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

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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. nuneztovari;* 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 albitarsis, argyritarsis, braziliensis* 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., Brazil; 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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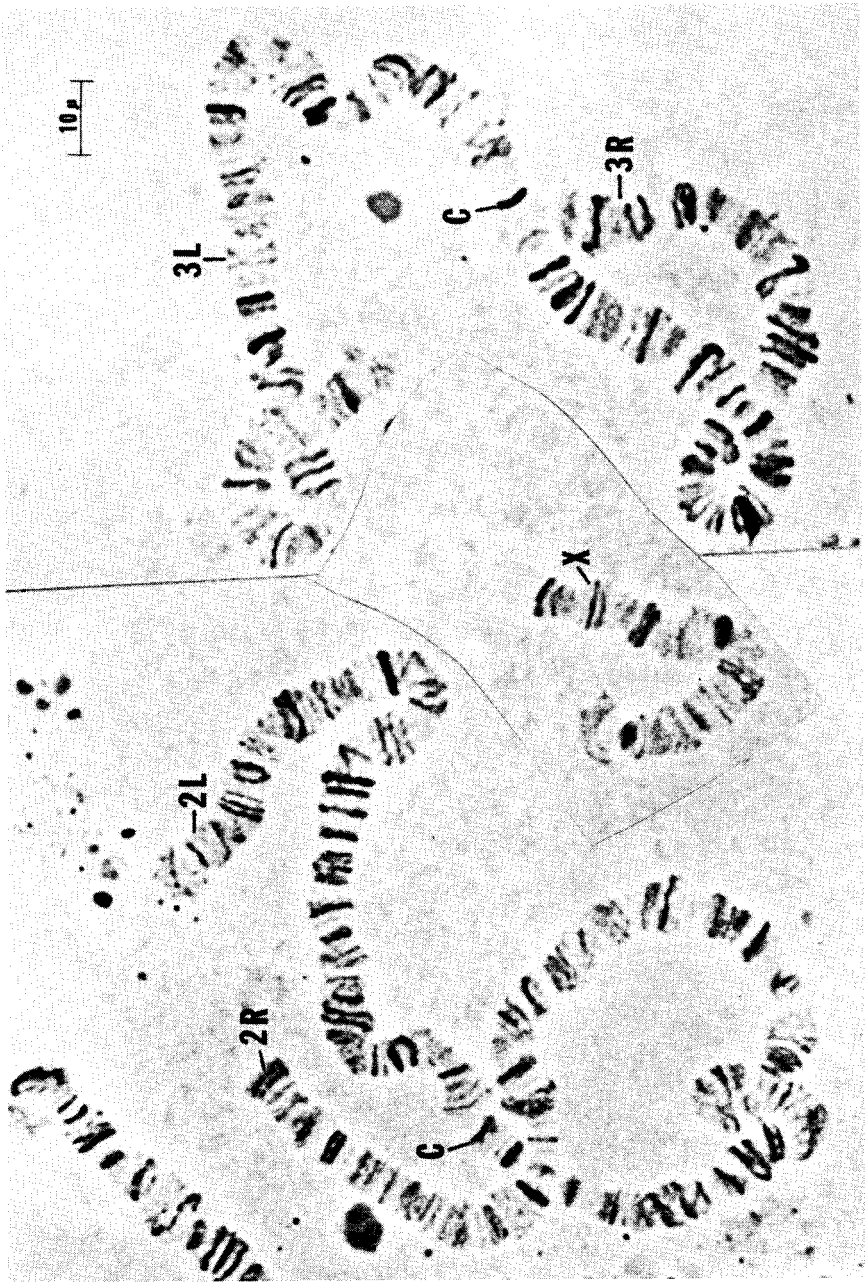


Fig. 1. Salivary gland chromosome complement of *Anopheles argyritarsis*.

