INVERSION POLYMORPHISM IN THE SALIVARY GLAND CHROMOSOMES OF *ANOPHELES DARLINGI* ROOT

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Anopheles (Nyssorhynchus) darlingi Root, is a widespread and important vector of malaria in South America from Colombia and Venezuela to Argentina. It has also been reported from Central America.

Salivary gland chromosome maps are now available for seven neotropical species in the subgenus *Anopheles*: *atropos, aztecus, hectoris, neomaculipalpus, pseudopunctipennis, punctimacula,* and *vestitipennis* (Kitzmiller *et al.* 1967). Five other species, *bradleyi, crucians, punctipennis, quadrimaculatus,* and *walkeri,* primarily North American but with some extension into Mexico and Central America, have also been mapped. Within the subgenus *Nyssorhynchus,* maps have been published for *albimanus* and *aquasalis.* Maps are currently in preparation for several other *Nyssorhynchus* species (*albitarsus, argyritarsus, braziliensis, evansae, noroestensis, nuneztovari oswaldoi, and triannulatus*).

The salivary gland chromosomes of *Anopheles darlingi,* herein described, show many banding pattern homologies with the chromosomes of other species in the subgenus. The most striking feature of the populations studied was the presence of large amounts of inversion polymorphism leading to a number of different arrangements in the X chromosome and in all four autosomal arms.

MATERIALS AND METHODS. Salivary gland preparations were made from fourth instar larvae (French, *et al.,* 1962) collected during the summers of 1969 and 1971. Population samples were taken from Manaus, Alixia, and Itacoatiara in the state of Amazonas, and from Araraquara, state of São Paulo, Brasil. Preparations were examined, usually at 1000X, under a Zeiss phase contrast system. Permanent preparations were made using the dry-ice method.

DESCRIPTION OF THE CHROMOSOMES. In common with all anophelines thus far described, the salivary chromosome complement consists of a short X chromosome and two longer autosomes (Figs. 1, 2). Average measurements: X, 72 microns; 2R, 212 microns; 2L, 158 microns; 3R, 140 microns; 3L, 140 microns. Individual chromosomes may show considerable lengthening due to pressure, but the above values would apply to preparations which do not appear unduly stretched. The right arm of chromosome two is about 1⅓ times the length of the left; chromosome three is almost exactly metacentric.

Because of the banding pattern similarities to other species of the subgenus, the same numbering system has been employed. The X contains zones 1–5; 2R, zones 6–15; 2L, zones 16–25; 3R, zones 26–35; and 3L, zones 36–45. The lettered zones are completely arbitrary, but have been placed in certain areas to correspond with similar areas on the maps of *aquasilis* and *albimanus.* Figure 3 is the "standard" arrangement, drawn from the Manaus population.

X-CHROMOSOME. The telocentric X (Figs. 1, 2, 3, 4) is the shortest arm of the complement, about 75 microns. Noticeably thinner than the autosomes, it often stains weakly, in females as well as males. At the free end the staining intensity varies, but the single dark band at the end of 1A, and the two dark bands at the beginning of 1C are recognizable. The three slightly less intense bands at the end of 1B often are quite dark, as dark as the two following in 1C. The three bands at the start of 2A are characteristic although somewhat variable; the center one of the three bands

is usually thicker and sometimes appears double. The three dark bands in the middle of the puff in region 2 sometimes appear as an indistinct dark area. 2B is always dark, with some variability in staining intensity. The three thin dark bands

