THESIS APPROVAL FORM

THESIS TITLE: A COMPARISON OF THE SALIVARY GLAND CHROMOSOMES OF ANOPHELES EISENI AND ANOPHELES PERYASSUI

AUTHOR: Tammi Huggins

DEGREE: Master of Science in the Biology Program

ADVISOR: Dr. Richard D. Kreutzer

| COMMITTEE MEMBERS: | ACCEPT | REJECT |
|---|---|---|
| [signature] | ✓ | |
| James R. Taylor | ✓ | |
| Paul VanBrandt | ✓ | |
| [signature] | ✓ | |
| | | |

DATE 7/27/82

1 copy to accompany library copy

1 copy to advisor

1 copy to Departmental Files

ABSTRACT

A Comparison of the Salivary Gland
Chromosomes of Anopheles eiseni
and Anopheles peryassui

Tammi Huggins

Master of Science

Youngstown State University, 1982

The salivary gland chromosomes of many members of the subgenus Anopheles have been mapped, and their evolutionary relationships examined. The standard arrangements for two additional species Anopheles eiseni and Anopheles peryassui were proposed. There are two pairs of autosomes and a pair of sex chromosomes in each species. The X chromosome of An. eiseni is sub-metacentric. The X chromosome of An. peryassui is sub-telocentric. The two pairs of autosomes of each species are metacentric. There was one rarely occurring inversion found in the right arm of chromosome three in An. eiseni.

The chromosomal banding patterns of the two species show similarities to each other most closely in the third pair of autosomes. The banding pattern of An. eiseni resembles that found in An. punctipennis, An. earlei, and An. aztecus most closely. The greatest degree of similarity of An. peryassui exist with An. punctipennis, An. perplexensand An. aztecus. These comparisons suggest a possible evolutionary relationship among the aforementioned species.

ACKNOWLEDGEMENTS

I would like to express my appreciation to Dr. Richard D. Kreutzer. His assistance and guidance in obtaining the data, developing photographs and writing the final paper was invaluable.

In addition I would like to thank Anna Pizzoferrato whose artistic talents drew the final salivary gland chromosome maps and Carol McGuinness for typing the final draft of the thesis.

TABLE OF CONTENTS

# LIST OF FIGURES

# CHAPTER I

## INTRODUCTION

Significant amounts of additional data have been compiled since the initial cytogenetic studies by Frizzi on anopheline chromosomes. Much of these data are concerned with evolution, speciation and taxonomy within a large species group referred to as the "Maculipennis Complex" (Kitzmiller, Frizzi and Baker, 1967).

The classical "anophelism without malaria" situation in some areas of Southern Europe gave impetus to these studies. The major vector, Anopheles maculipennis, is widely distributed throughout the entire region, but there was irregular malarial transmission.

Distinct ecological differences were shown to exist among the various groups, but differences in egg morphology were among the first relatively consistent differences noted. Crossing experiments demonstrated distinct differences with respect to crossability. These crossing experiments, discussed below, demonstrated clearly that groups were at least partially reproductively isolated, and that gene flow between them was absent or reduced. Bates (1940) recognized five species and two subspecies, a classification based on reproduction isolation, which has generally been accepted.

Although the ecological, physiological, and reproductive differences were clear and distinct, the members of the various races (adult males, females and larvae) could not be separated morphologically. Thus the "Maculipennis Complex" became one of the classic examples of a group of sibling species--species which were morphologically similar but with well defined ecological, physiological and genetic differences.

Additional studies on sibling species and a summary of similar data have been made by Mayr, 1963.

Frizzi (1947a, b, c, d, 1949, 1950, 1951, 1952, 1953a, b) was able to demonstrate that these sibling species could be separated on the basis of the banding pattern in the salivary gland chromosomes, each population of these sibling species had its own characteristic inversions and rearrangements. These differences in banding pattern morphology are important as a taxonomic tool and the study of evolutionary mechanisms in natural populations.

Chromosome Studies of Palearctic Species

The "Maculipennis Complex" may be divided into at least two geographical groups, Palearctic and Nearctic. The original studies were made with Palearctic species Anopheles labranchiae (subspecies An. labranchiae and An. atroparvus), An. maculipennis, An. messeae, An. melanoon (subspecies An. melanoon and An. subalpinus), and An. sacharovi. Work by Kitzmiller and Baker has chiefly concerned the nearctic species Anopheles aztecus, An. earlei, An. freeborni and An. occidentalis which constitute a closely-related group. Cytogenetic studies at first suggested that Anopheles quadrimaculatus and An. punctipennis also might belong to the Nearctic branch of the complex.

Another group of Nearctic species is found chiefly in the southeastern portion of the United States. Although not considered part of the "Maculipennis Complex", these species, Anopheles bradleyi, An. crucians, and An. geogianus form a species group with distinct morphological similarities. There are also three additional species found in the Southeastern United States, Anopheles atropos which prefers slightly

saline water for development, Anopheles perplexens which is morphologically similar to An. punctipennis and Anopheles walkeri.

Frizzi (1947) which reported that the salivary gland cells in An. atroparvus show five chromosome elements connected by a chromocenter. The first of the two V-shaped chromosomes consists of a "right" arm (2R) measuring 96 micra and a "left" arm (2L) measuring 102 micra. The second V-shaped chromosome consists of a "right" arm (3R) measuring 116 micra and a "left" arm (3L) measuring 78 micra. The X-chromosome appears thicker and better defined in salivary glands of female larvae than male.

The An. atroparvus pattern was selected by Frizzi as the "standard" chromosome banding pattern. Various chromosome rearrangements are shown by An. labranchiae, An. maculipennis, An. subalpinus, An. melanoon, and An. sacharovi from the standard pattern of An. atroparvus. An. maculipennis, An. subalpinus, and An. melanoon have a rearrangement in the right arm of the third chromosome, An. sacharovi has a rearrangement in 3R as well as an extensive rearrangement in the X-chromosome, and An. labranchiae shows only eight differences.

An. atroparvus and An. labranchiae show physiological, morphological, crossing and chromosome patterns which indicate a close relationship. Bates' designation of them as subspecies has been justified indicated by these similarities and their distinct geographic distributions. An. maculipennis, An. messeae, An. subalpinus, and An. melanoon appear more closely related among themselves than to the others, and An. messeae appears more distinct because of the inversion of the X-chromosome using chromosome studies.

Anopheles claviger (Kitzmiller and Coluzzi, in preparation) and Anopheles algeriensis (Kitzmiller, 1966) chromosomes were mapped. These species do not belong to the complex taxonomically, although they exhibit clear chromosomal similarities with An. atroparvus.

Chromosome Studies of Nearctic and Neotropical Species

The banding patterns of the Nearctic members of the "Maculipennis Complex," An. freeborni, An. occidentalis, An. aztecus, and An. earlei, show definite similarities in banding patterns to that of An. atroparvus. Several long areas near the centromeres, large internal areas of each arm, and all chromosome ends can be homologized with An. atroparvus. The X-chromosome, however, is quite different in each species, and all anophelines thus far studied can be identified by using the banding patterns of the X-chromosomes.

