L are identical in both species; however, the X-chromosome and the center sections of these arms are difficult to homologize with darlingi. Similarities in breakpoints of observed and suggested inversions in both species are additional evidences of chromosomal relationships. Distinct homologies exist with *Anopheles aquasalis* in the free and centromere ends of 2R, at the centromere end of 2L and at the free and centromere ends of chromosome three. Some homologies with chromosome two of *nuneztovari* are inferred.

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INHERITANCE OF MALATHION RESISTANCE IN AEDES TAE NIORRHYNCHUS (WIEDEMANN) ¹

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ABSTRACT. Malathion resistance in Aedes taeniorhynchus (Wiedemann) was studied to determine its mode of inheritance. Dosage-mortality curves were calculated for a resistant (R) strain, a susceptible (S) strain, and reciprocal hybrids [F₁ (R x S) and F₁ (S x R)] from data obtained by topical application of malathion. These data were used to analyze the progeny of genetic crosses. The R males and R females were found to be 31- and 46-fold resistant at the LD₉₀, respectively. The results of genetic crosses suggested that malathion resistance was inherited, primarily as a single dominant, autosomal gene.

Malathion resistance in populations of Aedes taeniorhynchus (Wiedemann) in Florida have become a problem of increasing concern in recent years. The first reports of resistance were made by Glancey et al. (1966), Gahan et al. (1966), Rathburn and Boike (1967), and Lofgren et al. (1967). These reports indicated that F₁ larvae and/or adults of field-collected A. taeniorhynchus were resistant (≥4×) to malathion in 4 counties on the west coast and in 5 counties on the east coast of Florida. Furthermore, the results of field applications by aircraft in 1966 showed a 10-fold increase in the amount of malathion needed for 90 percent reduction of adults of A. taeniorhynchus when compared to results obtained in 1959 (Glancey et al. 1966). Additional reports of malathion resistance in this species have since been reported by Boike and Rathburn (1968, 1969, 1972, and 1974) and Mount et al. (1971 and 1974). A review of these reports indicated that resistance is present along most of the east coast, the lower half of the west coast, and in the Florida Keys (Monroe County). No cross resistance to other organophosphate insecticides was observed in these studies.

Mount et al. (1974) reported the selection of a strain of A. taeniorhynchus that was highly resistant to malathion, but showed no cross resistance to other adulticides. The origin of this strain was a native population that was heterogeneous in susceptibility to malathion.

The objective of this present study was to determine the mode of inheritance of malathion resistance in A. taeniorhynchus. Although some generalizations can be made based on surveys for the detection of resistance, we believe that the mode of inheritance merits priority in assessing the

¹ This paper reflects the results of research only. Mention of a pesticide or a commercial or proprietary product in this paper does not constitute a recommendation or an endorsement of this product by the USDA.