KARYOTYPES OF SELECTED BATS

(ORDER CHIROPTERA)

by

Jerry Lee Osborne

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SIGNED: Jerry L. Osborne

APPROVAL BY THESIS DIRECTOR

This thesis has been approved on the date shown below:

E. Lendell Cockrum
E. L. COCKRUM
Professor of Zoology

5 aug 1965
Date

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Abstract

Karyotypes of fourteen species of the order Chiroptera are presented. A comparative study of these karyotypes indicates that centric fusion has played a major role in the evolution of Chiroptera and that chromosome evolution in at least two species has progressed away from the main line while morphological and behavior parallelism has been maintained.

While the data in most cases support the present classification and phylogeny of Chiroptera, it also indicates that some revision may be necessary.

Evolutionary trends and interrelationships are discussed for the three families: Vespertilionidae, Molossidae, and Phyllostomatidae.

Introduction

Recent developments in cytological techniques have caused an increased interest in the study of mammalian chromosomes. Yet, the use of these techniques as a taxonomic aid has not become widespread among mammalian taxonomists. This can be attributed to the fact that cytological methods are not usually needed for identification of mammalian species and ordinarily will simply substantiate the judgements of an experienced systematist. However, the importance of these techniques in interpreting the phylogeny of the mammalian species and their interrelationships should not be ignored. Cain (1958) gives an excellent summary of the use and importance of cytology in taxonomy.

Nuclear cytology has played only a slight role in the study of the mammalian order Chiroptera. Prior to 1948, the information pertaining to the chromosomes of bats was very inconsistent. Van der Stricht (1910) reported a haploid number of 9 or 10 for Vesperugo noctula. Athias (1912) reported an approximate haploid number of 16 for Rhinolophus hipposideris. Jordan (1912) and Hance (1917) both report chromosome counts of unknown species. The only reliable information during this period prior to 1948 was reported by Painter (1925). In his study, he correctly determined the diploid number of Nyctinomus mexicanus (=Tadarida brasiliensis).

Makino (1948), using testicular material, determined the diploid number of Pteropus dasymallus inopinatus to be 38. The chromosome complement was reported to consist of thirty-six metacentric and two terminal chromosomes. This is the only member of the suborder Megachiroptera that has been examined cytologically. The Japanese horseshoe bat, Rhinolophus ferrum-equinum, was also included in his study and a haploid number of 29 was reported for the species.

In 1949, Bovey studied fourteen species of Old World bats representing three families: 1. Rhinolophidae, three species; 2. Nycteridae, one species; and 3. Vespertilionidae, nine species. His study represents the most recent investigation of chromosomes in bats that has come to the attention of the author.

The present investigation has three objectives: 1. to make available to other workers cytological information regarding additional species of Chiroptera; 2. to elucidate a few problems in the taxonomy and phylogeny of Chiroptera; and 3. to present data that will be useful in future studies concerning the evolution of mammalian chromosomes.

In Chiroptera, the phylogeny is complex and present interpretations are often ambiguous. Cytotaxonomy can play a major role in clarifying the relationships of many groups within this Order. In the present study: four species in the family Phyllostomatidae, seven species in the family Vespertilionidae, and two species in the family Molossidae have been examined by cytological techniques. The chromosome morphology of each is described and illustrated in karyogram form and selected photomicrographs are included.

Since only a few scattered species within the Order have been studied by cytological methods, only trends in phylogeny can be shown. The results generally verify the classical taxonomic classification but in some instances show that minor revisions may be necessary.

Materials and Methods

With the advent of the colchicine-hypotonic citrate method of Ford and Hamerton (1956) the study of mammalian chromosomes has been greatly advanced. The action of colchicine is three-fold: 1. it acts to prevent spindle formation and thereby causes an accumulation of cells in metaphase; 2. it causes the chromosomes to shorten; and 3. it causes the sister chromatids to separate, showing the exact position of the centromere. The action of the hypotonic citrate solution complements the action of the colchicine by expanding the cell.

To date there are several good techniques utilizing the colchicine-hypotonic citrate method. The one chosen for this study is described by Patton (1965) and involves the following steps:

1. Inject live animals intraperitoneally with colchicine (0.05 grams percent), 0.01 ml. per gram body weight.

2. Sacrifice the animal after seven hours and immediately remove the complete humerus. The epiphysis is cut off and the bone marrow removed from the shaft by flushing with 3 ml. of 1.0 percent sodium citrate. Pipette solution vigorously to break up any cell clumps.

3. Incubate the resultant cell suspension at 37° C for 30 minutes.

4. Filter solution through two layers of cheese cloth and centrifuge at 500 rpm for five minutes.

5. Pour off supernate without disrupting the button, add 3 ml. of freshly prepared Carnoy's fixative and allow cells to fix in a cold

box for 30 minutes.

6. Centrifuge at 500 rpm for five minutes and then wash several times without disrupting the button.

7. Pour off supernate and add about 0.7 ml. of fixative. Resuspend cells by pipetting vigorously.

8. Pipette droplets of cell suspension onto chemically clean slides and ignite the suspension immediately, following the blaze-dry method of Scherz (1962). Allow slides to dry.

9. Stain in aceto-orcein for one and one half hours.

10. Dehydrate in three, 30 second changes of absolute methanol and mount.

After the slides were prepared each was scanned for well spread metaphase plates. Those cells that appeared intact with the cell membrane undamaged by the preparatory technique and with chromosomes spread symmetrically were used to obtain the final diploid number of the species. Cells that showed chromosomes widely separated and with other cells in the vicinity were disregarded. Any spreads that showed chromosomes representing different mitotic stages or reacting in different ways to the stain were also disregarded. The cells selected to be counted were then placed under 1280 X magnification and counted several times by visually dividing the chromosome complement into several groups. Photomicrographs were taken of at least six spreads for each species and then the chromosomal complement was again counted.

A Zeiss microscope equipped with a 35 mm. camera was used and photomicrographs were taken on Kodak high contrast copy film.

For karyogram analysis, intact cells were chosen based on the following criteria: 1. cells showing the least amount of overlapping of chromosomes; 2. cells exhibiting the highest degree of chromosome morphological acuity; and 3. cells representing mitotic metaphase. In describing the karyogram, the standardized terminology of Patton (1965) was used. In his description, there are four types of chromosomes based on arm ratios. The median chromosome possesses a median centromere and has an arm-ratio between 1:1.0 and 1:1.09; the sub-median chromosome has a submedian centromere and an arm-ratio between 1:1.10 and 1:1.99; the sub-terminal chromosome has its centromere subterminal and an arm-ratio 1:2 or greater; the terminal chromosome has no visible second arm. Metacentrics refers to both median and submedian chromosomes while acrocentrics refers to both terminal and subterminal chromosomes.

For karyogram analysis, the chromosomes were separated into groups according to their morphology and comparative size. In many instances, a group contained a series of chromosome pairs that gradually decreased in size without any obvious breaks in the series; thus the group was not further subdivided.

The sex chromosomes were determined on the basis of the heteromorphic pair found in the males. The non-homologous pair was designated as the sex chromosomes and X- and Y- determination was decided from the female karyotype which consists of a homomorphic X-complement.

Live bats used in this study were collected in Japanese "mist" nets and hand nets. The Japanese "mist" nets were employed over water holes and in the entrances of mine tunnels and caves. Hand nets were employed inside mine tunnels and caves where bats were found clinging to the walls.

Specimens were identified on the basis of external morphology and skull characteristics. They are all now located in the museum at the University of Arizona as part of the permanent collection. Localities and catalog numbers are listed in Appendix C.

The taxonomy and classification of the bats used in this study was based on Hall and Kelson (1959), Simpson (1945) and Miller (1906).

Results

Fourteen species (including four species in the family Phyllostomatidae, eight species in the family Vespertilionidae, and two species in the family Molossidae) have been examined for chromosome morphology and diploid number. The numerical results from this study are listed in Table 1, and the morphological description of each species karyotype is given below. Representative karyotypes and photomicrographs are presented in Appendix A and B respectively.

Species Examined in the Family Phyllostomatidae

Pteronotus davyi: Subfamily Chilonycterinae

Two male individuals were examined and the species' diploid number was determined to be 40. A representative karyotype (Plate 1, Appendix A) shows three groups into which the chromosomal complement was separated based on chromosome morphology and size.

Group A consists of six large pairs of approximately equal sized metacentric chromosomes with either medial or submedial centromeres.

Group B consists of five pairs of medium sized to small metacentric chromosomes with either medial or submedial centromeres.

Group C consists of seven pairs of small to very small terminal chromosomes.

Since only male specimens were studied, X and Y designation of sex chromosomes was not possible. Structurally, one of the sex

Table I. Summary of diploid numbers, autosomal types, sex chromosomes, and fundamental numbers of the species studied in the order Chiroptera.

Family and Species	2n	M	SM	ST	T	X-Y	NF*	♂	♀
Phyllostomatidae									
Pteronotus davyi	38	4	18	--	14	(M T)	56	2	0
Macrotus waterhousii	40	2	18	2	16	SM T	60	3	2
Leptonycteris nivalis	32	14	16	--	--	(SM SM)	60	0	3
Choeronycteris mexicanus	16	4	4	4	4	? ?	(26)	0	1
Vespertilionidae									
Myotis thysanodes	44	2	6	--	34	SM SM	50	4	1
Myotis volans	44	2	6	--	34	M T	50	1	1
Myotis fortidens	44	2	6	--	34	(SM T)	50	1	0
Pizonyx vivesi	44	2	6	--	34	(SM T)	50	2	0
Pipistrellus hesperus	28	14	8	--	6	(SM T)	(48)	0	1
Eptesicus fuscus	50	--	2	--	48	(SM T)	(48)	0	2
Lasiurus cinereus	28	14	6	--	6	SM T	46	2	1
Lasionycteris noctivagans	20	4	6	2	6	SM T	30	2	0
Molossidae									
Tadarida brasiliensis	48	--	6	40	--	M T	(54)	2	2
Tadarida femorosacca	48	--	8	4	36	(SM T)	(58)	0	1

* M = medial centromere; SM = submedial centromere; ST = subterminal centromere; T = terminal centromere; NF = fundamental number.

chromosomes is a small metacentric type with a submedial centromere belonging to group B and the other a medium sized terminal chromosome belonging to group C.