Another species, An. quadrimaculatus, is sometimes included in the complex on purely taxonomic grounds (Bates, 1940). Chromosome studies (Klassen, et al., 1965) tended to verify this placement. The free ends of the autosomes and long areas within them are quite similar to An. atroparvus. Chromosomal studies show autosomal homologies between An. punctipennis and An. atroparvus although most taxonomists consider An. punctipennis widely separated from the complex. These similarities plus certain larval taxonomic similarities prompted a tentative inclusion of An. punctipennis in the "Maculipennis Complex".

This study deals with two additional species of the genus Anopheles which are distributed primarily in the northern portion of the Neotropical region. An. eiseni is found in Central America, Mexico, Colombia, Venezuela, Trinidad, Tobago, Surinam, French Guiana, Brazil, Bolivia, Peru and Ecuador.

An. peryassui is found in Brazil, Guianas, Venezuela, Colombia, Peru, and Bolivia.

An. eiseni and An. peryassui differ in larval form in that the frontal head hair is palmate with leaflets serrated in An. eiseni and leaflets smooth in An. peryassui. The wings of adult An. eiseni are almost entirely dark while in An. peryassui the wings are mostly pale.

Some additional species of Anopheles, namely An. pseudopunctipennis, An. vestitipennis, An. hectoris, An. neomaculipalpus, An. punctimacula and An. apicimacula from Mexico and Central America show striking autosomal similarities not only among themselves, but to the North American "maculipennis" forms, especially to An. quadrimaculatus and An. freeborni.

All of the above-mentioned species, both palearctic and nearctic have a basic taxonomic similarity in addition to the chromosome one, they belong to the same subgenus, Anopheles. There exists a common autosomal pattern in the subgenus, Anopheles, with specific pattern differences in the interior of the autosomes the result of rearrangements, mostly paracentric inversions.

The data from salivary chromosomes indicates that the banding pattern of the autosomes is subgeneric in character and chromosomal differentiation within the subgenus has been accomplished by means of inversions and other rearrangements. The "Maculipennis Complex" designation for these chromosomally-related species should probably be replaced by a subgeneric one.

# CHAPTER II

## MATERIALS AND METHODS

### Collection of Specimens

Larvae of An. eiseni were collected in the Mojinga Swamp near Colon, Republic of Panama. These larvae are commonly found in pot-holes, rockholes, streamed pools, ditches and bromeliads. The larvae can be identified by the branched lateralhair, number six, on the third abdominal segment, palmate hairs present on the second abdominal hair and the palmate hair with serrated leaflets.

The adults of An. eiseni are a common jungle species that rarely enter homes. They attack man during the early evening hours. The adults have four white spots on the wing of an otherwise dark scaled wing and fringe. A pale spot or band is present on the hind tibia.

The larvae of An. peryassui were collected near Villavicencio, State of Meta, Colombia. These larvae often inhabit shaded water of fresh water jungle pools, swamps and stagnant streams. The larvae can be identified by the presence of dichotomous branching of inner and outer clypeal hairs, the hairs of the inner clypeal are well separated, and the palmate hairs have smooth leaflets.

The adults feed on man when the jungle habitat is invaded. They are most prevalent at the end of the rainy season and are malaria vectors. An. peryassui adults can be identified by study of the hind tarsal segments and wings. Hind tarsal segments three-five are not entirely white, hind tarsal segment two is 9-20% dark. The wings are largely pale.

Larvae were identified and a few from each species were allowed to mature to the adult stage. The adults were then also specifically identified allowing for a check of the larval identification.

Preparation of Chromosomes

The salivary gland dissections were made in 45% acetic acid. To remove the salivary glands pressure was applied just posterior to the thorax, the head removed and the dissecting needle inserted under the cuticula from the anterior. The incision was made by rubbing a second needle over the inserted one. The interior of the thorax was then exposed by opening the incision, removing the gut and the salivary glands were removed. After removal from the thoracic cavity, the salivary glands were placed in a drop of lacto-aceto-orecin on a siliconized coverslip. Depending on the species, the concentration of stain and the staining period varies. These variations are to be expected when dealing with different species, and have also been observed with a number of other Neotropical anophelines (French et al., 1967, Coluzzi, 1968). An additional procedure that varies with the species is the pressure applied to the coverslip needed to rupture the nuclear membrane and spread the chromosomes.

The "dry-ice" method was used to make the preparations permanent. The slides were placed on dry-ice for about 10 minutes, the siliconized coverslip "flipped off" with a single edge razor blade and the tissue then dehydrated for a period of one minute each in two changes of absolute alcohol. Zeiss "Eischlussmittel L 15" was used as a mounting medium. After drying, the slides were ready to be studied.

Chromosome Study

Preparations were examined usually at 1000X using a Zeiss phase contrast system. The map was diagrammed from standard photographic enlargements, and details of the banding patterns were filled in by direct observation at 1000X with the Zeiss phase contrast system.

# CHAPTER III

## DESCRIPTION OF CHROMOSOMES

### Anopheles eiseni

All species thus far mapped belonging to the subgenus Anopheles show clear homologies in each of the autosomal arms, particularly at the free ends and at the centrome regions. The internal part of each arm may be modified, but the changes which have occurred may be postulated at least theoretically, by comparison of banding patterns.

The salivary gland complement consists of three pairs of chromosomes, a short pair of sex chromosomes and two pairs of longer autosomes (Figure 1). The chromosomes are weakly attached to each other by connectives that join at the centromere regions. It is rare to find the chromosomes radiating from a single point. The various arms are seen with the centromere regions in close proximity.

The chromosome complement consist of a short subtelecentric, XR and XL, chromosome, 63 micra. The right arm, 2R, of the metacentric second arm averages 150 micra, the left arm, 2L, of the second arm averages 135 micra, the right arm of the submetacentric third arm, 3R, averages 215 micra, and the left arm of chromosome number three, 3L, averages 100 micra (Figure 2). These chromosomes are approximately the same length as An. perplexens.

The numbering system for the arms of An. eiseni is the same one used for other anopheline mosquitoes. The X contains zones 1-5; 2R, zones 6-14; 2L, zones 15-21; 3R, zones 22-32; and 3L, zones 33-39. The lettered zones are completely arbitrary. They have been placed