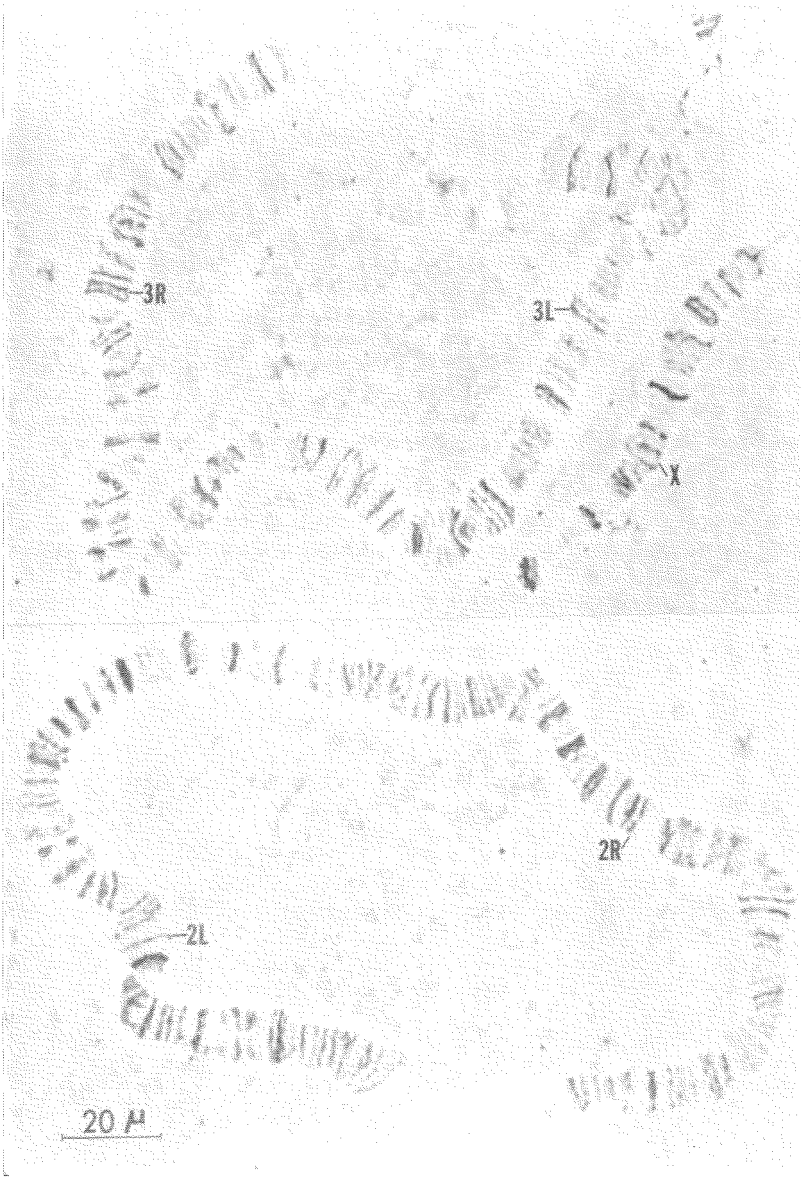

FIG. 1.—Chromosome complement of *Anopheles darlingi*, Manaus population.