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

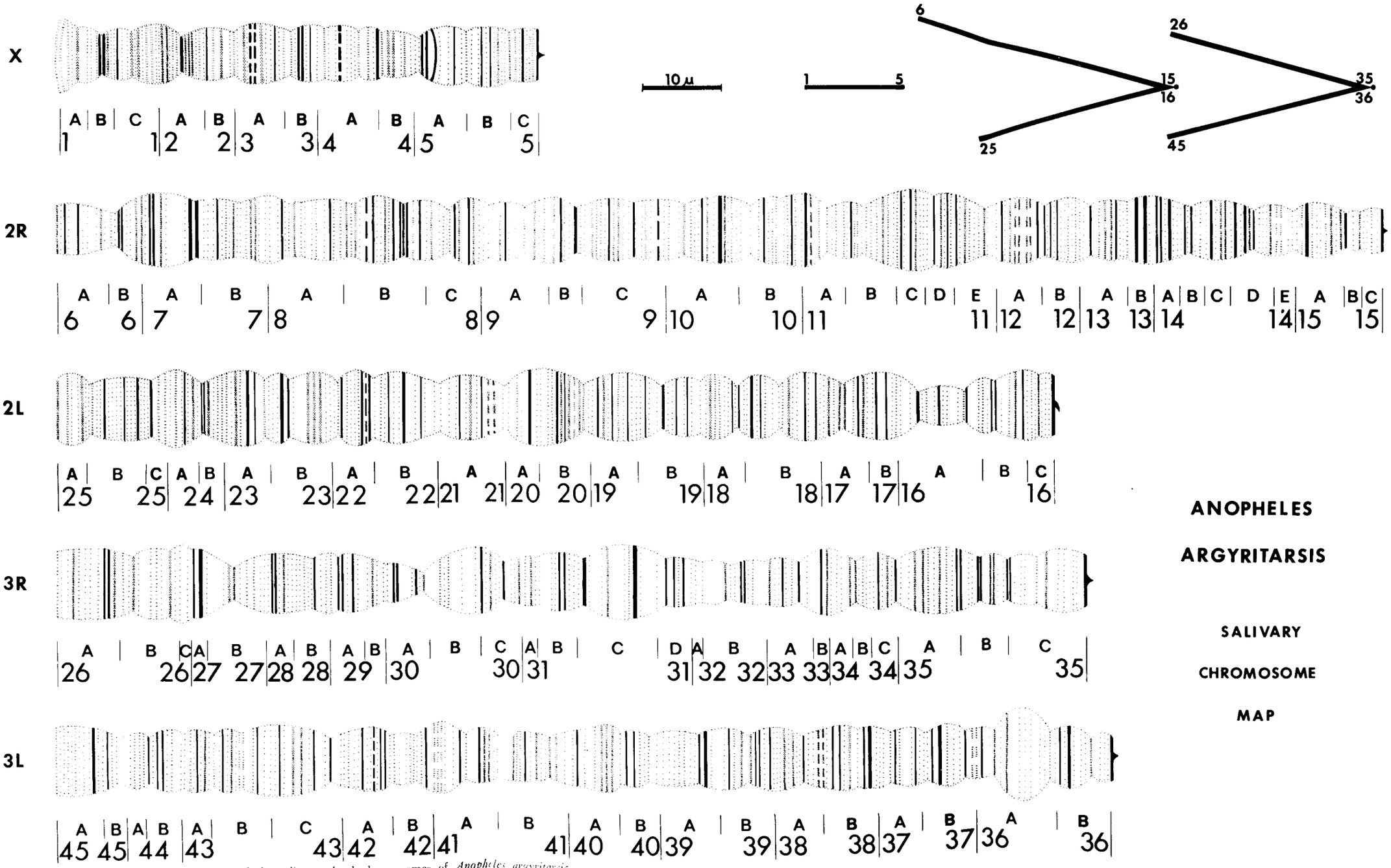


Fig. 2. Proposed map of the salivary gland chromosomes of *Anopheles argyritarsis*.