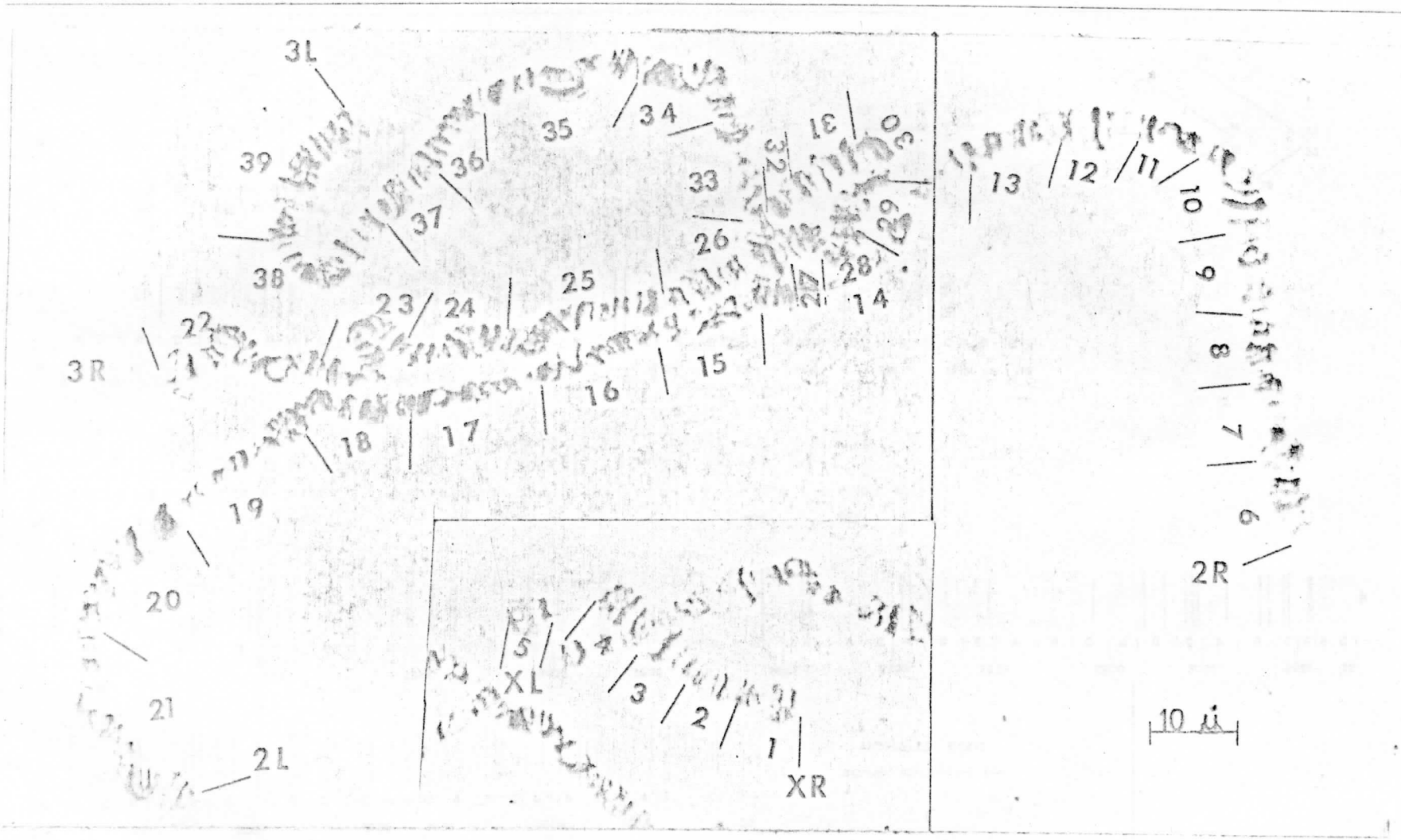


Figure 1. Salivary Chromosomes of Anopheles eiseni

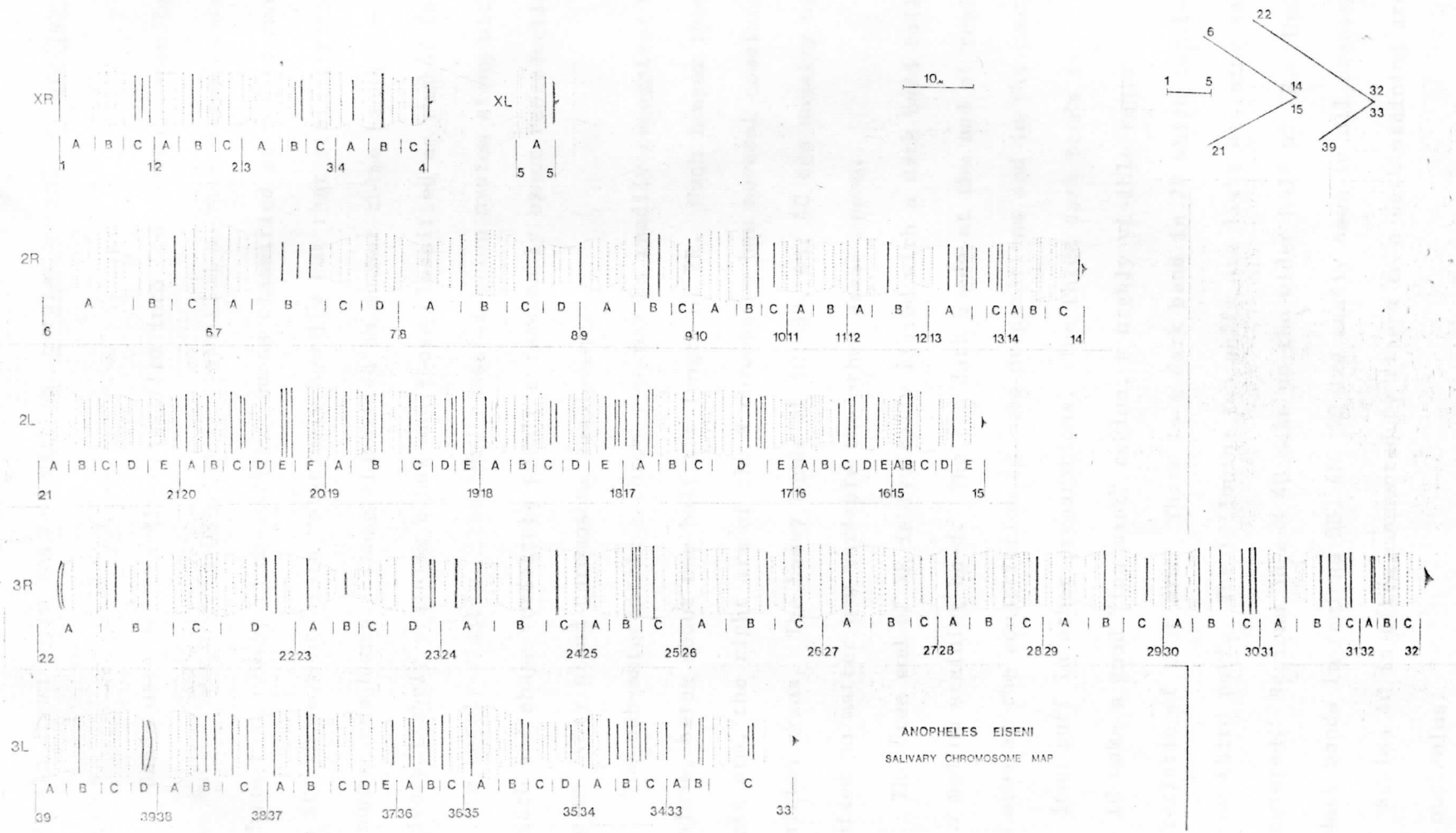


Figure 2. Anopheles eiseni Salivary Chromosome Map

in certain areas to correspond with An. perplexens and An. bradleyi sequences.

Bands were arbitrarily classified into four types, depending on the intensity of staining. Bands classified as number one bands are those that always stain darkly. Bands classified as number two bands are those that stain relatively heavily but that stain lighter than number one bands. Those classified as number three bands are relatively lightly staining bands and those classified as number four bands are barely visible. The band classification system along with the width and shape variations permit a reasonably exact reproduction of the salivary gland chromosome complement.

The sub-metacentric X chromosome may be readily recognized by size alone, being about one-half the length of 2R. Much longer than the left arm, the right arm of the X chromosome has several consistent recognition areas. The heavy bands of 1C, 3B, 4B, 4C can usually be recognized no matter how variable the other areas appear.