Macrotus _waterhousii_: Subfamily Phyllostominae

Three specimens, one male and two females were studied; the species' diploid number was determined to be 40. Five morphological groups can be easily separated in the karyotype (Plate 2, Appendix A).

Group A includes eight pairs of large to medium sized metacentric chromosomes. Pair 4 is the only chromosome in the karyotype with a medial centromere.

Group B includes one small pair of submedial chromosomes.

Group C includes one characteristic pair of large subterminal chromosomes.

Group D includes five medium sized terminal chromosomes.

Group E includes three pairs of small to very small terminal chromosomes.

The X-chromosome was determined to be a small submedian chromosome belonging to group B. The Y-chromosome, not shown in the female karyotype, is a terminal chromosome.

Choeronycteris _mexicanus_: Subfamily Glossophaginae

One female specimen was examined from which the species diploid number of 16 was determined. The karyotype (Plate 3, Appendix A) is easily divisible into four groups.

Group A consists of two large pairs of chromosomes; pair 1 includes two large subterminal chromosomes and pair 2 includes two large submedial chromosomes.

Group B consists of two pairs of medium size chromosomes; one subterminal pair and one submedial pair.

Group C consists of two small pairs of medial chromosomes.

Group D consists of two pairs of terminal chromosomes; one large pair and one smaller pair.

Since only one female was examined the sex chromosomes could not be determined.

Leptonycteris nivalis: Subfamily Glossophaginae

Three female specimens were examined and a diploid number of 32 was determined for the species. The karyotype (Plate 4, Appendix A) consists of all metacentric chromosomes and is divisible into two groups based on size.

Group A includes five pairs of large metacentric chromosomes with either medial or submedial centromeres.

Group B includes the remaining eleven pairs of metacentric chromosomes which range from medium to small in size.

Sex chromosomes could not be determined because only females were examined.

Species Examined in the Family Vespertilionidae

Myotis thysanodes:

Three individuals were examined, two males and one female. A diploid number of 44 was determined for the species. The karyogram (Plate 5, Appendix A) shows three morphological groups that are easily discernable in the karyotype.

Group A is composed of three large pairs of metacentric chromosomes with either submedial or medial centromeres.

Group B contains a single pair of small metacentric chromosomes with medial centromeres.

Group C includes seventeen pairs of medium to small sized terminal chromosomes.

The submedial X-chromosome is smaller in size than the chromosomes of group A and larger than the chromosomes in group B. The Y-chromosome is the smallest metacentric chromosome in the karyotype and has a medial centromere.

Myotis volans:

Two individuals of this species were examined, one male and one female. A diploid number of 44 was determined for the species. As found in Myotis thysanodes, the autosomal complement could be easily divided into three morphological groups as shown in the species' karyogram (Plate 6, Appendix A).

Group A includes three pairs of large metacentric chromosomes with either medial or submedial centromeres.

Group B contains a single pair of small metacentric chromosomes with medial centromeres.

Group C includes seventeen pairs of large to small terminal chromosomes.

The X-chromosome is a medium sized metacentric type with a medial centromere and is easily recognized in the karyotype because of its characteristic size, smaller than those of group A and larger than those of group B. In contrast, the Y-chromosome is a small terminal chromosome of group C.

Myotis fortidens:

The karyotypes of all three *Myotis* studied are morphologically indistinguishable from each other. The karyogram of *Myotis fortidens* (Plate 7, Appendix A) shows chromosomes slightly larger than those of the other two species of *Myotis* but this difference is due to the use of an earlier stage in mitotic division.

The diploid number is 44 and the karyotype is divided into three groups.

Group A consists of three pairs of metacentric chromosomes with the largest, pairs 1 and 2, having submedial centromeres and the smallest, pair 3, having medial centromeres.

Group B is composed of a single small pair of submedial chromosomes.

Group C includes seventeen pairs of terminal chromosomes.

Since no female specimens were examined the X- and Y-chromosomes were arbitrarily assigned based on the findings of other species. The X-chromosome is a medium sized metacentric type with a slightly submedial centromere and is easily distinguished, because of size, from the remaining chromosomes. The Y-chromosome is a small terminal type.

Pizonyx vivesi:

Two male specimens were examined and the species diploid number was determined to be 44. The karyotype (identical to the *Myotis* karyotype) was divided into three groups, (Plate 8, Appendix A).

Group A includes three large pairs of metacentric chromosomes with either medial or submedial centromeres. All three pairs are of approximately the same size.

Group B includes a single small pair of submedial chromosomes.

Group C consists of seventeen pairs of terminal chromosomes varying from the largest (pair number 5) to the smallest (pair number 21).

The sex chromosomes are recognizable morphologically but could not be designated as X or Y since no females have yet been examined cytologically. If, however, it is assumed that the same pattern is followed as was found for the genus Myotis, from which they can not be karyologically distinguished, we can arbitrarily designate the medium sized metacentric sex chromosome as X and the smaller morphologically undefined chromosome as Y.

Pipistrellus hesperus:

One female specimen was examined from this species and a diploid number of 28 was determined. The species karyotype (Plate 9, Appendix A) was divided into four morphological groups.

Group A includes two large pairs of metacentric chromosomes; pair 1 has medial centromeres and pair 2 has submedial centromeres.

Group B contains eight medium sized pairs of metacentric chromosomes with either medial or submedial centromeres. There is a slight decrease in size from pair 3 to pair 10.

Group C includes a single small pair of medial chromosomes.

Group D includes three pairs of terminal chromosomes that exhibit a slight decrease in size.

Sex chromosomes could not be determined since only females were examined.

<u>Eptesicus</u> <u>fuscus</u>:

Two females were examined. The species diploid number was determined to be 50. The karyotype of the species (Plate 10, Appendix A) could only be divided into two morphological groups.

Group A consists of one large pair of medial chromosomes which represents the only metacentric type found in the karyotype.

Group B is composed of the remaining twenty-four pairs of terminal chromosomes. There is a gradual decrease in size of the chromosome pairs without showing any breaks for further subdivision of the group.

Sex chromosomes could not be distinguished since only females were investigated.

<u>Lasiurus</u> <u>cinereus</u>:

Results obtained from two specimens, one male and one female, show a diploid number of 28 for the species. The representative karyotype is illustrated (Plate 11, Appendix A) showing the three morphological groupings into which the autosomal complement is divided.

Group A includes six large pairs of metacentric chromosomes with medial or submedial centromeres. All pairs are of the same relative size with the exception of pair number 6 which is slightly smaller.

Group B includes four small pairs of metacentric chromosomes with medial or submedial centromeres.

Group C includes three pairs of terminal chromosomes.

The X-chromosome, as illustrated in the karyogram, is a large metacentric with a submedial centromere and could be included in group A based on size and morphology. The Y-chromosome, not shown in the female karyogram, was determined to be a small terminal chromosome.

Lasionycteris noctivagans:

Two individuals, both males, were studied and the species diploid number was found to be 20. The karyotype (Plate 12, Appendix A) was divided in four autosomal groups.

Group A consists of two large pairs of submedial chromosomes which can be distinguished from each other by the more distal location of the centromeres in pair 1 and the more medial location of the centromeres in pair 2.

Group B includes three pairs of medium sized metacentric chromosomes which can be individually identified by the location of the centromeres. Pair 3 is subterminal; pair 4 is medial and pair 5 is submedial.

Group C consists of an extremely small pair of medial chromosomes.

Group D contains three small pairs of terminal chromosomes.

Sex chromosomes of this species are determined morphologically but at this time cannot be designated X and Y since no females were examined. One is a medium sized submedial chromosome and is easily identified due to its unique size, smaller than the chromosomes in group B and larger than those of group C. The other sex chromosome appears as a stained "spot" and is assumed to be a terminal chromosome representing the smallest member of group D.

Species Examined in the Family Molossidae

<u>Tadarida brasiliensis</u>:

Two specimens, one male and one female, have determined the species diploid number to be 48. The karyotype (Plate 13, Appendix A) can be separated into three groups according to size.

Group A consists of a single large pair of submedian chromosomes.

Group B includes three medium sized pairs of submedial chromosomes.

Group C includes nineteen pairs of acrocentric autosomes with subterminal centromeres.

The Y-chromosome, not shown in the representative female karyotype, is a medium sized subterminal acrocentric type. The X-chromosome as shown in the karyogram is a medium sized metacentric chromosome with a submedial centromere.

<u>Tadarida femorosacca</u>:

The karyotype of this species (Plate 14, Appendix A) was determined from a single female individual. The species diploid number is 48. The karyotype is divided into four morphological groups which easily distinguishes it from the karyotype of the related species, <u>Tadarida brasiliensis</u>.

Group A is composed of a single large pair of submedial chromosomes.

Group B consists of four medium sized pairs of chromosomes with both submedial and subterminal centromeres.

Group C is composed of a single pair of small subterminal chromosomes.

Group D consists of eighteen pairs of terminal chromosomes.

Since only a female specimen was examined, the sex chromosomes of this species could not be determined.

Discussion

Mechanisms of Chromosomal Evolution Affecting the Karyotype

The various mechanisms of karyotype evolution were discussed extensively by White (1945) and Swanson (1957). In this study, we were only concerned with those relevant to the changes in chromosome numbers and/or affecting the karyotype.

A. Fusion and Fragmentation (Robertsonian Variation). Robertson (1916), in his studies on insects, first suggested the phenomenon of fusion. He suggested that the metacentric chromosomes may have arisen by the apical fusion of two acrocentric chromosomes or according to the formula: $V = 1 + 1$. This implies that the reverse process, fragmentation or fission, is also possible but further investigations have shown little supporting evidence in mammals.

Matthey (1945, 1949) has shown that centric fusion accounted for a large part of the visible chromosome changes between different karyotypes of allied species of lacertilian lizards. In his work he coined the term <u>nombre fondamental</u> (N.F.) or fundamental number for the total number of chromosomal arms in a complement.