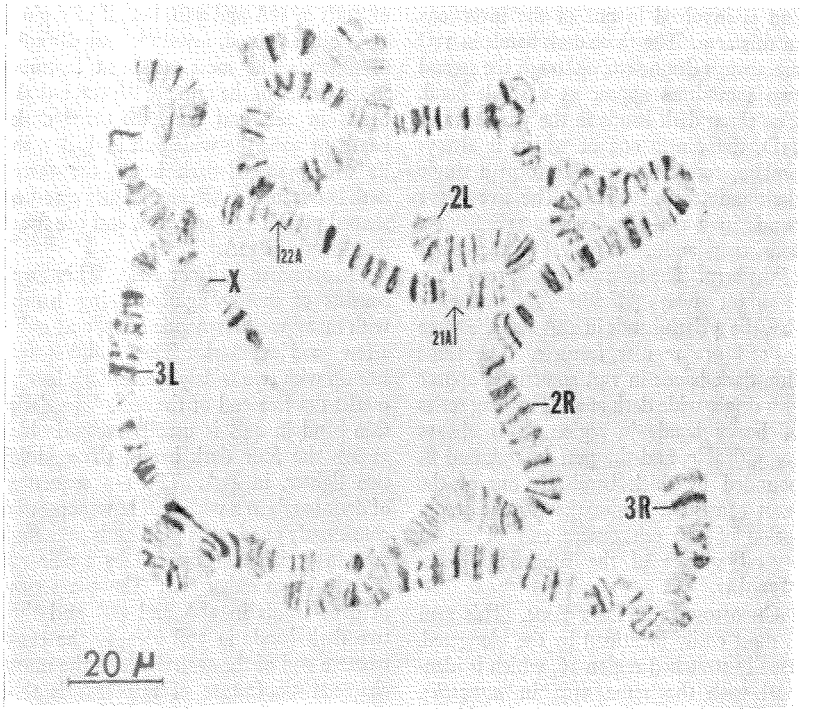

FIG. 2.—Chromosome complement of *Anopheles darlingi*, Araraquara population. Arrows indicate regions 22A through 21A which is found inverted in this population (In (2L)1).

in 3A often are very close and may appear as a single heavy dark area. The puff in 4A is uniformly of poor quality, twisted, lightly staining, asynaptic, but the two dark bands in this puff are almost always broken, as shown.

The dark band in 5A, preceded by a somewhat lighter one, is characteristic of the centromere end of the chromosome, as is the lightly-stained, stretched neck-like area in 5B. The three dark bands in a 1–2 pattern in 5B and 5C are always recognizable.

CHROMOSOME 2, RIGHT ARM. This arm is clearly the longest of the complement, about 1½ times the length of each of the other three autosomal elements. The centromere end is identical with *aquasalis* in regions 14 and 15, and with only a few differences in band intensity, the free end is also identical with *aquasalis* in region 6 and 7. This long arm is marked with several characteristic areas, as well as with several "weak" areas which appear to be break points for inversions. At the free end the puff in 7A with three thin dark bands at its beginning and two heavy dark bands at its end is typical. The three dark bands at the beginning of 6A are variable, and sometimes appear as a condensed, indistinct dark area. The five dark bands in 8A are followed by a wide light area, which terminates in a heavy narrow band. This area is uniformly of poor quality, and the following area 8C, tends to be the same. The two dark bands in 9A and the two in 9B are consistent landmarks, but the rest of area 9 is twisted, asynaptic, indistinct and difficult to follow. Region 10 is also often weakly-staining

and is involved in one of the inversions in this arm. The three dark bands in 11C are always distinctive, although the second two sometimes appear as a single band. The three dark bands in the wide area in 11D, the center one of which is always broken, sometimes seem to form a single dark indeterminate area. The five heavy bands in a 1–1–3 pattern in 11E form a consistent landmark, typical of the center of the arm. The following area, region 12, is almost always difficult to follow, except that the 4 dark bands in 12A and the three in 12B are regular features. The three thin dark bands in 13A sometimes appear as a single wide dark area. The long series of heavy bands in region 14 is always recognizable, and the puff in 15A and B, bounded by thick bands, is one of the most consistent landmarks for the recognition of the centromere end of 2R in several species of the subgenus *Nyssorhynchus.*

CHROMOSOME 2, LEFT ARM. This arm is most easily identified by the elongated, usually stretched region 16, which is identical with the same area in *aquasalis.* Several other easily recognizable blocks of bands are present, along with several indistinct areas, evidently associated with inversion breakpoints. At the free end of the arm a series of dark bands in regions 25 and 24 are separated by variable light areas. The three dark bands in 22A, the middle one broken, and the 7 dark bands in 22B and 21A are consistent in the Manaus arrangement, but are involved in two inversions in other populations. The two wide puffs in 21B through 19A, each with a pair of widely spaced heavy bands are usually distinctive, as are the three heavy bands in 19B. Regions 18 and 17 are typically composed of a long series of dark bands, in the pattern shown.

CHROMOSOME 3, RIGHT ARM. The right arm of chromosome three is about the same length as 2L and 3L. It can be most readily recognized by the lightly staining free end, with a pair of dark bands in 26A and 27A. At the centromere end the most easily identified region is the pair of puffs in 35A and 35B, but all of region 35 is often twisted, asynaptic and difficult to follow. The most consistent features in the center of the arm are the three dark bands in 28B and 29A, the three dark bands in 30A, the wide puff in 30B with its widely spaced dark bands, the three dark bands in 31A, the consistently spindle-shaped puff in 31B and 32A, and the four dark bands in 32A.