The free end of XR is typically flared with a dark band followed by a medium staining band. The two dark bands at the end of region 1A are seen at the constriction in some preparations and in the center of one long puff in other preparations. The three dark bands in region 1C make a good "landmark" region, a highly visible region characteristic of that arm. There is a dark band in 2B which terminates the third puff. The following two puffs are light staining and often twisted. Regions 3B and 4C make up the wider half of XR. The thick dark bands in regions 3B, 4B, 4C are easily seen in all preparations. Region 4C is often connected by fibers to a dark staining area in the nucleolus.

The shorter arm of the X chromosome, XL, consists of a dark band followed by 5 light bands. One region, 5A, makes up this flared piece, the XL chromosome.

The right arm of chromosome 2 contains several distinctive, consistent regions which make its identification certain. Perhaps the most prominent features are triplet bands in 7B, the pair of heavy bands in the center of the puff of region 9B; and the series of dark bands in regions 13A, B, and C. There is considerable variation in puffing patterns among preparations of the same species. The free end unlike that of most other members of the subgenus Anopheles is not expanded into a bulb-like puff but rather is slightly flared and lightly staining. The most prominent band of the free end is in region 6C. Regions 6C to 8B are often elongated and twisted. 7B is always a dark staining region because there is a triplet of three heavy bands. The puff in regions 7C and D is a difficult one to identify because it is lightly staining and elongated. The two dark bands in the center of the puff in 9B also make a good landmark. The puff of regions 10A and B is highly visible because it begins with a pair of dark bands in a 2-1 series of region 9C and ends with two dark bands in 10B and C. The pair of dark bands in the center of this puff stains somewhat lighter. The dark bands in regions 12A and B sometimes stain with medium intensity. Regions 13A through 13C are consistently dark staining and appear as shown on the map. The centromere region, 14C, consist of a triplet of medium bands followed by a closely spaced light band and this region terminates with a single dark band.

The left arm of chromosome two contains several distinctive, consistent regions which make its identification certain. The most distinctive features are the puff in 20E banded by three heavy bands; the three heavy bands separated by regions 19D and E; and the series of dark bands in 18E and 17A. Identification of the free end, the center of the arm and the centromere end can begin from these points. The pair of dark bands in a constriction at the beginning of 21B sometimes appears as one band. The light bands of regions 21A through 21E appear with variable intensities; in some preparations they are darker than indicated on the map. The most prominent feature of the free end is the series of dark bands of 20A through 20C. The puffs in these regions are often variable. The three dark bands in region 20E often appear as one thick, dark staining area. The three dark bands in 19C are also commonly seen as one dark staining region. There is a prominent puff in region 19D, E and 18A. The ends of this puff are dark staining composed of a series of dark bands while the middle of the puff is of medium staining intensity. This puff makes an excellent landmark. The puff in 18A and B is variable in width and staining intensity. Regions 18E and 17A are easily recognized due to the intensity of staining, although it is difficult in some preparations to distinguish the number of bands present. The triplet of bands in 17C often appear as broken dark bands. The three heavy bands in the small puff of 17C appear at times to be two heavy thick bands. These bands can only be distinguished in good preparations. The bands of 17E, 16A and B are closely spaced and difficult to determine intensity, but they usually appear as shown on the map. The

series of heavy bands in region 16C make a good landmark. There is a closely spaced pair of two bands in 15A followed by a closely spaced pair of dark bands of 15B which often appears as one band. 15C is consistently a clear area. Region 15F is the light staining centromere region.

The right arm of chromosome 3 is the longest arm in the complement, as it is in all other members of the subgenus Anopheles studied thus far. It shows many areas which are consistently found in other species of the subgenus. The free end, starting at region 22 has a typical flared tip with two heavy bands, followed by three characteristic puffs. Regions 22A through 23A are very similar to the corresponding regions of An. punctipennis. The closely spaced bands of 22C can be seen in detail in only good preparations. The pair of dark bands in 22D are consistently prominent; while the dark band in 23A is not as consistently prominent, in some preparations it appears much lighter. The single dark band at the constriction of 23B is easily recognized. The puff that begins in region 23C and D is a light one but as this puff expands it stains increasingly darker. 23D contains three dark bands that are easily recognized. The pair of dark bands at the end of 23D often appear as one thick dark band. The same phenomena occurs in 24A with a triplet of dark bands. The thick band in 24B is an excellent landmark. The center band of the puff in 24C is a thick heavy band also an excellent landmark, which is one of the best places to begin identification of this area of the arm. Region 25B contains a triplet of thin dark bands that often appears as a single dark staining band. This triplet also appears in An. quadrimaculatus. The bulbous puff of 26C with the single dark band in the center of the puff is an excellent

landmark. The two dark bands of 28A are a prominent pair seen in all preparations. Regions 28B and C are often twisted and folded. The following regions 29A through 30B are also twisted, folded, and indistinct. These regions can be seen in a few preparations as an inversion (Figure 3). The inversion heterozygote occurs infrequently. The break points of the inversion are between the pair of medium bands in 29A and between the pair of light bands that are split between regions 30B and 30C. A triplet of heavy bands follows and terminates the indistinct folded area of the inversion. These three bands can be seen in the corresponding region of An. atropos. There are two dark bands in 31B and two dark bands in 31C, these often appear as one band. Regions 32A, B, and C are consistently weak. Only in good slides do the bands appear as shown. Atypical of the subgenus Anopheles the centromere region is one of lightly staining intensity.

The left arm of chromosome three follows the general conservative pattern for this arm exhibited by other members of the subgenus Anopheles. The some what flared free end consists largely of medium to light staining bands. The most easily recognized region of the free end lies in 39D. There is a characteristic "birdseye" region in 39D and 38A. This region consists of a medium staining band followed by a dark band at the constriction and a pair of concave bands in the next puff. The first of the pair of concave bands is the darker of the two. The rest of the puff in 38A is often indistinct. The next prominent area is in 37A and B. Here there is a triplet of dark bands in 37A followed by a pair of heavy bands in 37B. The puff of regions 37C, D, and E is often twisted and indistinct. The indistinct area ends with a thick dark band in 37E. Regions 36A through 35B form a diagnostic