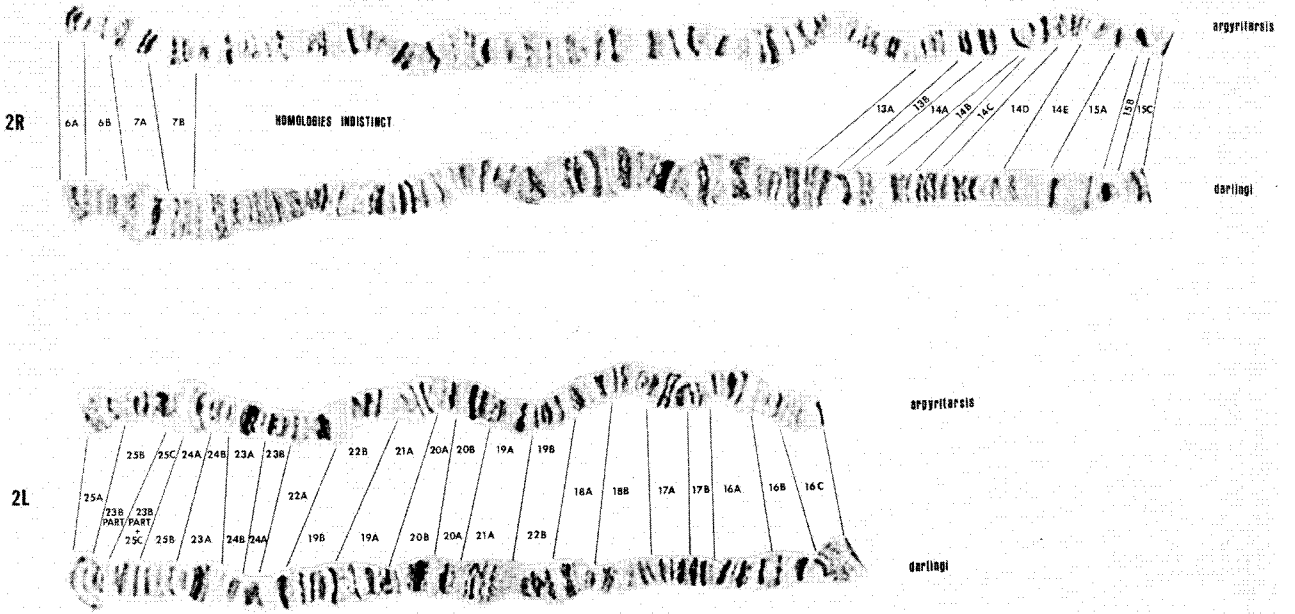


Fig. 3. Chromosome two of *argyritarsis* compared with that of *darlingi*. Regions which contain only one numbering unit are identical and in the standard *argyritarsis* sequence in both species. In regions which contain two numbering units the upper number refers to the *argyritarsis* standard region, 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

Table 1. 3L Aberration Data

	Standard	Inversion Hetero-zygote	Inversion Homo-zygote
Estado de Rio de Janeiro, Brasil	100	25	12
Estado de Guanabara, Brasil	37	5	1
Districto Federal de Rio de Janeiro, Brasil	60	5	2
Colombia	6	2	..
Venezuela	13	..	..
Panama	50	..	..

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 anophelines 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* arangement 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, reinverts 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 reinverting 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-