CHROMOSOME 3, LEFT ARM. This arm consists of mostly lightly-staining bands with many weak asynaptic areas, and only a few good landmarks. The usually spatulate tip with two widely spaced dark bands marks the free end of the arm. The dark thin band in 44B is usually recognizable as are the four dark bands, the middle two lighter, in 43A. The 1–2 sequence of dark bands in a wide puff in region 41 is consistently good, as is the lightly staining area in region 40, bounded by a pair of dark bands at either end. The 3–2 series of dark bands in 38A and 38B and the two dark bands in 37B identify the centromere end of the arm. Perhaps the most characteristic feature of this arm is the occurrence of several weak, asynaptic, lightly-staining areas, usually indicative of break points for inversions. The most prominent of these areas occur in 44B, 43C–42A, 42B–41A, 41B–40A, 39A–39B, and 36A–36B.

ABERRATIONS. In the limited number of populations which have been studied, nine separate inversions have been recovered; one in the X chromosome, three in 2R, one in 2L, and two each in 3R and 3L. The inversion in the X chromosome (In (1)1) is extensive and includes most of the chromosome beginning at 2A and ending before the last band in 4B (Fig. 4). This inversion has been found at a low frequency in the material collected in and around Manaus and near Alixia, Amazonas. The only arrangement found in the population from Araraquara, São Paulo, is the one shown in the map.

Three inversions have been recovered in 2R, all from the northern material. The longest, In (2R)1, involves regions 8A-8C;

FIG. 4.—Inversion in the X chromosome (In (1)1).

the shortest, In (2R)2, 10A–10B; and the third involves 12A–12B (In (2R)3) (Figs. 7 and 8). Inversion (2R)1 is not common in the heterozygous condition and the homozygous arrangement of this inversion (inverted from the arrangement shown on the map) is rare. Several, but not all, of the 27 possible combinations involving these three inversions have been recovered. No heterozygous inversions have been found in the Araraquara material, but the 2R arrangement there contains In (2R)1 homozygous.

In 2L, a complex inversion heterozygote presumably the result of two overlapping inversions, is quite common in the northern material (Fig. 5), but has not been found in the Araraquara specimens. Thus far only the complex inversion heterozygote and both homozygotes have been recovered, in a 1:2:1 ratio in field

FIG. 5.—Complex inversion heterozygote, Standard/Manaus, Manaus population.

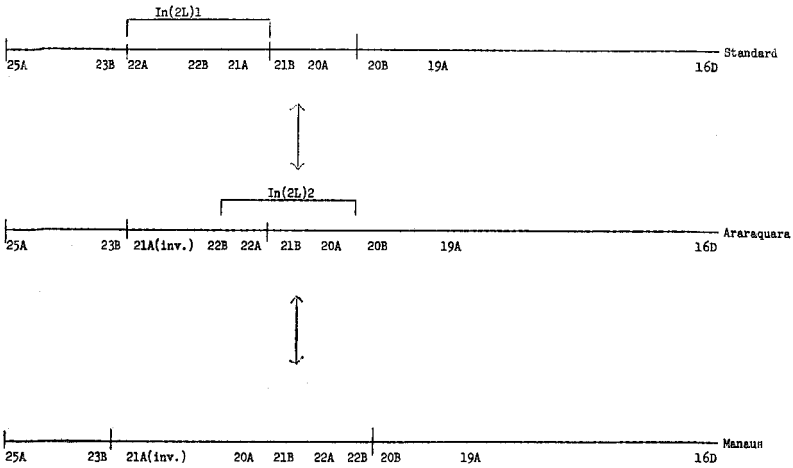

FIG. 6.—Genesis of the three arrangements in 2L.