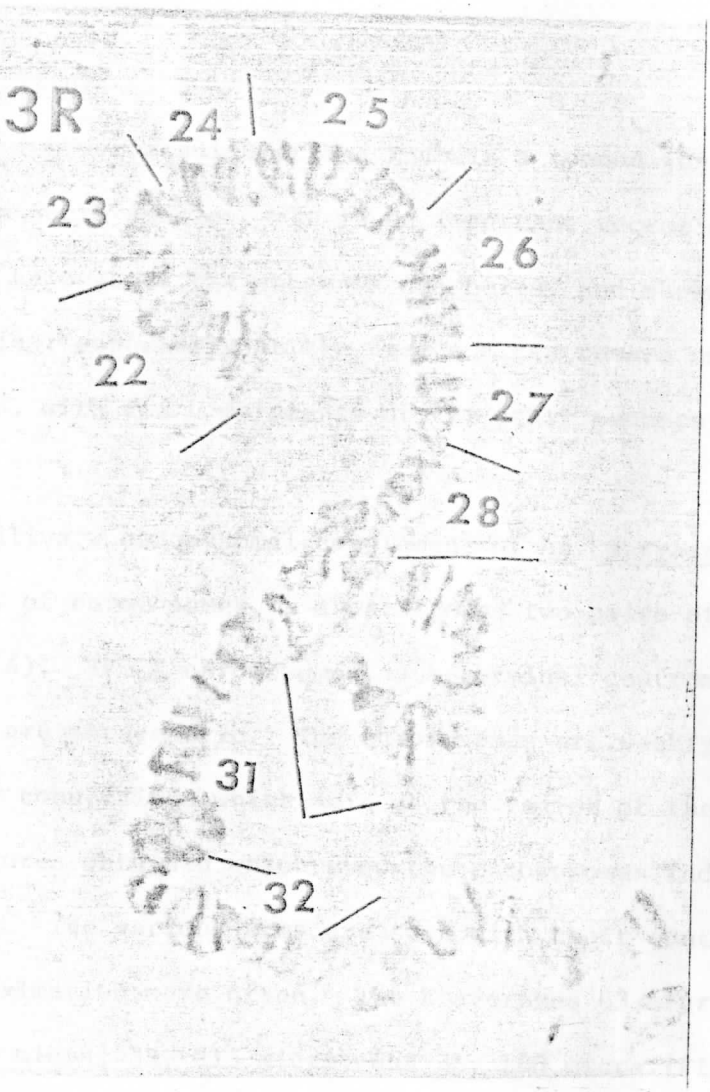


Figure 3. 3R Inversion of Anopheles eiseni

area for the center of the arm. The following regions 35C-33C are often stretched, variable and indistinct. The dark bands present in these regions are few and thin. The centromere region of 33C consists of a 2-3-2 pattern of band followed by a pair of heavy bands and finally a pair of light bands.

Anopheles peryassui

Anopheles peryassui Dyar and Knab is a common jungle species of Central and South America. It is an important vector of malaria. An. peryassui belongs to the subgenus Anopheles, and shows clear autosomal homologies, especially at the free and centromere ends of the autosomal arms, with the complements of the other members of the subgenus.

The salivary chromosomal complement of An. peryassui consists of three pairs of chromosomes, a short X, and two pairs of longer autosomes (Figure 4). The X chromosome has a terminal centromere, while the autosomes are metacentric. The chromosomes are weakly attached to one another by connectives which join at the region of the centromere. Rarely are figures obtained which show the chromosomes radiating from a single point. The various arms are seen with their centromere ends in close approximation more often. The X averages 63 micra; 2R, 150 micra; 2L, 135 micra; 3R, 215 micra; and 3L, 160 micra (Figure 5). The same numbering system was employed because of similarities in banding pattern with other members of the subgenus Anopheles. The X contains zones 1-5, 2R, zones 6-14; 2L, zones 15-21; 3R zones 22-32, and 3L, zones 33-39. Lettered subdivisions of the numbered zones are arbitrary.