B. Non-Robertsonian Variation. In this group are all other mechanisms affecting the structural changes of chromosomes including the various types of translocations, inversions, and deletion-duplication factors. In this study, only pericentric inversions, ones which could transform one metacentric chromosome into an acrocentric chromosome or vice-versa, would cause a noticeable affect in the species karyotype.

C. Polyploidy and polysomy are not thought to have played a significant role in mammalian chromosomal evolution and no evidence for either case has been found in the evolution of the Chiroptera.

Interpreting Data Derived From Karyotypes

Before interpreting the accumulative data in Table 2, a brief discussion concerning the reliability of fundamental numbers is necessary. The nombre fondamental can be determined in two ways: (1) by counting all arms in the complement including the sex chromosomes as Bovey has done in his study; or (2) by counting only the chromosomal arms of the autosomes, excluding the sex chromosomes, as Matthey later defined the term.

Another difficulty in determining the N.F. is often encountered in the study of mammalian chromosomes when subterminal chromosomes are involved. In some karyotypes, the complement includes chromosomes with extremely small arms which are difficult to detect by the present techniques. Therefore, the establishment of N.F. becomes a very delicate task and the value can be determined only in an approximate and often arbitrary manner.

In the present study the fundamental number has been determined according to the system proposed by Matthey in which all autosomal chromosome arms are counted. In cases where only female specimens were examined, two was subtracted from the total number in the assumption that the Y-chromosomes were terminals. With only one exception, where all chromosomes of the complement were of the metacentric type, all determined Y-chromosomes were terminal chromosomes.

In interpreting the ranges of diploid and fundamental numbers in Table 2, it was assumed that all Robertsonian variation (fusion) reduces the total number of chromosomes without changing the total number of arms. This is to say, if all species in a family underwent only Robertsonian variation we would expect to find a wide range in diploid numbers but the fundamental number of each species would remain equal and unchanged. If any other structural change played a role in the chromosomal evolution of the group, we would expect to find either a decrease or increase in the fundamental number. For example, if a metacentric chromosome was formed by a pericentric inversion instead of by the centric fusion of two rods, there would be an increase of two arms in the karyotype without altering the diploid number. This can obviously work in the reverse manner and create one rod shaped chromosome from a former metacentric type thereby decreasing the N.F. by two in the karyotype. In either case, the N.F. is changed by two.

Therefore, changes in the N.F. can only be created by non-Robertsonian variation but, on the other hand, it is possible for non-Robertsonian variation to counteract itself and not change the N.F. in the resultant karyotype, i.e. one pericentric inversion creating a metacentric chromosome and one creating a terminal type.

Assuming that both Robertsonian and non-Robertsonian variations have played a role in the karyotype evolution, we can express numerically the degree to which each has been involved based on the range of N.F. within the family and the range of the diploid numbers. If the

Table 2. Summary of chromosome numbers and fundamental numbers in Chiroptera

Species	2n	Mt	Ac	NF	Investigator
Megachirptera					
Pteropodidae					
Pteropus dasymallus inopinatus	38	?	?	72	Makino '48
Microchiroptera					
Nycteridae					
Nycteris sp.	42	?	?	79	Bovey '49
Rhinolophidae					
Rhinolophus euryale	58	?	?	62	Bovey '49
Rhinolophus ferrum-equinum	58	?	?	62	Bovey '49
Rhinolophus ferrum-equinum	58	?	?	?	Makino '48
Rhinolophus hipposideros	54	?	?	60	Bovey '49
Phyllostomatidae					
Chilonycterinae					
Pteronotus davyi	38	22	14	56	Osborne '65
Phyllostominae					
Macrotus waterhousii	40	20	18	60	Osborne '65
Glossophaginae					
Leptonycteris nivalis	32	30	--	60	Osborne '65
Choeronycteris mexicanus	16	12	4	(26)	Osborne '65
Vespertilionidae					
Myotis myotis	44	?	?	54	Bovey '49
Myotis mystacinus	44	?	?	52-54	Bovey '49
Myotis emarginatus	44	?	?	52	Bovey '49
Myotis thysanodes	44	8	34	50	Osborne '65
Myotis volans	44	8	34	50	Osborne '65
Myotis fortidens	44	8	34	50	Osborne '65
Myotis daubentoni	42	?	?	54	Bovey '49
Pizonyx vivesi	44	8	34	50	Osborne '65
Pipistrellus pipistrellus	42	?	?	(51)	Bovey '49

Table 2. Summary of chromosome numbers and fundamental numbers in Chiroptera. (continued)

Species	2n	Mt	Ac	NF	Investigator
Pipistrellus hesperus	28	22	6	(48)	Osborne '65
Pipistrellus nathusii	44	?	?	?	Bovey '49
Plecotus auritus	32	?	?	54	Bovey '49
Barbastella barbastellus	32	?	?	54	Bovey '49
Eptesicus fuscus	50	2	48	(48)	Osborne '65
Miniopterus schreibersii	46	?	?	52	Bovey '49
Lasiurus cinerius	28	20	6	46	Osborne '65
Lasionycteris noctivagans	20	10	8	30	Osborne '65
Molossidae					
Tadarida brasilienses	48	?	?	?	Painter '25
Tadarida brasilienses	48	6	40	(54)	Osborne '65
Tadarida femorosacca	48	12	36	(58)	Osborne '65

Note: Mt = metacentric chromosomes; Ac = acrocentric chromosomes; NF = fundamental number range of N.F. is zero then Robertsonian variation has contributed 100 percent, on the other hand if the range of N.F. is equal to the range of diploid numbers then each type of variation has contributed exactly 50 percent. By interpolating the proportional degree of variations in N.F. we can obtain the approximate percentage of Robertsonian variation (fusion and/or fragmentation) and of non-Robertsonian variation.

Evolutionary Trends and Relationships Within the Family Vespertilionidae

Looking at the family Vespertilionidae as a whole, one can only conclude that it represents a very heterogeneous group. The diploid numbers of the seventeen species studied vary from the lowest count of 20 in _Lasionycteris noctivagans_ to the highest count of 50 in _Eptesicus fuscus_, giving a diploid range of 30 for the family. By using the N.F. as a criterion for analysis, a range of 26 is observed. In either case, the data indicate a very diverse group, at least karyotypically.

If one separates the family into two parts, one part including all the species except _Lasionycteris noctivagans_ and the second part including only _L. noctivagans_, one arrives at a very different conclusion. We can justify this separation of _L. noctivagans_. First, anatomical and morphological studies by Winge (1941) indicate that the genus _Lasionycteris_ is a very divergent member of the family Vespertilionidae and secondly, the cytological evidence presented here indicates that the genus occupies an isolated position at least karyotypically from the remaining genera studied.

By analysing the cytological data as previously described, the results in Table 3 were obtained.

Table III. Evolutionary trends in the family Vespertilionidae based on diploid and fundamental numbers.

Family Vespertilionidae	2n	N.F.	% R.V.	% Non-R.V.
Group 1	20-50 (range 30)	30-54 (range 24)	44.7	55.3
Group 2	28-50 (range 22)	46-54 (range 8)	81.8	18.2

Note: 2n = diploid number; N.F. = fundamental number; R.V. = percent of Robertsonian variation; Non-R.V. = percent of non-Robertsonian variation. Group 1, includes all the studied vespertilionid species; Group 2, includes all the studied vespertilionid species except for *L. noctivagans*.

The cytological evidence indicates that Robertsonian variation has played the major role in the evolution of the vespertilionid bats with the exception of the single genus *Lasionycteris*. The latter appears to be karyotypically divergent and occupies a position isolated from the remaining genera in the family.

Further evidence of this hypothesis can be shown in the genus *Pipistrellus*. Bovey's study of *Pipistrellus pipistrellus* shows that the karyotype of the species includes nine metacentric chromosomes and thirty-three rod shaped chromosomes for a diploid number of 42. Since his study involved the male sex, we can subtract one X-metacentric and one Y-acrocentric chromosome from the total and have remaining eight metacentric and thirty-two acrocentric chromosomes for the species autosomal karyotype. *Pipistrellus hesperus*, a New World representative of this genus, has an autosomal karyotype consisting of twenty-two metacentric and four acrocentric chromosomes (see Table 4). By the mechanism of centric fusion we can convert twenty-eight acrocentric chromosomes to fourteen metacentric chromosomes in *P. pipistrellus* and theoretically evolve *P. hesperus* which has a 2n number of 28, (twenty-two metacentric and four acrocentric chromosomes).

Table IV. Chromosome counts in the genus *Pipistrellus*.

Species	2n	Met.	Acr.
P. pipistrellus	42	8	32
P. hesperus	28	22	4

Note: Met. = metacentric chromosomes
Acr. = acrocentric chromosomes

Cytological Evidence Suggests the Taxonomic Revision of *Pizonyx vivesi*

Miller (1906) changed the generic name of *Myotis vivesi* to its present taxonomic status *Pizonyx vivesi*. This change in generic status was based on the following facts: 1. the size of the foot relative to the tibia exceeded that of any of the large-footed species of *Myotis* and the extreme compression of the claws was unlike any member in the related genus; 2. a glandular mass is present near the middle of the forearm and is not found in any other closely related species. All other characters fall within the *Myotis* range. The cytological data presented here indicates that the change by Miller in the generic status of *Myotis vivesi* was not valid and that *Pizonyx vivesi* should be put back with the genus *Myotis* as first described by Menegaux (1901). As shown in the karyograms of the three species of *Myotis*, *M. thysanodes*, *M. volans* and *M. fortidens*, and the karyogram of *Pizonyx vivesi*, these four individuals have identical karyotypes and cytologically are inseparable. Each species has a diploid number of 44 and identical chromosomal complements composed of two median, six submedian, and thirty-four terminal chromosomes. The size relationships between the four chromosome complements appear approximately equal when the karyotypes from the same mitotic stages are compared.

Evolutionary Trends and Relationships Within the Family Molossidae

Only two species of the genus *Tadarida* were studied, both showin a diploid number of 48. However, both karyotypes show morphological differences which can easily be used for species identification. *Tadarida brasiliensis* shows a unique karyotype consisting of eight submedial, thirty-eight subterminal and no terminal chromosomes. The subterminal chromosomes all have extremely small second arms which makes the concept of N.F. difficult to apply. If all of the arms were to be counted, the resultant N.F. would equal 92 as compared to the N.F. of *T. femorosacca*, (58). By not counting the subterminal arms, an N.F. of 54 is obtained and is more closely related to that found in *T. femorosacca* which has only four subterminal chromosomes. This striking difference may simply be due to the technique used in preparing the chromosome material or might be explained by a series of pericentric inversions. In either case further study on the cytology of the molossids is needed for a better understanding of the mechanisms involved in their evolution.