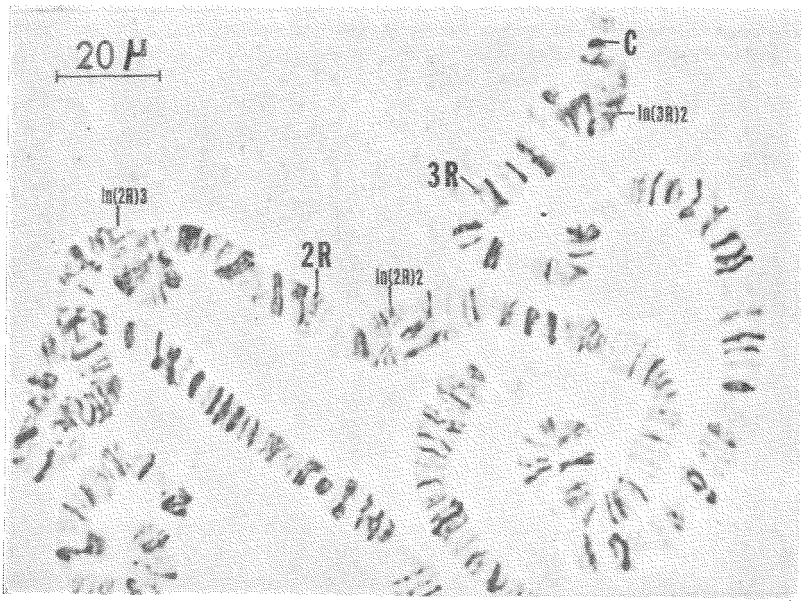

FIG. 7.—Inversions in 2R (In(2R)2), (In(2R)3) and inversion in 3R (In(3R)2). C is the centromere.

collected material from Manaus. Neither of the heterozygotes for the two simple inversions tentatively designated In (2L)1 and In (2L)2, has been found. No inversion heterozygotes have been recovered from the Araraquara material, but these specimens always show In (2L)1 in the homozygous inverted arrangement, and inversion In (2L)2 in the homozygous standard arrangement.

Two inversions occur in 3R. The first, In (3R)1 involves regions 28A–32A and the second, In (3R)2, regions 34B–35A (Figs. 7 and 9). In the Manaus population the standard arrangement as shown on the map occurs in about one-third of the slides, the ST/In (3L)1 heterozygote in about two-thirds. Inversion homozygotes are rare. Most specimens are as shown on the map for the In (3L)2 arrangement, but heterozygotes occur rarely and inversion homozygotes have not been found. No heterozygotes have been recovered for either arrangement from the Araraquara material, but In (3L)1 appears to be homozygous in the inverted order in all specimens.

Similarly, two inversions occur as heterozygotes in 3L. The first of these, In (3L)1 extends from 43A to 43C and the second, In (3L)2, from 40A to 38A (Fig. 9). In (3L)1 occurs in both the Amazonas and Araraquara populations with high frequencies but In (3L)2 has been found only in Manaus.

DISCUSSION. The most striking feature of the *darlingi* slides from Amazonas is the high frequency of inversion heterozygotes. Approximately 90 percent of the slides examined in Amazonas were heterozygous for one or more inversions. This abundant polymorphism was found only in the populations near Manaus, and was clearly absent in the limited sample from the south, at Araraquara. The latter sample was admittedly restricted but it should be interesting to sample populations from other parts of the range. The Amazonas populations, from near the center of the range of the species show an elevated polymorphism and the Araraquara sample, near the southern border of the geographic distribution, appears to show fixation of