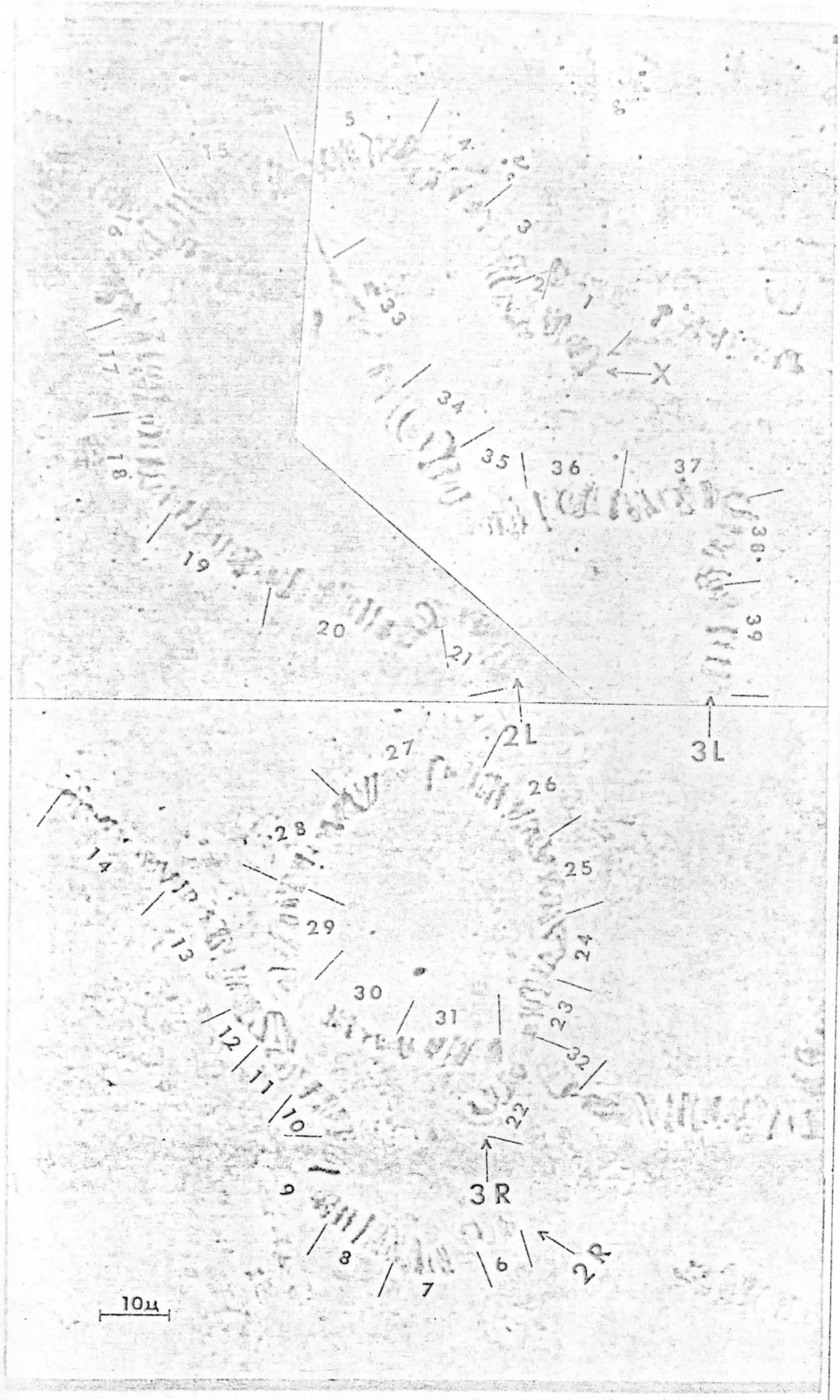


Figure 4. Salivary Chromosomes of Anopheles peryassui

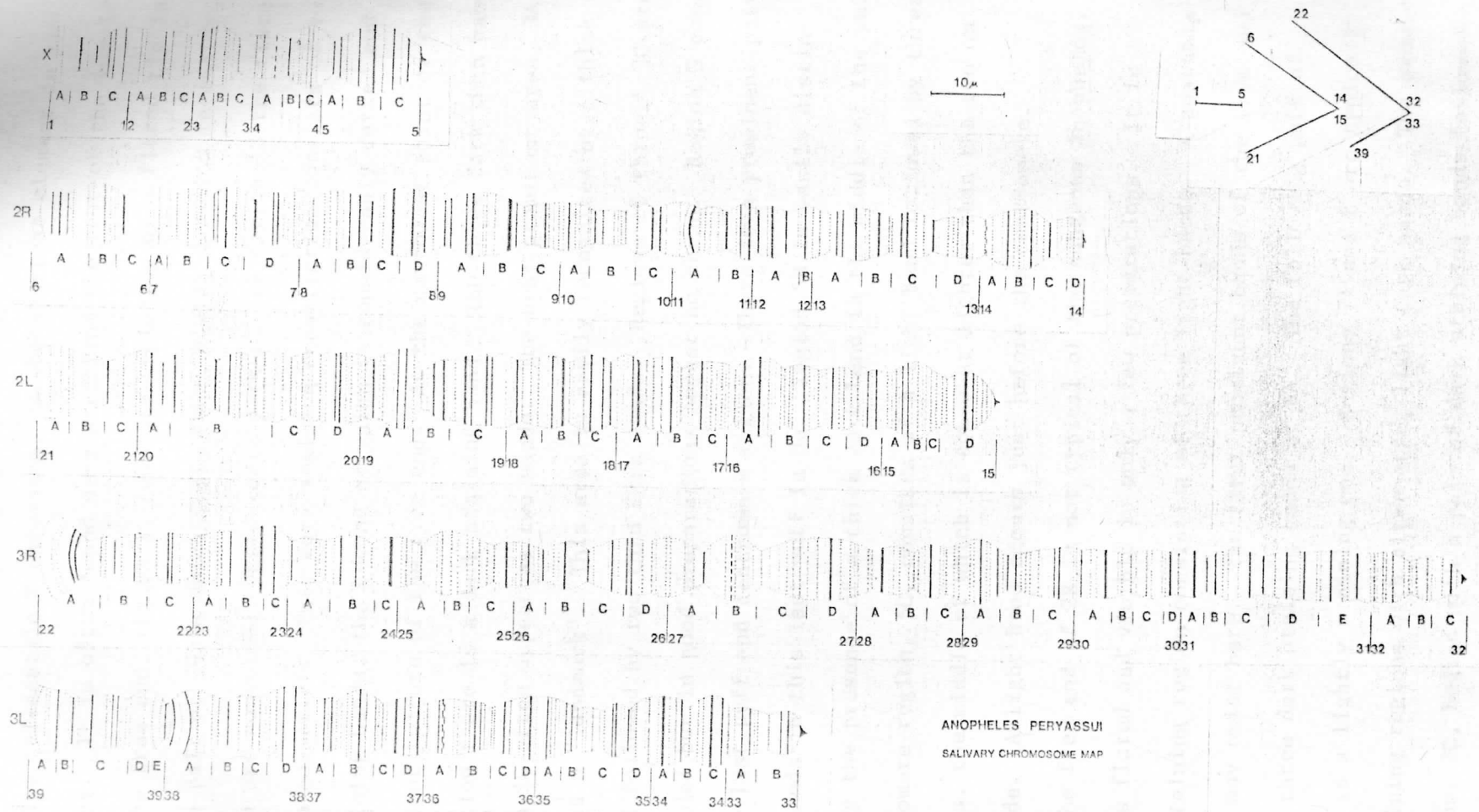


Figure 5. Anopheles peryassui Salivary Chromosome Map

The telecentric X chromosome is the shortest element in the complement. It is often found separated from the rest of the complement. The free end, region 1, consists typically of a flared tip in 1A with a pair of bands which stain usually with a medium intensity but which are sometimes considerably darker. In region 1B the flare begins to constrict. This dark staining constriction consists of two dark staining bands, the second of which is considerably darker and is a consistent feature of the free end. At the narrowest point of the constriction there is a dark staining band. The three dark thin bands in region 1C often appear as two bands. The next prominent area, 3A represents a "landmark". This area is easily recognized by a thick dark band followed by two thin dark bands. Regions 1C through 3C are often folded but in good preparations appear as shown. Region 5 contains the last puff and centromere region. There is a prominent pair of dark bands in this last puff in 5A. Region 5B is easily distinguished by the presence of a thick dark band in the middle of the puff. The centromere region, 5C, consists of a light band followed by three dark bands, the middle of which is somewhat lighter than the two on either side. A light band occurs just before the centromere.

The free end of 2R is not typical of the subgenus Anopheles. The end is flared and visible in only a few preparations. It is a lightly staining region consisting of three light bands. A possible inversion may exist here. The first prominent bands of the free end are those three dark staining bands of 6A. The following puff of region 6B is a lightly staining one. Regions 7A and B are typically light staining regions with alternating light dark bands. The prominent region, 7C, begins with a pair of dark staining bands followed

by a third thicker dark band. Region 7D, also distinct, consists of a thick dark staining band between two puffs followed by a pair of dark staining thin bands. Regions 8B, C and D contain prominent dark staining bands. The thick dark band, the first of a pair of dark bands in region 9B, is a good landmark. Regions 8C through 9B are consistently very wide puffs. These regions are reminiscent of those found in An. punctipennis. The pattern is not similar but the dark bands are there. Region 9C is the beginning of a much thinner puff. 10B is also a thin region. Regions 9C through 10B are often twisted and indistinct. There are two dark bands in the wider puffed region of 10C. Region 11A is characterized by a pair of dark staining convex bands, the first of which is the lighter of the two. There is a short wide puff in 11B with three dark staining bands. Region 12A consists of a puff with three dark bands. The middle dark band is the most obvious of the three. The broken dark band in 14A is easily recognized. Region 14B is highly visible and here there are two dark bands engaging three lighter bands. The lightly staining centromere, region 14D, is often not seen at all.

The free end of 2L is typically spatulate with a single heavy band in 21C. There is also a single dark band in region 21B. 21C begins with a prominent band at the constriction. Regions 21A through 20B are very similar to An. punctipennis, An. atroparvus and An. perplexens. Region 20D is easily recognized by the pair of dark bands flanked on both sides by a pair of light bands. Region 19A is expanded into a wide puff with two dark thin bands forming an always recognizable area. There are two pairs of dark staining bands, in each the first is

the thicker of the two in the easily recognized area of 19C. There is a triplet of dark staining bands in 18C. 17B contains a prominent dark band. Region 16A is expanded into a wide puff with a prominent pair of dark bands. Regions 16B through 16D are usually twisted and indistinct. The centromere region, 15D, is similar to that of An. perplexens. 15D begins with three light bands followed by a wide heavy band, terminating with several light bands.

The right arm of chromosome three is generally similar to 3R in all other species of the subgenus Anopheles. The free end and the centromere of the longest arm are clearly homologous with several other species.

At the free end of the arm, the characteristic pattern of a flared tip, followed by three small puffs is present. A pair of light bands followed by a pair of convex dark bands make up the flared end of 22A. There are two distinctly dark bands in 22B. The thick dark band in region 23B is an excellent landmark. Region 24A is always a poor region appearing to be a constricted region of light staining bands; although a closer look demonstrates a series of 3-1-4-1-4-3 bands in this region. There is a thick dark band in region 24C that is characteristic in all specimens. Region 26C begins a puff that appears like two puffs in many slides. Region 27B contains a very obvious region that consists of a ball-shaped puff and a broken dark band through the center of the puff. This band is surrounded on both sides by two number 2 bands. There are two light bands that terminate the puff. Banding pattern is difficult to determine in regions 27C through 29B because there are many bands that are closely spaced. Region 27D contains the most obvious portion of an elongated puff

with a dark thick band in the center of the puff. The dark band of region 28A can be used as a good landmark because of its thickness. Region 28C sometimes appears constricted and sometimes as a puffed region. An elongated region of the chromosome arm begins in region 29B and continues through the centromere of the arm. The puffs are often indistinguishable in regions 30A-centromere. The bands are consistently as shown on the map. The heavy bands in regions 30 and 31 are easily recognized. Region 32 is similar to the corresponding region of An. punctipennis. There are a series of number two bands terminating in a constriction of 32A. 32B consists of several medium staining bands while 32C has one dark staining band in the centromere region.