Evolutionary Trends and Relationships Within the Family Phyllostomatidae

Within the family Phyllostomatidae, Choeronycteris mexicanus exhibits a unique karyotype with a diploid number of 16. This represents the lowest diploid number that has thus far been determined in the order Chiroptera. Leptonycteris nivalis, the only other species studied belonging to the same subfamily, Glossophaginae, has a diploid number of 32 and the entire complement is composed of metacentric chromosomes. The karyotype of Choeronycteris mexicanus is composed of six pairs of metacentric, two pairs of subterminal, and two pairs of terminal chromosomes. Pteronotus davyi and Macrotus waterhousii, the two remaining phyllostomatids studied, each representing separate subfamilies, show diploid numbers of 38 and 40 respectively. From this data, we can only assume that Choeronycteris mexicanus represents a karyotypically divergent form in the family Phyllostomatidae while maintaining similar morphological characteristics. The other cytological explanation is that this species represents a different evolutionary lineage and shows extreme morphological convergence in adaptation and behavior mechanisms.

By assuming that Choeronycteris mexicanus is a divergent form and represents an isolated position in the evolution of the phyllostomatids, we can speculate as to the mechanism involved in the evolution of the remaining phyllostomatids by examining the karyotypes of the other three species studied. This will give us the result in Table 5 which indicates that Robertsonian variation has played the major role in the evolution of the phyllostomatids and that *Choeronycteris mexicanus* probably represents an isolated position within their evolution.

On the other hand, if we do not accept the theory that *Choeronycteris mexicanus* represents a divergent form then the results are reversed and non-Robertsonian variation has played the major evolutionary role.

Table V. Evolutionary trends in the family Phyllostomatidae based on diploid and fundamental numbers.

Family Phyllostomatidae	2n	N.F.	% R.V.	% Non-R.V.
Group 1	16-40 (range 24)	26-60 (range 34)	29.3	70.7
Group 2	32-40 (range 8)	56-60 (range 4)	75.0	25.0

Note: Group 1, includes all the studied phyllostomatid species; Group 2, excludes *Choeronycteris mexicanus*.

Summary and Conclusions

Improved cytological techniques have been used in a study of fourteen species of the order Chiroptera representing the families: Phyllostomatidae, Vespertilionidae, and Molossidae. Diploid numbers, fundamental numbers, and chromosome morphology have been determined for each species and corresponding karyograms have been illustrated.

The results from this study have provided the basis for the following conclusions:

1. Cytological information has in most cases agreed with and further substantiated previous work in the taxomony and phylogeny of Chiroptera.

2. Karyotype differences can be seen in all species examined with the exception of two genera, *Myotis* and *Pizonyx*.

3. The three species of *Myotis*: *M. thysanodes*, *M. volans*, and *M. fortidens* have similar karyotypes and can not be distinguished from each other.

4. Cytological evidence suggests that *Pizonyx vivesi* should be *Myotis vivesi*.

5. Centric fusion has played the major role in the evolution of Chiroptera.

6. Centric fusion can be demonstrated theoretically as the mechanism for the evolution of the New World *Pipistrellus hesperus* from the Old World *Pipistrellus pipistrellus*.

7. *Lasionycteris noctivagans* represents a divergent genus occupying an isolated position in the phylogeny of the family Vespertilionidae.

8. *Choeronycteris mexicanus* shows extreme karyotypic divergence in the family Phyllostomatidae.

Appendix A

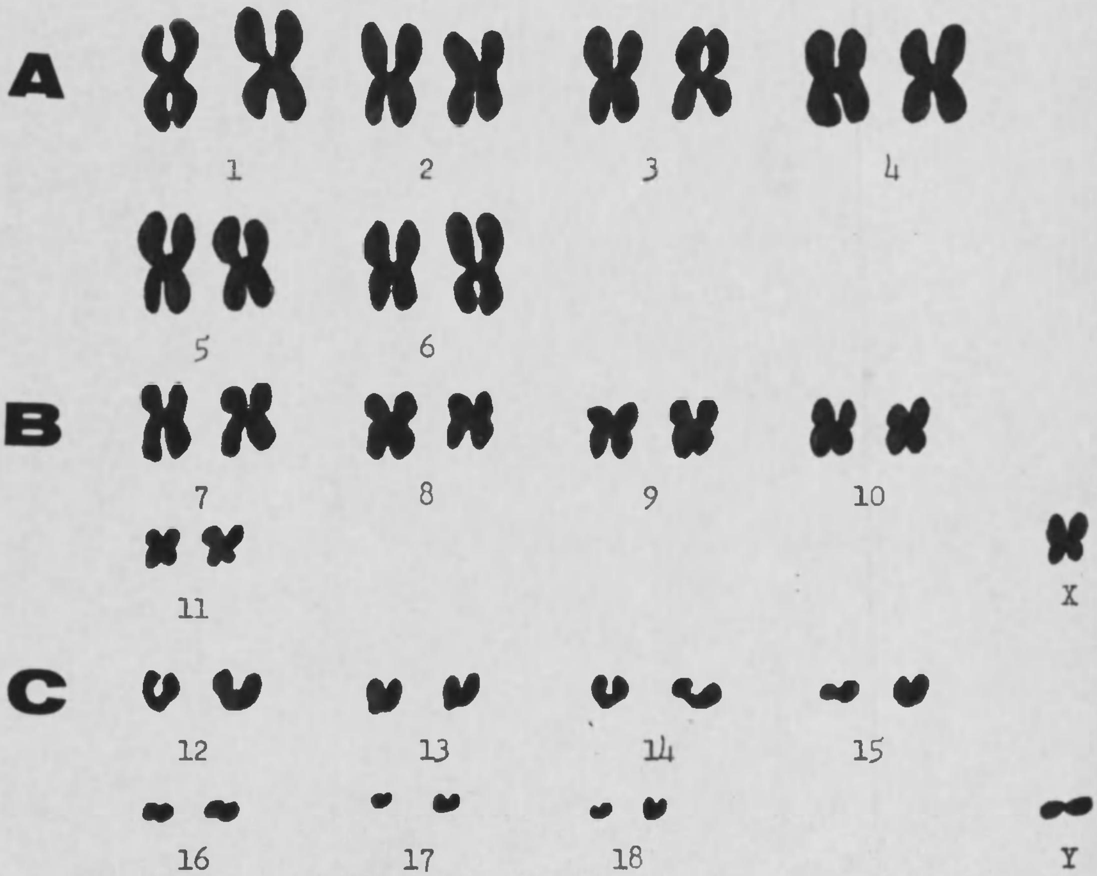

Plate 1. Representative male karyotype of <u>Pteronotus davyi</u> (24,000X)

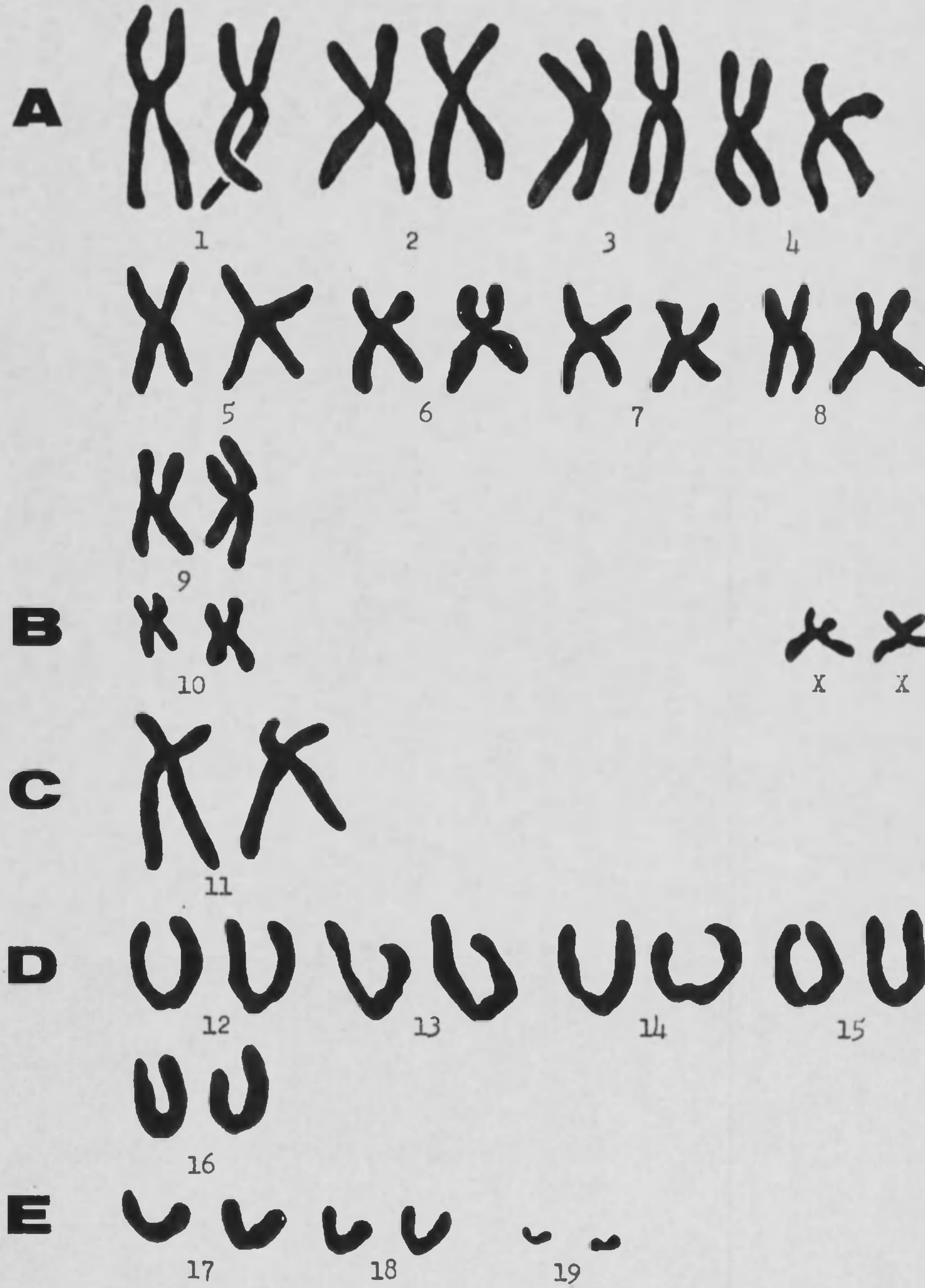

Plate 2. Representative female karyotype of <u>Macrotus waterhousii</u> (24,000X)