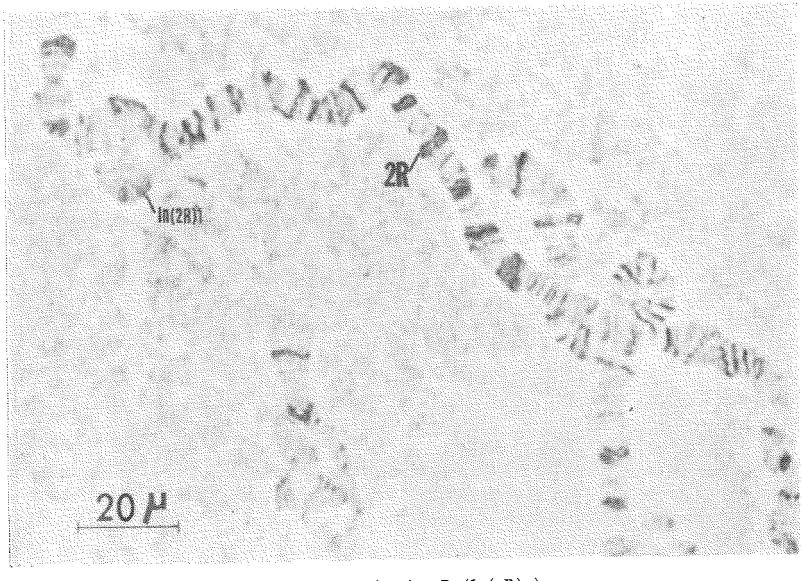

FIG. 8.—Inversion in 2R (In(2R)1).

certain arrangements, with reduced polymorphism.

Anopheles darlingi is an efficient vector of malaria in South America. Perhaps the chromosomal polymorphism is one factor which permits its widespread distribution (Central America to Argentina), its ecological ubiquity and its vector capacity under a wide range of conditions. One immediate problem which suggests itself is the examination of inversion frequencies in populations which are locally responsible for transmission.

The aberrations listed above have resulted in several different banding arrangements which differ markedly in the northern and southern populations. In the X chromosome, the arrangement shown on the map exists in both the Manaus and Araraquara populations, but In (1) 1 has been found only in Amazonas, not yet in the southern population. In 2R, three separate paracentric inversions result in eight different banding patterns. The sequence shown on the map is most common in the Amazonas material. In the specimens from Araraquara In (2R)1 has apparently become fixed so that these specimens all show In (2R)1 homozygous, inverted, from the sequence shown on the map, and In (2R)2 and In (2R)3 as shown.

In 2L, three different arrangements have been recovered; "Standard," "Araraquara"

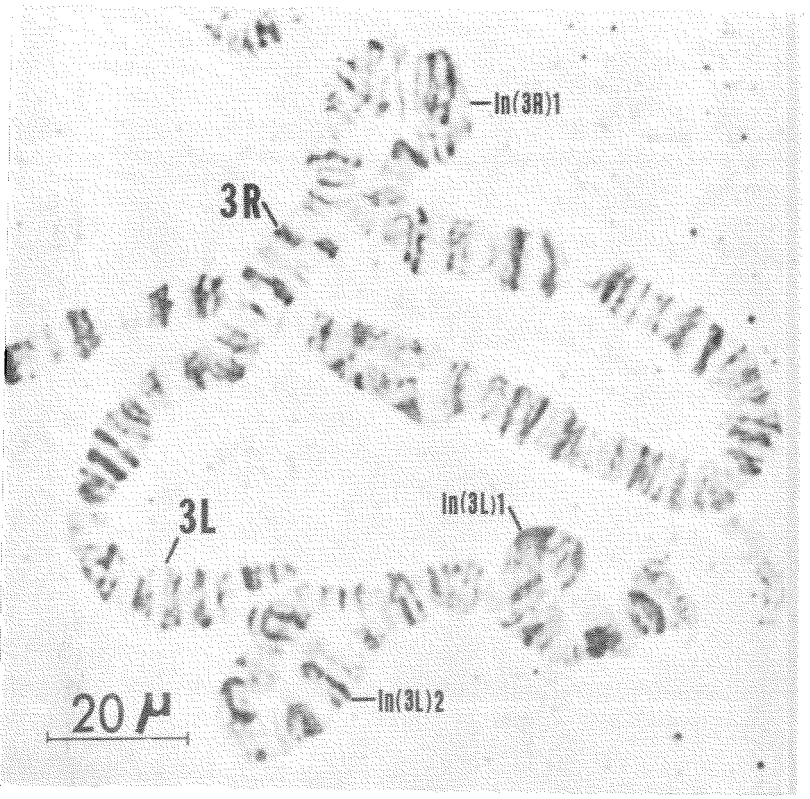

FIG. 9.—Inversion in 3R (In(3R)1) and inversions in 3L (In(3L)1), (In(3L)2).