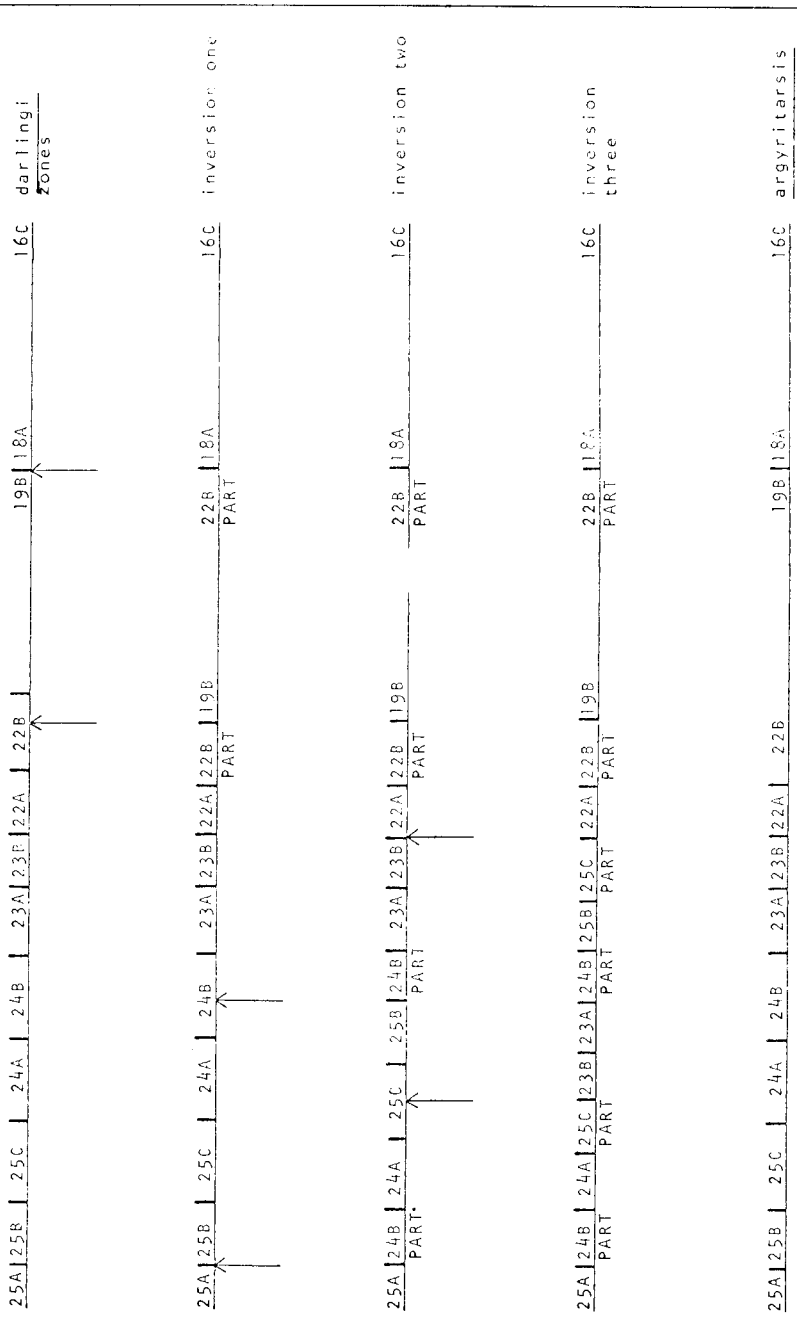


Fig. 4. Probable derivation of *argyritarsis* 2L from that of *darlingi*. Arrows indicate inversion breakpoints.

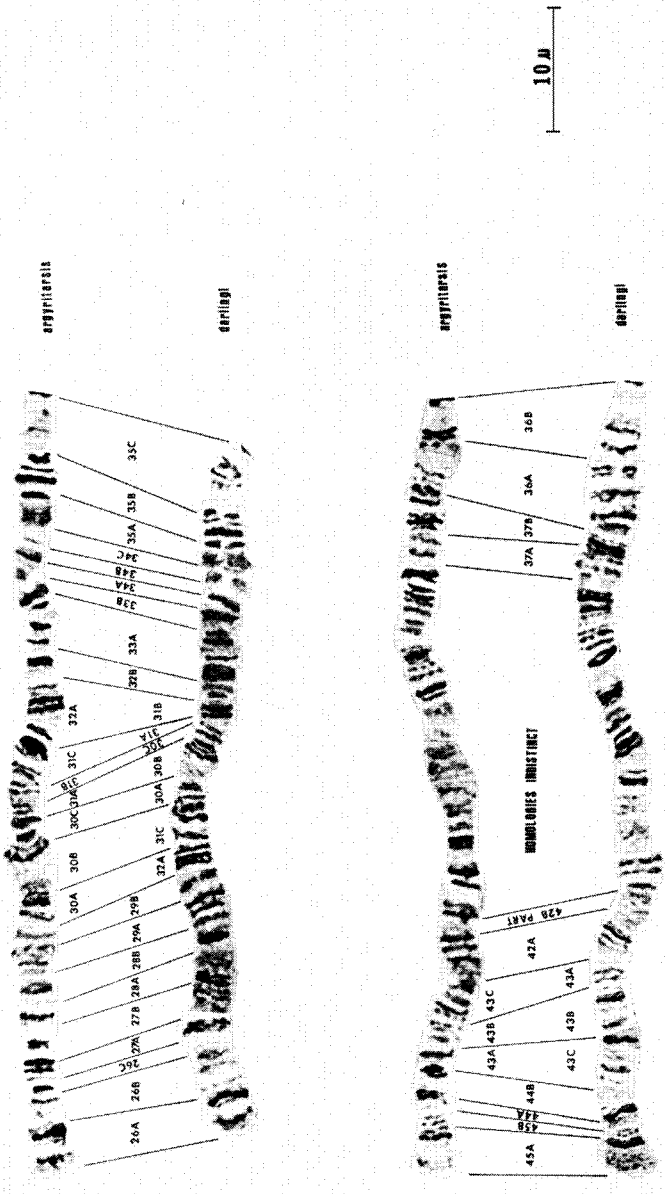


Fig. 5. Chromosome three of *argyritarsis* compared with that of *darlingi*. Refer to figure 3 for explanation of the numbering.