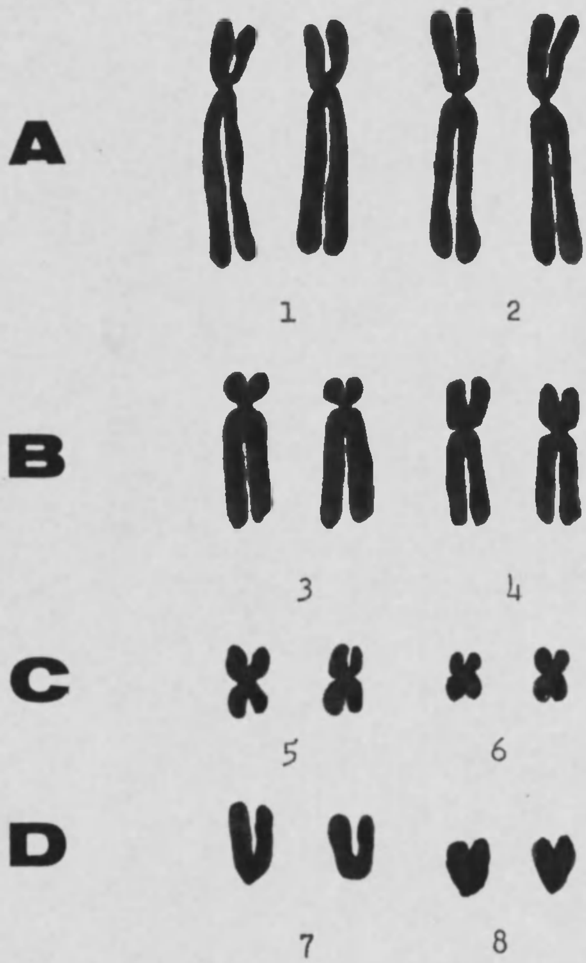

Plate 3. Representative female karyotype of Choeronycteris mexicanus (24,000X)

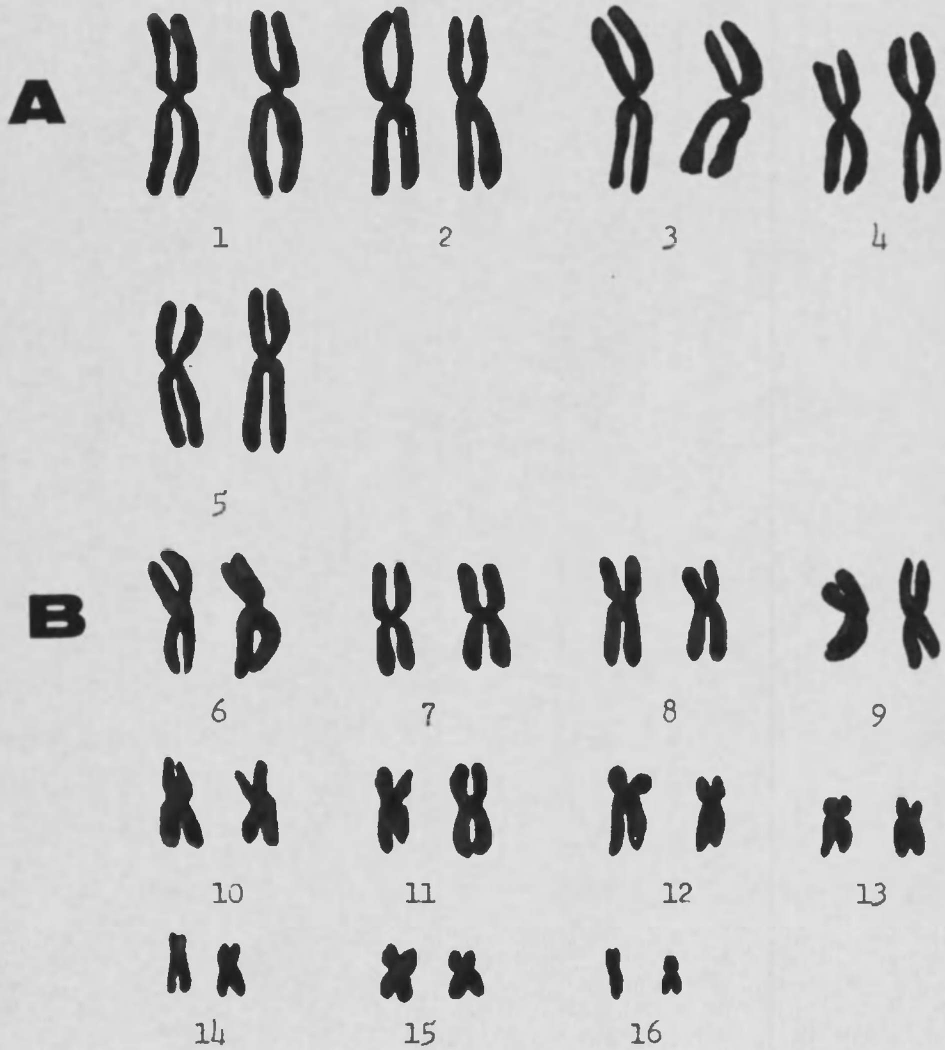

Plate 4. Representative female karyotype of <u>Leptonycteris nivalis</u> (24,000X)

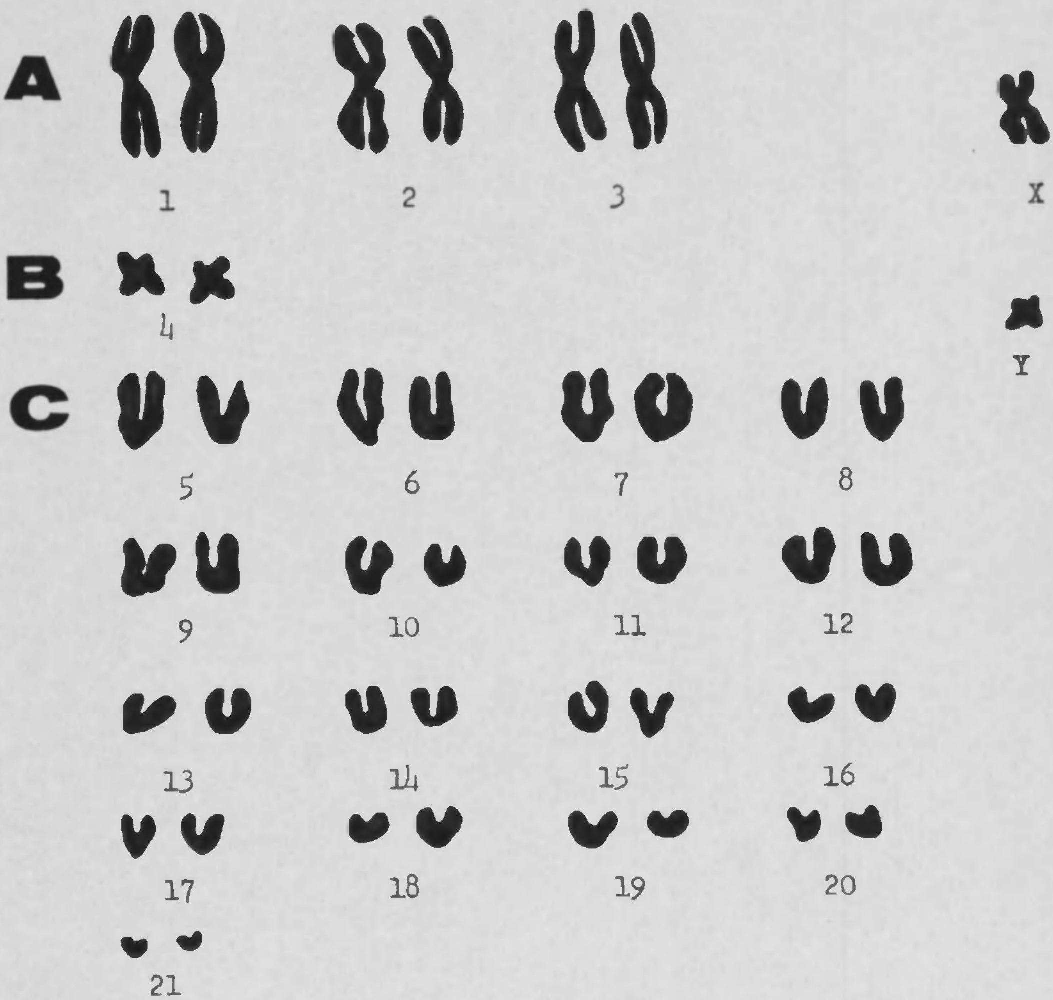

Plate 5. Representative male karyotype of <u>Myotis thysanodes</u> (24,000X)