and "Manaus." The Araraquara arrangement has been recovered thus far only from the southern population in the state of São Paulo; it may be derived from the standard arrangement by a simple paracentric inversion, herein designated as In (2L)1 (Fig. 6). The Araraquara arrangement might have also been derived from the Manaus arrangement by a different paracentric inversion, In (2L)2 (Fig. 6). The Araraquara arrangement is found only in the southern material, thus far it has not been recovered in Amazonas. The Standard and Manaus arrangements are found with approximately equal frequencies in the Amazonas slides, and the ST/ST, ST/M and M/M genotypes occur in about a 1:2:1 ratio. The heterozygotes, ST/M (Fig. 5). show a complex figure, the result of an overlapping inversion. The probable genesis of these arrangements is shown in Fig. 6. The Standard and Manaus arrangements may be derived, in either direction, only through an intermediate arrangement as shown in Fig. 6, but this intermediate arrangement has not as yet been recovered as a simple inversion in Amazonas. The inverted arrangement of In (2L)1, however, is the only sequence found in the Araraquara specimens. Two possible modes of origin of these three arrangements of 2L are possible. If the arrangement which we are calling Araraquara were the primordial one, then the Standard arrangement could have been derived by a simple paracentric inversion In (2L)1, involving 22A–21A of the map. Similarly the Manaus arrangement could have been derived by a paracentric inversion, In (2L)2, Zones 22B–20A. If, however, either the Manaus or Standard were the ancestral arrangement, then both inversions would have been required, one overlapping. The sequences, assuming a Standard-Araraquara direction would be as follows: (1) Inversion of 22A–21A (In(2L)1) to produce the Araraquara sequence (2) Inversion of 22B–20A (In(2L)2) to produce the Manaus arrangement. This sequence would leave 21A following 23B, and in an inverted order. The Manaus arrangement would be as indicated in Fig. 6.

Manaus and Araraquara are 1800 miles apart. The fixation of the In (2L)1 arrangement in the Araraquara population is understandable, but the absence of it in the north is strange. It would not be surprising to find this arrangement in Amazonas, as well as the other two. As yet neither the ST or M arrangements have been found in the south, nor is In (2L)1 heterozygous in this material.

In 3R, the Araraquara material shows only In (3R)1, homozygous and inverted from the mapped sequence. No standard or heterozygous arrangements have been found. Inversion In (3R)2 is always as shown. In the Manaus material heterozygotes for In (3R)1 are common, as is the standard arrangement, but homozygous inverted sequences are rare. In (3R)2 is almost always present in the arrangement shown on the map. Heterozygotes are rare, and the inverted homozygote has not been found.

The 3L inversions are interesting. In (3L)1 heterozygotes occur in about half the specimens in both northern and southern populations, most of the rest are the standard arrangement, and the homozygous inversion is rare in the north, apparently absent in the south. In (3L)2 occurs only in Manaus, is frequent in heterozygotes, rare as the homozygous inversion. It is apparently absent in Araraquara.

The subgenus *Nyssorhynchus* is subdivided in several ways by different authors. One commonly accepted scheme consists of two principal series, the *albimanus* and *argyritarsus* (Schreiber and Guedes, 1961, Cova-Garcia, 1961) series and their subseries. In this scheme *argyritarsus, albitarsus* and *braziliensis* are classified along with *darlingi* in the *argyritarsus* series. In the *albimanus* series, *albimanus* is in one subseries, *aquasalis, nuñeztovari* and *oswaldoi,* among others, are in another subseries. At the present time the exact relationships of the 20 species which comprise the subgenus

Nyssorhynchus are not clear; it becomes of interest therefore to examine the chromosomal homologies which are thus far available. Study of our *argyritarsus* slides from Brazil show a close relationship in banding pattern to *darlingi*. The free and centromere ends of all autosomal arms are similarly banded, and large sections of the interiors of both 3R and 2L are similar in the two species. Preliminary examination of our *albitarsus* slides shows definite similarities at the ends of all autosomal arms, but the central portions of the arms are less securely homologous. No study has as yet been made of our *braziliensis* material.