The left arm of chromosome three retains, in An. peryassui, the essentially conservative pattern found in other members of the subgenus Anopheles. Regions 39A-39C are similar to that of An. punctumacula. The free end is somewhat flared beginning with a pair of broken medium staining bands followed by a pair of light staining bands terminating with a thin dark staining band. The first puff begins in region 39D. The pair of two bands of 39E, the dark band at the constriction and the pair of concave bands of region 38A form an area, "the birdseye," which appears to be a consistent feature in 3L of all members of the subgenus. There is a large puff with a prominent pair of dark bands in region 38D. The ball-shaped puff in 37B is similar to that in several other anophelines. In the center of this puff is a pair of dark bands, the first of which stains more darkly, that form an easily recognized area. The two dark bands in region 37D,

the first of which is thicker, also forms an easily recognized area. Region 36A contains two dark bands, the second is consistently broken. Region 36C begins an expanded region and contains two dark bands, the first of which is darker. A thick dark band, a good landmark, is found in region 36D. This region is followed by 35A which contains a triplet of dark bands, the first of which is the darkest. Regions 35B and C are often not seen clearly. These regions are usually folded and stain lightly. Regions 35D through 34C are consistently very wide. A large ball-shaped puff begins in 35D containing 4-1-4-3-4-2 series. There is a prominent thick band in 35A at the center of the puff. This band is flanked by medium staining bands. There is a two band followed by a pair of dark bands terminating with a light band in the centromere region.

# CHAPTER IV

## DISCUSSION

The salivary gland chromosomes of the members of the subgenus Anopheles offer an excellent opportunity to examine various aspects of the evolution within the group. Many blocks of genes common to all members of the subgenus are seen in the banding patterns. Some of these arrangements are found unaltered in sequence in several of the species. Inversions can often account for the differences noted in banding patterns. Inversion heterozygosity permits an assessment of the numbers and types of chromosomal events that have resulted in the present gene arrangements and thereby an estimate of the degree of evolutionary relationship among the species.

Such chromosomal banding relationships have been made not only among Anopheline (Kitzmiller, Frizzi, Baker and Coluzzi), but also among members of the genera Drosophila and Chironomus. Because Anopheles freeborni has been used as a standard type banding pattern to assess relationships among certain Anophelines (Kitzmiller, Frizzi and Baker, 1967), the following discussion will examine some of the relationships of An. eiseni and An. peryassui with An. freeborni (Kitzmiller, Baker, 1963), and where possible with other related species.

### Anopheles eiseni

The salivary chromosomes of Anopheles eiseni show many similarities to the salivary chromosomes of other members of the subgenus Anopheles. The ends of the chromosomes are similar to An. freeborni and An. quadrimaculatus (Frizzi, 1953a).

The X chromosome is similar to that of An. nuneztovari (Kitzmiller, et al., 1973). Both species have a right and left X arm. The banding and puffing patterns are not similar.

The right arm of chromosome two appears to be very distinctive and quite unlike that of any other Anopheline thus far studied. Although there are some similarities of the free ends of An. eiseni and An. peryassui, the tip of both is somewhat flared followed by a slight constriction stained more darkly. In both cases region 6C begins with a heavy band.

The left arm of chromosome two shows some similarity in shape of the free end to An. earlei (Kitzmiller and Baker, 1965). Regions 21 and 20, of the free end show some similarity in intensity with An. earlei. In both species region 21 stains lightly while region 20 stains darkly. Region 20C of An. aztecus (Baker and Kitzmiller, 1946b) resembles regions 20C, D, and E of An. eiseni. The triplet of heavy bands in 20E of An. eiseni is found in the far end of 20C of An. aztecus. The rest of the arm except the centromere region does not homologize well with previously studied Anophelines. The centromere region resembles that of An. earlei in staining intensity with light bands found on either side of the single dark band.

The right arm of chromosome three clearly shows banding affinities with other members of the subgenus Anopheles. Similarities are especially striking when this arm is compared to the third right arm of An. punctipennis (Baker and Kitzmiller, 1963a). Basically the banding pattern of this arm is "standard" at the free end and close to the centromere, that is, it closely resembles most of the other species of the subgenus in these areas.

The banding patterns of regions 22A through 23B are essentially similar to An. punctipennis and An. aztecus. The triplet of bands in region 25B in An. punctipennis is found in region 25B of An. eiseni and 25B of An. quadrimaculatus. Regions 29A-31A have undergone in a few preparations an inversion. The right arm of chromosome three, the longest arm in all species studied, appears quite susceptible to inversion rearrangements in some species. Several inversions are known to exist in various species in this arm, and hypothetical rearrangements, postulated to account for comparisons among species, add many more. The centromere region of 3R most closely resembles An. occidentalis (Baker and Kitzmiller, 1965) in staining intensity.

The left arm of chromosome three is very similar to 3L of other Anophelines. The somewhat flared shape of the free end is similar to the free end of An. aztecus. The large puff in regions 36B through 35B is similar to the puff in the corresponding regions of An. atropos (Kreutzer, et al., 1969a). The "birdseye" region, typical of all Anophelines, is present in region 39D. This is similar to An. peryassui where the "birdseye" is found in regions 39E and 38A. Although this is not the typical location of the birdseye. In most Anophelines it is found in region 35.

Anopheles peryassui

The flared free end of the X chromosome is similar to that of An. crucians and An. bradleyi (Kreutzer, et al., 1970). The centromere is similar to the staining intensity of the centromere region in An. aztecus.

The free end of the right arm of chromosome two of An. peryassui is not typical of the subgenus Anopheles. The end is flared and clear in only a few preparations. A possible inversion may exist here. The wide puffs in regions 8C through 9B are reminiscent of those found in An. punctipennis. The pattern is not similar but the dark bands are there. At a glance the banding pattern of the arm most resembles An. aztecus.

The left arm of chromosome two resembles several other members of the subgenus Anopheles. The free end, regions 21A through 20B, is very similar to An. punctipennis, An. atroparvus and An. perplexens. The pair of dark bands in region 18B is similar to that of An. perplexens (Kreutzer and Kitzmiller, 1971). The puff in regions 19B and C which consists of three pairs of heavy bands is similar to that of regions 19D and E in An. aztecus. Regions 20D and 19A of An. punctipennis resemble corresponding regions of An. peryassui. The bands of the centromere region resemble those of An. perplexens although this region of An. perplexens is much more elongated than that of An. peryassui.