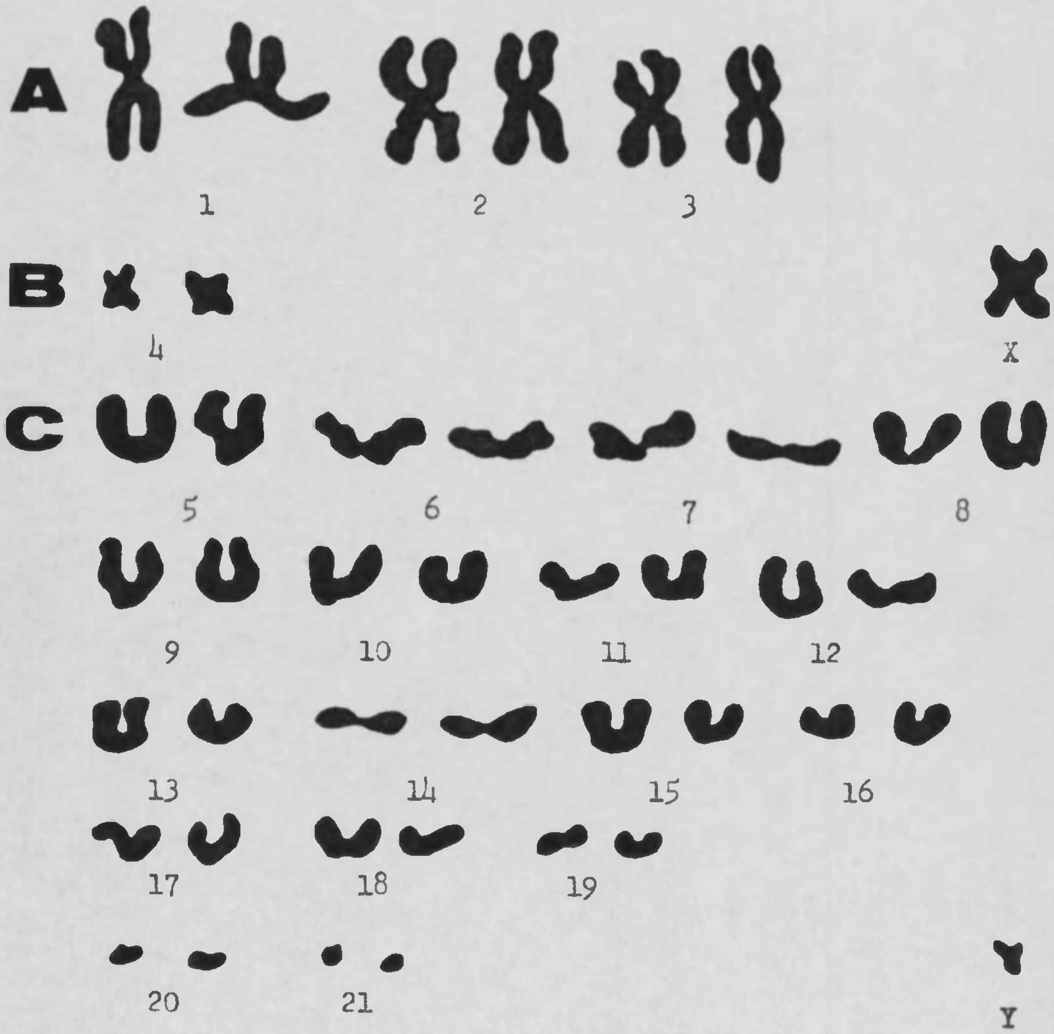

Plate 6. Representative male karyotype of Myotis volans (24,000X)

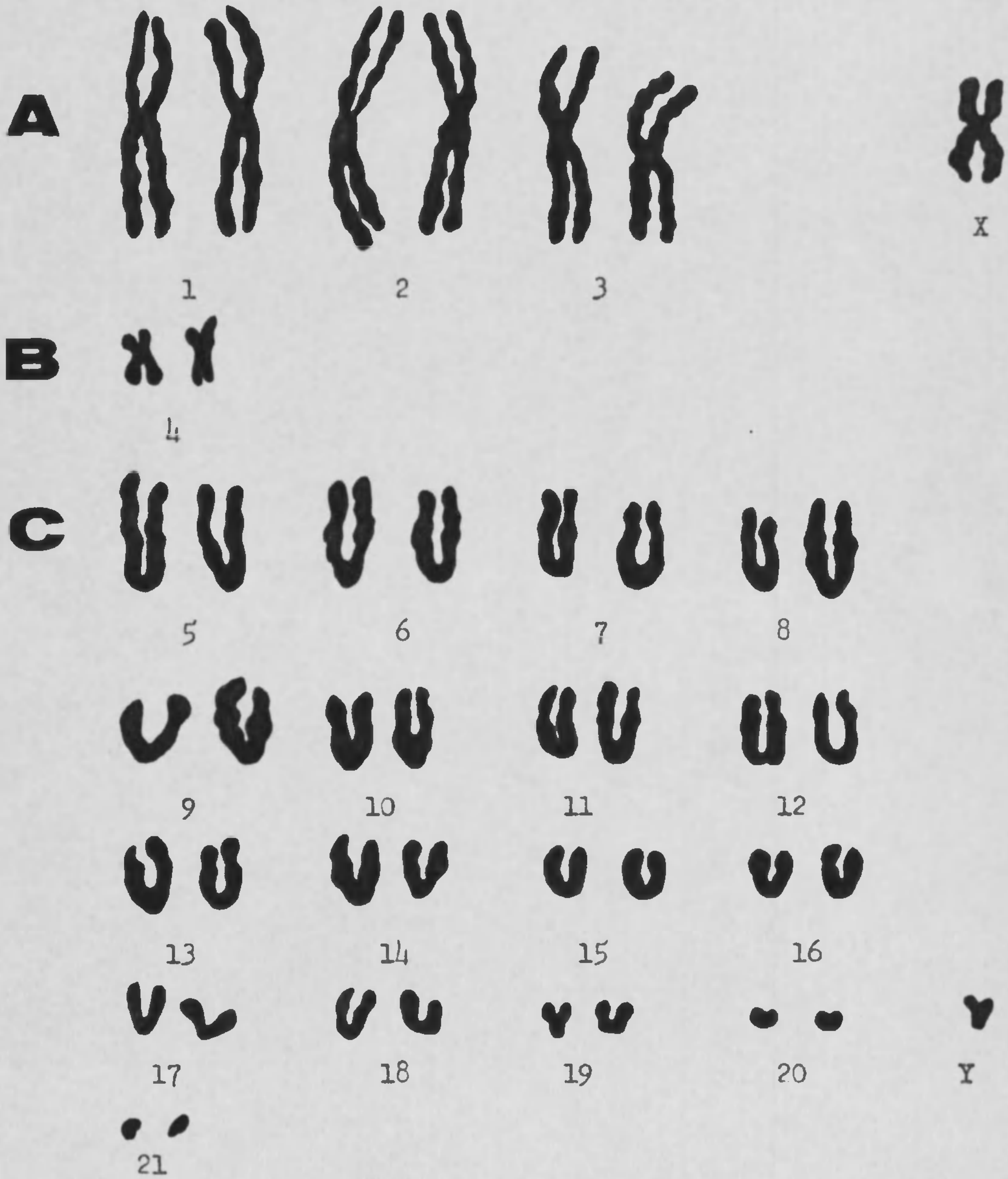

Plate 7. Representative male karyotype of <u>Myotis fortidens</u> (24,000X)

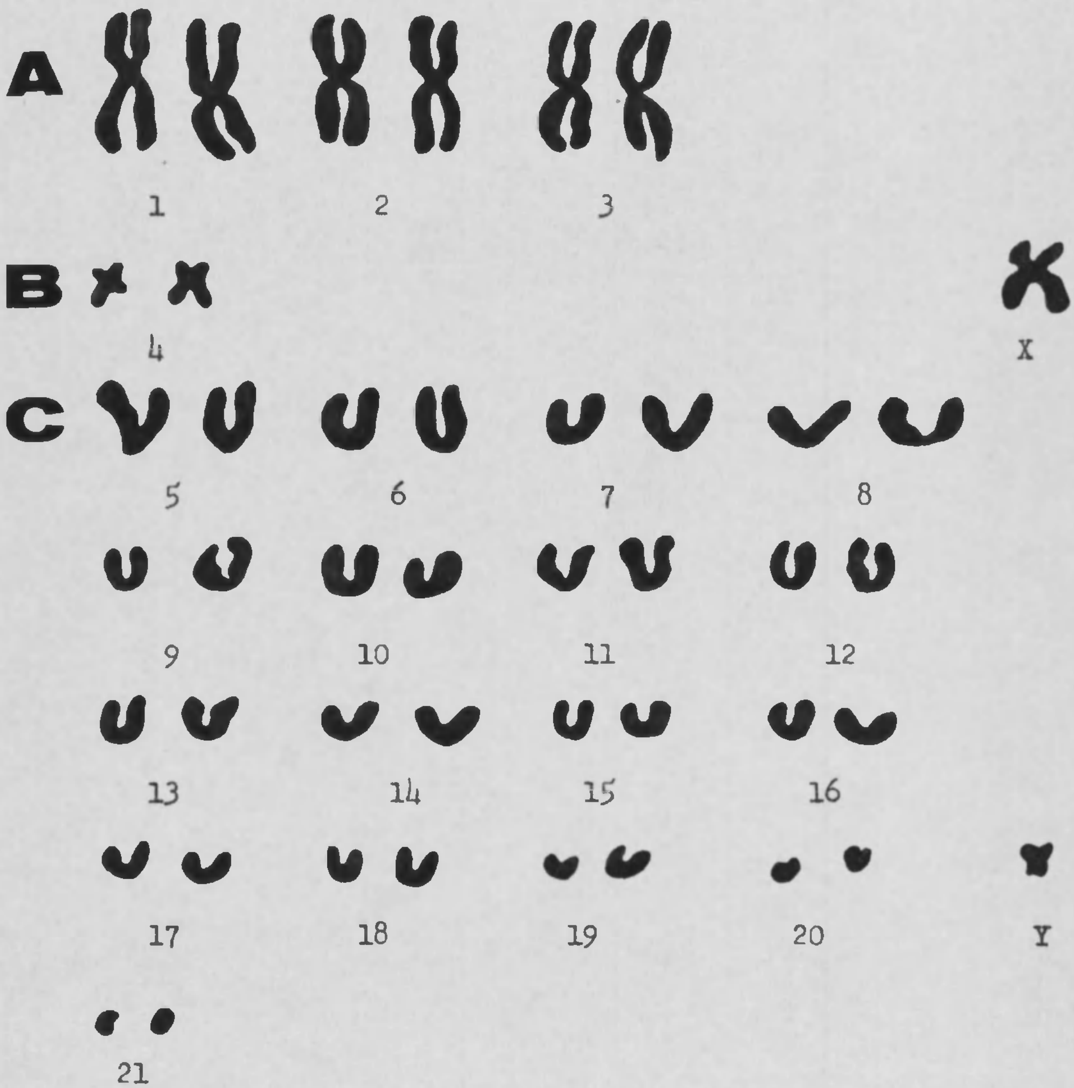

Plate 8. Representative male karyotype of <u>Pizonyx</u> <u>vivesi</u> (24,000X)