At the present time (April 1972) salivary chromosome maps are complete for *aquasalis, albimanus* and *nuñeztovari*. Careful comparison of *aquasalis* and *darlingi* shows some similarities, but not as extensive as ones with *argyritarsus*. In 2R the free ends correspond quite well with *aquasalis* through 7B with the exception that the three bands in the puff in 7A are always thinner in *darlingi*. The likeness may extend, with less certainty, through the first half of 8A. At the centromere end of the arm the two species are identical from 14A through 15C. This part of the arm contains several consistent recognition areas: a series of heavy bands in 14A; another heavy series in 14B, 14C and 14D; a stretched light area in 14E; and a typical puffed area in 15A and 15B, bounded by thick heavy bands at each end. This area, 14A–15C is usually so distinct in both species that it serves as the principal identification landmark for the centromere end of 2R. In *nuñeztovari* area 15 is undoubtedly the same as in *darlingi* and *aquasalis* and 14E is very similar. In *nuñeztovari* region 14 resembles closely an inversion of region 14 in both *darlingi* and *aquasalis*. At the free end of the arm, 6A–8A of *nuñeztovari* shows some similarities to the same region in the other two species, but not as clearly so. There are no similarities whatsoever between 2R of *albimanus* and 2R of *darlingi*. In 2L the only corresponding regions are 16A–16C in *darlingi* and *aquasalis*, possibly in *nuñeztovari*, none of the rest of the arm shows any likeness whatsoever. There are no apparent homologies in either 3R or 3L between *darlingi* and either *aquasalis, albimanus* or *nuñeztovari*. A few possibly inverted areas in the interior of 3L may be homologous between *darlingi* and *nuñeztovari* but must await further detailed study.

A preliminary calculation of the amount of homology between *darlingi* and the other three species is indicated below, assuming *darlingi* as 100 percent and the other values expressed as percent similarity in the species indicated.

	nuñeztovari	*aquasalis*	*albimanus*
X	0	0	0
2R	.22	.40	0
2L	.08	.14	0
3R	0	0	0
3L	0	0	0

These figures clearly suggest the most homology occurs in 2R, and that the order of genetic affinity would appear to be *darlingi—aquasalis—nuñeztovari—albimanus*.

SUMMARY. The salivary gland chromosomes of *Anopheles darlingi* are described and a standard map for the species is proposed. Nine inversions have been described. The genesis of an arrangement in 2L found only in material collected near Araraquara, São Paulo, Brasil, is described. This arrangement may tentatively be used to separate northern and southern populations of this species. The northern populations (Amazonas) are more polymorphic than the southern population; certain chromosomal arrangements are more common in the north, others are more common in the south. The inversion data support the hypothesis that chromosomal polymorphism is greater at the center of the range of the species and more limited at the periphery. The high amount of chromosomal polymorphism might possibly be linked with vector capacity.

ACKNOWLEDGMENTS. We wish to ac-

knowledge the generous support of the National Institutes of Health which supplied most of the funds for the work, including travel to Brazil, through Research Grant E-3486. The Pan American Health Organization provided invaluable local contacts in the field, and made arrangements in our behalf with the Campanha de Erradicacão da Malária in Brazil. Drs. G. Garcia-Martín and José Nájera of PAHO, Washington, D.C., were especially helpful. In Manaus, Dr. Agostinho Cruz Marques, Chief, Region II, SUCAM, provided laboratory space, vehicles, boats, field personnel, and unlimited cooperation. Dr. Glenn Fleming, PAHO entomologist at Manaus, aided in many professional and personal ways during both summers in Manaus. Mr. Nelson Ferreira Fé of the C.E.M. staff in Manaus, aided in many ways in field collections and in slide preparation. Dr. Ivan Ricciardi, PAHO, Rio, and Dr. Hesse Hobbs, AID, Rio, provided high level professional cooperation. Dr. Renato Corrêa, Chief of the service for the Eradication of Malaria and Prevention of Chagas Disease, State of São Paulo, provided access to *darlingi* areas near Araraquara, as well as transport and laboratory facilities. Miss Mary B. Knight, Miss Cristina Sena Mascarenhas and Mrs. Reva Beth Russell assisted in preparation of the slides. The drawings were made by Mr. Harold Meier.

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