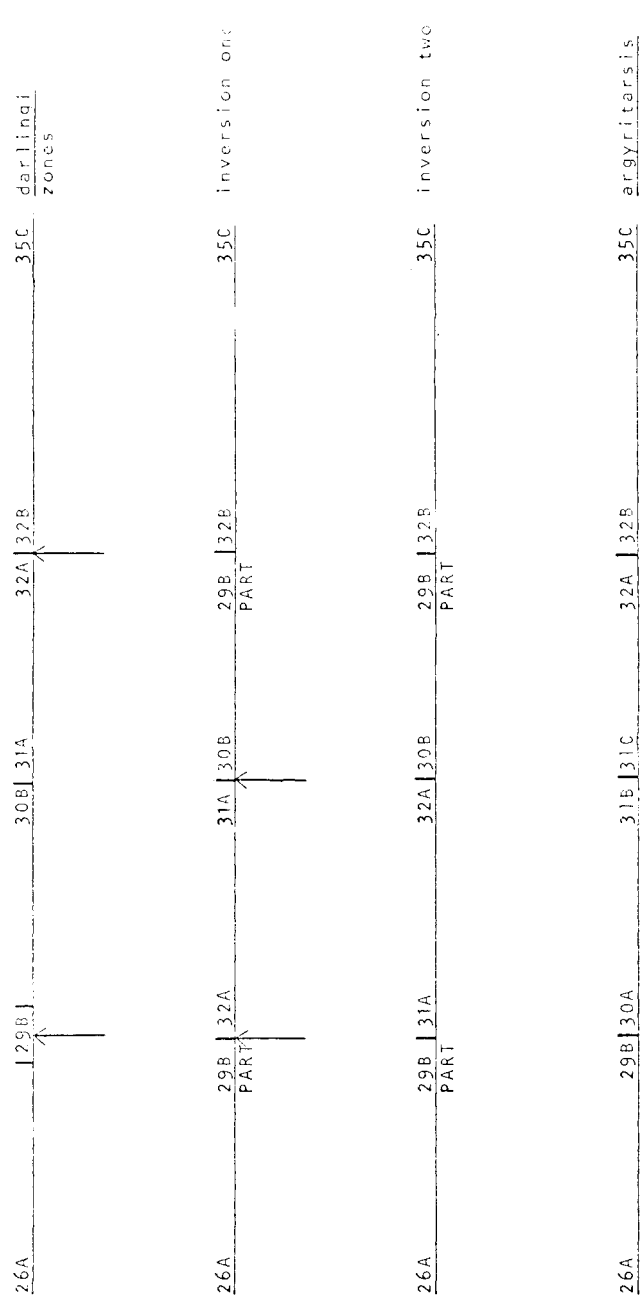


Fig. 6. Probable derivation of *argyritarsis* 3R from that of *darlingi*. Arrows indicate inversion breakpoints.