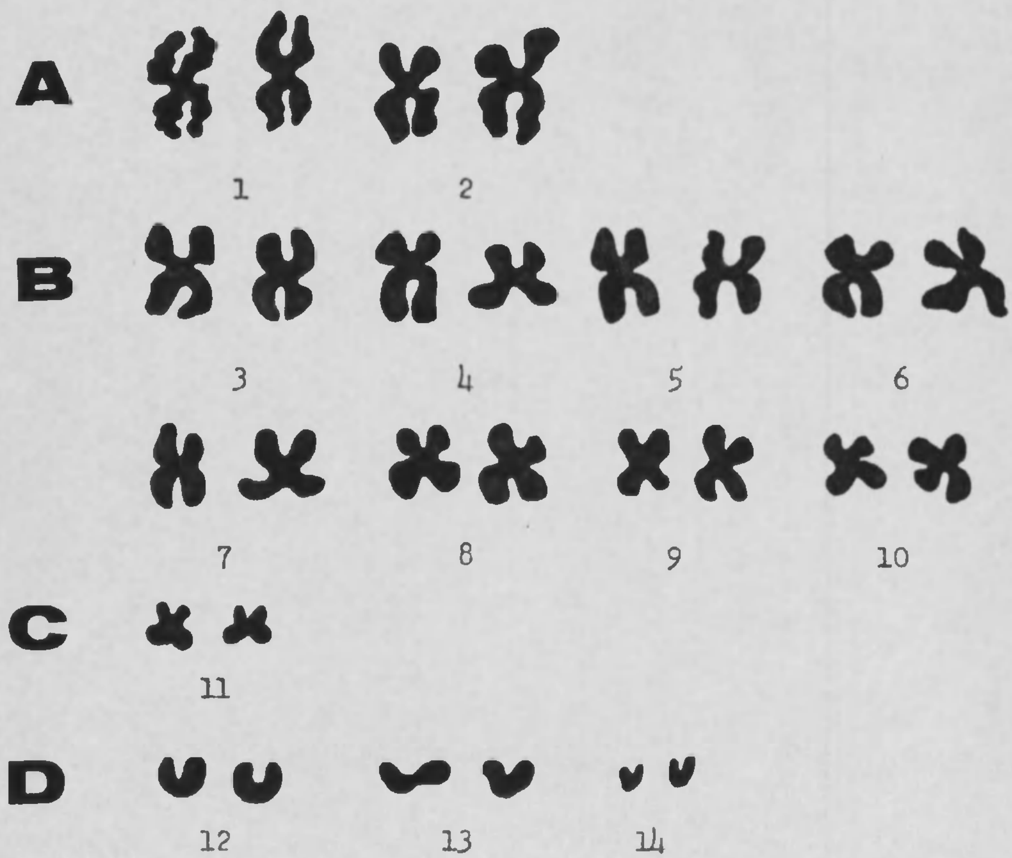

Plate 9. Representative female karyotype of <u>Pipistrellus hesperus</u> (24,000X)

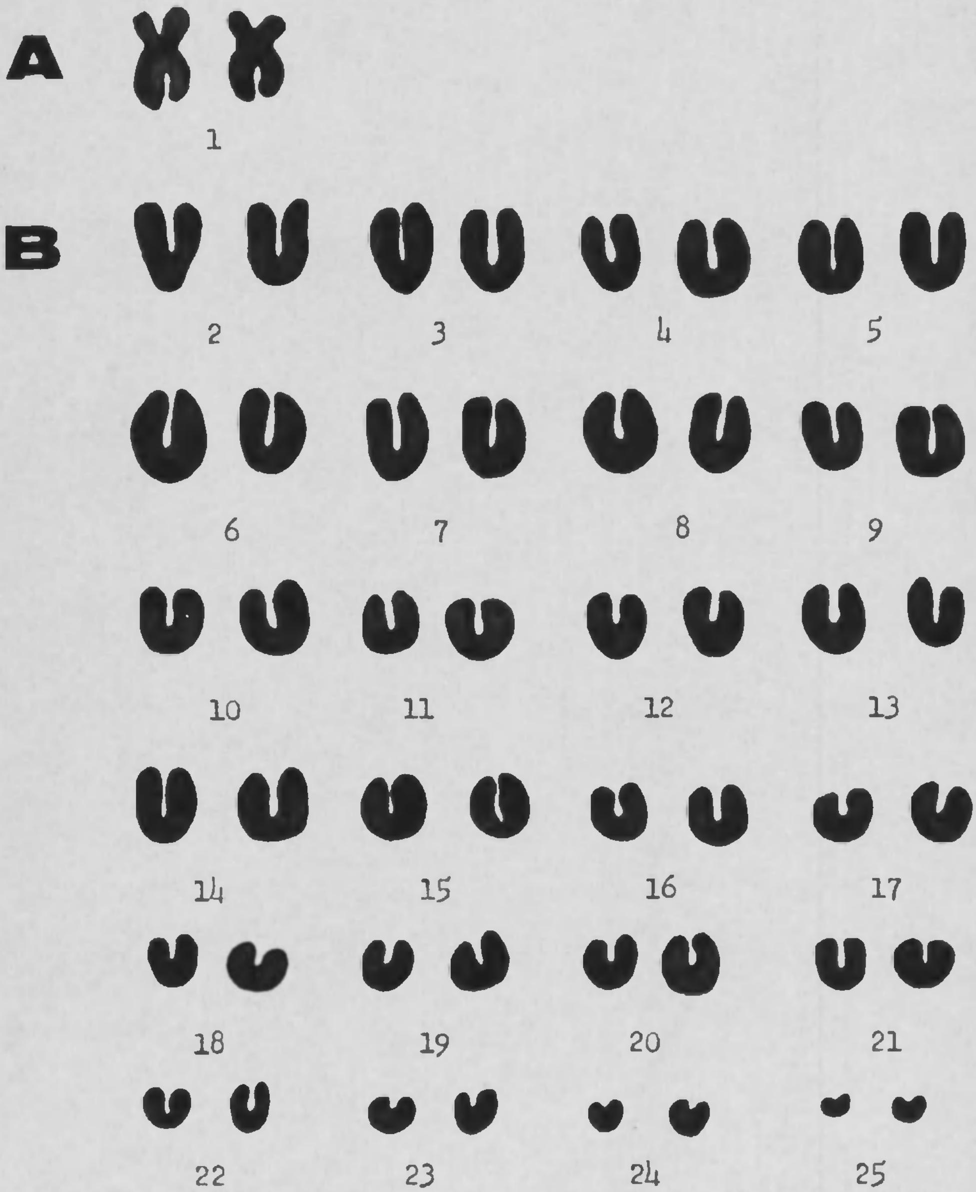

Plate 10. Representative female karyotype of <u>Eptesicus fuscus</u> (24,000X)

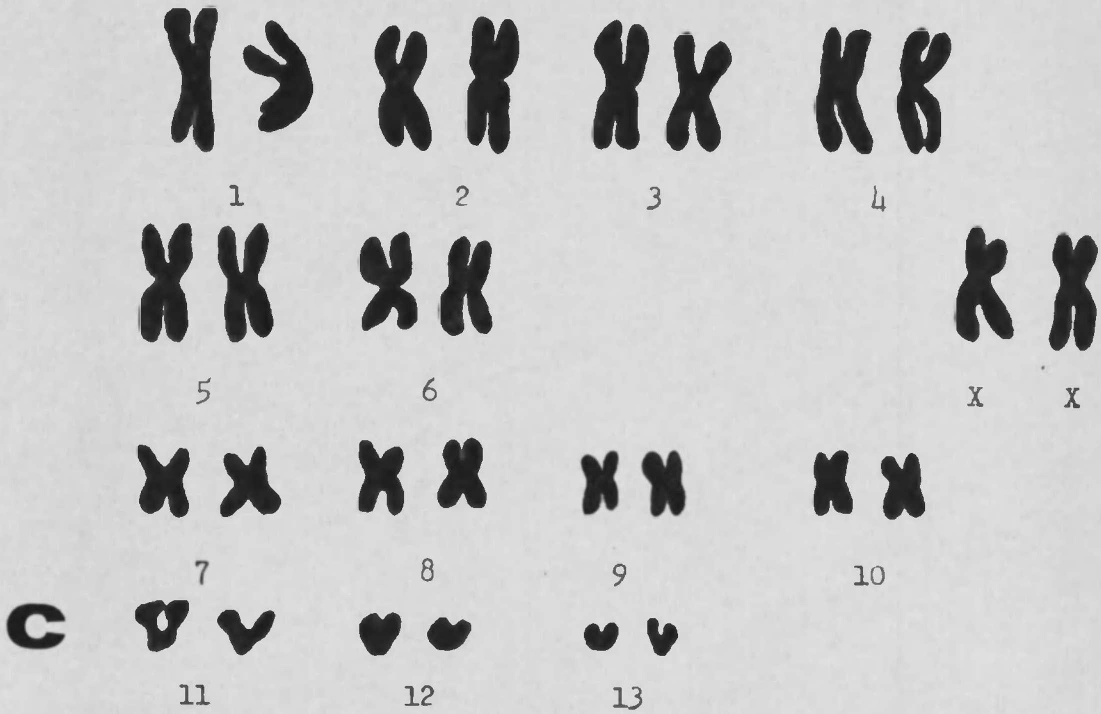

Plate 11. Representative female karyotype of <u>Lasiurus cinereus</u> (24,000X)