Generally the right arm of chromosome three is similar to all other species of the subgenus Anopheles. Region 22A through 22C are most similar to An. aztecus. Region 23C of An. bradleyi resembles that of An. peryassui. The ball-shaped puff of region 27B resembles the ball-shaped puff of region 27C in that of An. occidentalis. The dark bands of region 31 are reminiscent of those in the corresponding region of An. freeborni. Region 32 is similar to that of An. punctipennis.

The left arm of chromosome three retains the essentially conservative pattern found in other members of the subgenus Anopheles.

The free end, regions 39A-39C, is similar to that of An. punctumacula (Kreutzer, et al., 1969b), and An. freeborni. The birdseye appears in regions 39E and 39A. This is relatively early in the chromosome compared to other members of the subgenus Anopheles. Most Anophelines carry the birdseye in region 35. An. eiseni also has the "birdseye" relatively early in region 39D. There are two dark bands in region 38D in An. eiseni similar to those in An. perplexens, An. punctipennis and An. freeborni. The bulb-shaped puff in region 37B is also found in An. punctipennis and An. perplexens. The centromere region closely resembles that of An. bradleyi and An. crucians.

# CHAPTER V

## SUMMARY

The descriptions and drawings of the salivary gland chromosomes of the Neotropical anopheline species, An. eiseni and An. peryassui, have been presented. It is proposed that the described banding patterns be the "standard" arrangements of these species. The X chromosome of An. eiseni is sub-metacentric while the X chromosome of An. peryassui is sub-telocentric. The two pairs of autosomes of both species are metacentric. There was one inversion in 3R of An. eiseni.

When the salivary gland chromosomes of the species were compared to other members of the subgenus Anopheles, there were similarities at the free and centromere ends of the autosomes. The greatest degree of homology of An. peryassui was with An. punctipennis, An. perplexens and An. aztecus. The greatest degree of homology of An. eiseni was with An. punctipennis, An. earlei and An. aztecus. The two species, An. eiseni and An. peryassui, most closely resemble each other in the right and left arms of chromosome three. These comparisons suggest an evolutionary relationship among the aforementioned species.

More attention should be given to chromosomal polymorphism. Proper studies could lead to understanding the evolution, taxonomy and specialization of difficult complexes. The studies would clarify the position with regard to known "ecological races" and may contribute to the understanding of such phenomena as behavioristic resistance and transmission or non-transmission of diseases. If the Drosophila

pattern is followed by mosquitoes, we may find correlations of chromosomal patterns with altitude, temperature, salinity and many other ecological conditions.

# BIBLIOGRAPHY

Baker, R. H. and J. B. Kitzmiller. 1963a. "Cytogenetic studies on Anopheles punctipennis." Amer. Zool., 3, 535.

Baker, R. H. and J. B. Kitzmiller. 1946b. "The salivary chromosomes of Anopheles aztecus." Rev. Inst. Salubr. Enferm. Trop., 24, 43.

Baker, R. H. and J. B. Kitzmiller. 1965. "The salivary chromosomes of Anopheles occidentalis." Bull. Wld. Hlth. Org., 32, 575.

Bates, M. 1940. "The nomenclature and taxonomic status of the mosquitoes of the Anopheles malculipennis complex." Ann. Ent. Soc. Amer., 33, 343.

Coluzzi, M. 1964. "Morphological divergences in the Anopheles gambiae." Riv. Mal., 43, 197.

Dobzhanzky, T. and C. Epling. 1944. "Chromosomal races in Drosophila pseudoobscura and D. persimilis." Carnegie Inst. Wash. Publ., 554, 47.

French, W., R. Baker, and J. B. Kitzmiller. 1962. "Preparation of mosquito chromosomes." Mosq. News, 22, 377.

Frizzi, G. 1947a. "Cromosomi salivari in Anopheles maculipennis." Sci. Genet., 3, 67.

Frizzi, G. 1947b. "Determinazione del sesso nel genere Anopheles." Sci. Genet., 3, 80.

Frizzi, G. 1947c. "Salivary gland chromosomes of Anopheles." Nature, 100, 226.

Frizzi, G. 1947d. "Genetica di pupolazione in Anopheles maculipennis prime ricerche sperimentali." Ricerca Scient., 17, 894.

Frizzi, G. 1949. "Genetica dipopolazine in Anopheles maculipennis studi preliminari sui riodinamenti cromosomici del gruppo." Ricerca Scient., 19, 544.

Frizzi, G. 1950. "Studio sulla sterilita delgi ibridi nel genera Anopheles, I. Sterilita nell incrociofra Anopheles maculipennis, Anopheles atroparvus ed Anopheles maculipennis, Anopheles typicus e nel reincrocio del cromosomi salivari." Sci. Genet., 3, 260.

Frizzi, G. 1951. "Dimorfismo cromosomico in Anopheles maculipennis messeae." Sci. Genet., 4, 79.

Frizzi, G. 1952. "Nuovi contributie prospettive de ricerca nel gruppo Anopheles maculipennis in base allo studio del dimorfismo cromosomico (ordinamento ad X invertito e tipico) nel messeae." Symp. Genet., 3, 231.

Frizzi, G. 1953a. "Extension of the salivary chromosome method to Anopheles claviger, Anopheles quadrimaculatus, and Anopheles aquasalis." Nature, 171, 1072.

Frizzi, G. 1953b. "Etude cytogenetique d'Anopheles maculipennis en Italie." Bull. Wld. Hlth. Org., 9, 335.

Kitzmiller, J. B. 1966. "Subgeneric chromosomal patterns in the genus Anopheles." Wld. Hlth. Org. SC/VG, 67, 2.

Kitzmiller, J. B. and R. H. Baker. 1963. "The salivary chromosomes of Anopheles freeborni." Mosquito News, 23, 254.

Kitzmiller, J. B. and R. H. Baker. 1965. "The salivary chromosomes of Anopheles earlei." Canad. J. Genet. Cytol., 7, 275.

Kitzmiller, J. B., G. Frizzi, and R. H. Baker. 1967. "Evolution and speciation within the maculipennis complex of the genus Anopheles." In: Genetics of Insect Vectors of Disease. New York: Elsevier Publ. Co., Chapt. 5.

Kitzmiller, J. G., R. D. Kreutzer, and E. Talla Farro. 1973. "Chromosomal differences in populations of Anopheles nuneztovari." Bull. Wld. Hlth. Org., 48, 435.

Klassen, W., W. L. French, H. Laven and J. B. Kitzmiller. 1965. "The salivary chromosomes of Anopheles quadrimaculatus." Mosq. News, 25, 328.

Kreutzer, R. D., S. L. Narang, and J. B. Kitzmiller. 1970. "A comparison of the salivary gland chromosomes of Anopheles crucians and Anopheles bradleyi." Cytologia, 35, 355.

Kreutzer, R. D. and J. B. Kitzmiller. 1971. "Chromosomal similarities between Anopheles perplexens and Anopheles punctipennis." Mosquito News, 31, 3.

Kreutzer, R. D., S. L. Narang and J. B. Kitzmiller. 1969a. "The salivary gland chromosomes of Anopheles atropos." Mosquito News, 29, 223.

Kreutzer, R. D., T. Tadano, S. L. Narang and J. B. Kitzmiller. 1969b. "The salivary gland chromosomes of Anopheles punctimacula." Rev. Bras. Malar. Doevc. Trop., 21, 559.

Mayr, E. 1963. Animal Species and Evolution. Harvard University Press, Cambridge, XVI, pp. 797.