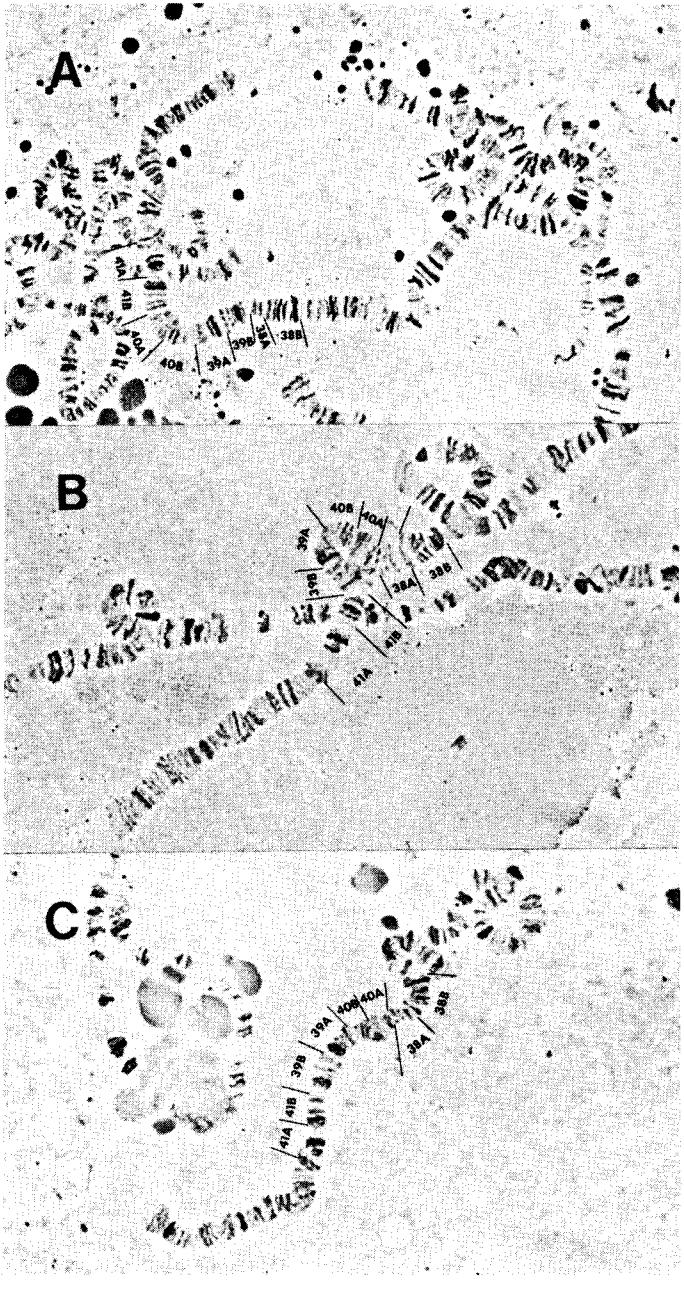


Fig. 7. Naturally occurring arrangements of 3L in *argyritarsis*. "A" standard sequence. "B" inversion heterozygote for region 40A–39B. "C" inversion homozygote.

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.

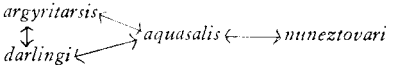
Although the data in Table 1 are limited, they indicate that the 3L inversion frequency is greater in the south and lesser in the north. In 2R, 6A–7B and 13A–15C are areas which are very similar in both species. In *darlingi* both 7B and 13A are breakpoints for naturally occurring inversions. The breakpoint 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.

	darlingi inverted and non-inverted sequences	*aquasalis*	*nuneztovari*
X	0	0	0
2R	0.45	0.40	0.22
2L	1.00	0.14	0.08
3R	1.00	some	0
3L	0.56	some	0

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:



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 argyritarsis* 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.

ACKNOWLEDGMENTS. Thanks are due to many colleagues in Panama, Colombia and Brazil. Col. Robert Altman and Lt. Col. Larry Johnston, Fort Clayton, Canal Zone provided laboratory space and facilities on several occasions. Dr. Marvin Keenan and Dr. Melvin Boreham aided in many collections. In Colombia the cooperation of the Servicio Nacional de Erradicación de la Malaria, especially of Dr. F. Ferro and Dr. H. Ferrer-Ferrer, Sr. Gilberto Garces and Sr. Huberto Cubrillos is sincerely appreciated. In Brazil, the field and laboratory work would not have been possible without the excellent cooperation of S U C A M, which provided laboratory space, facilities, transport and excellent cooperation throughout Brazil. Particular thanks go to Drs. Ivan Ricciardi and Ernani Ferreira and to Sr. Luzenario Patrioto de Nasciamento of S U C A M, Rio. In Manaus, Dr. Agostinho Cruz Marques provided excellent facilites and help in collection of specimens. Sr. Nelson Ferreira Fé worked with us for parts of three years, both in the field and in the laboratory. Professor L. Cavalcanti of the Federal University of Rio de Janeiro provided laboratory space for 4 months in Rio de Janeiro, Sr. Ricardo I. Rios, also of U.F.R.J. worked daily with us during 1972 in Rio. It is also a pleasure to acknowledge the continuing cooperation of the Pan American Health Organization, both in Washington and in South America. Without the contacts and active cooperation of P.A.H.O., this work simply could not have been done. Especial thanks go to Drs. G. García-Martín and J. Nájera in Washington and to Dr. Glenn A. Fleming in Manaus. This research was supported by the University of Illinois, and by Grant E-3486, U.S.P.H.S., and Youngstown State University Research Grants 184 and 191.

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