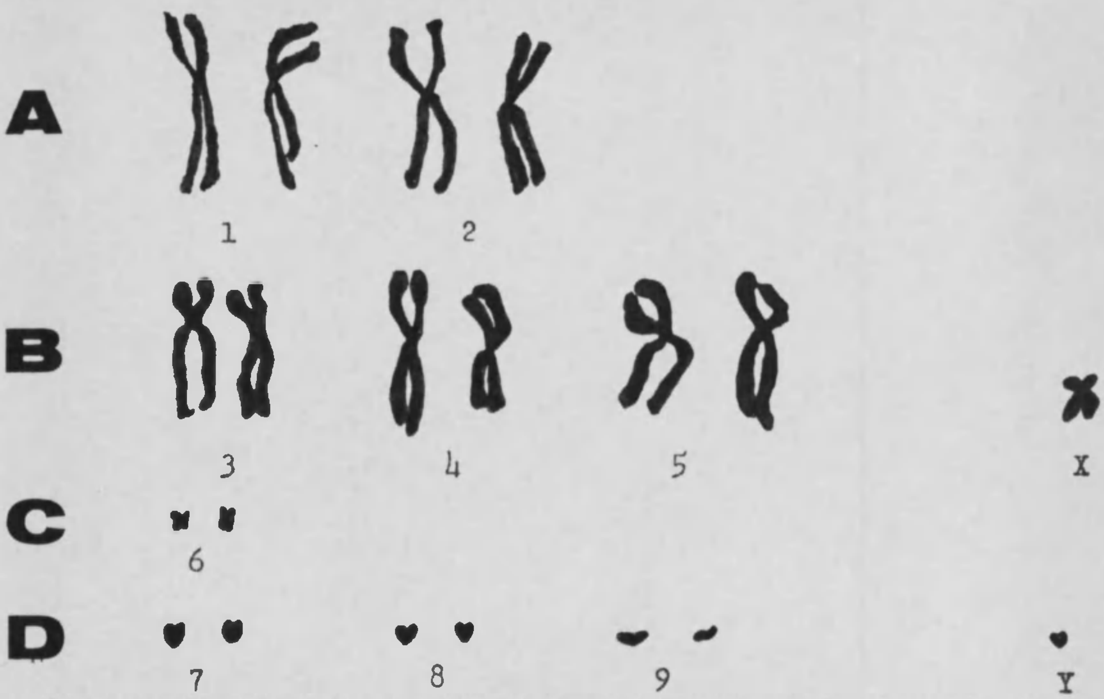

Plate 12. Representative male karyotype of <u>Lasionycteris noctivagans</u> (24,000X)

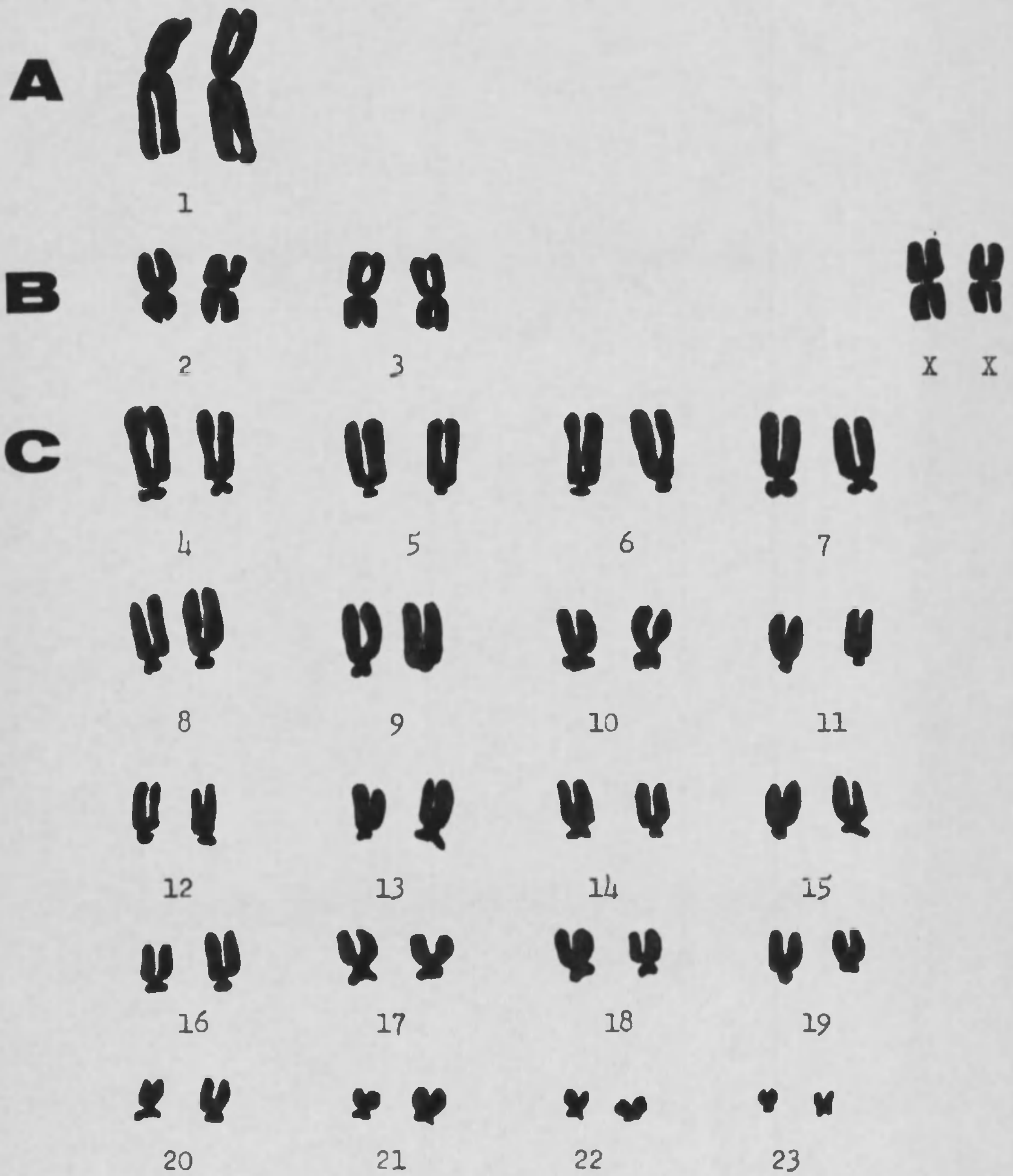

Plate 13. Representative female karyotype of <u>Tadarida brasiliensis</u> (24,000X)

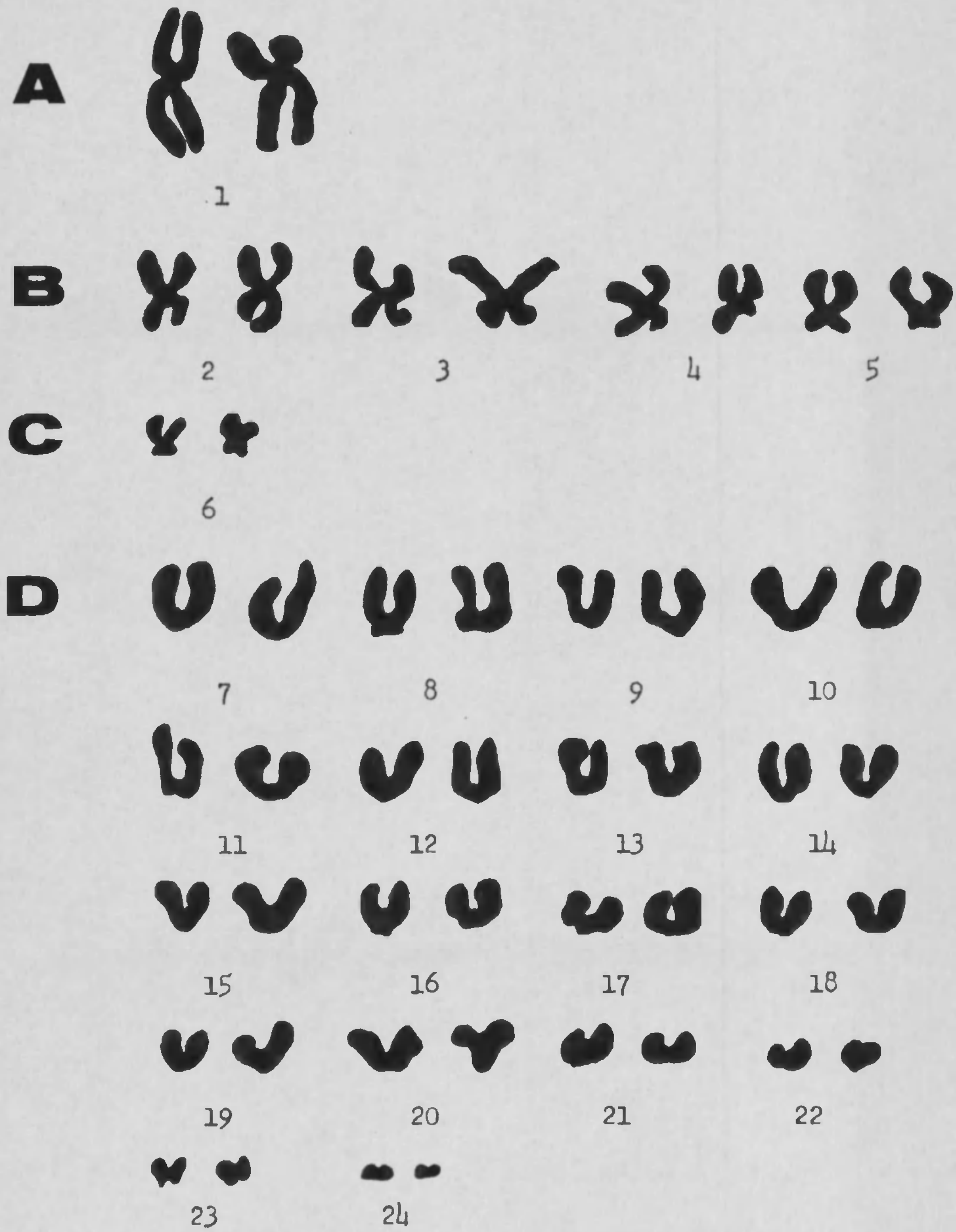

Plate 14. Representative female karyotype of Tadarida femorosacca (24,000X)

Appendix B

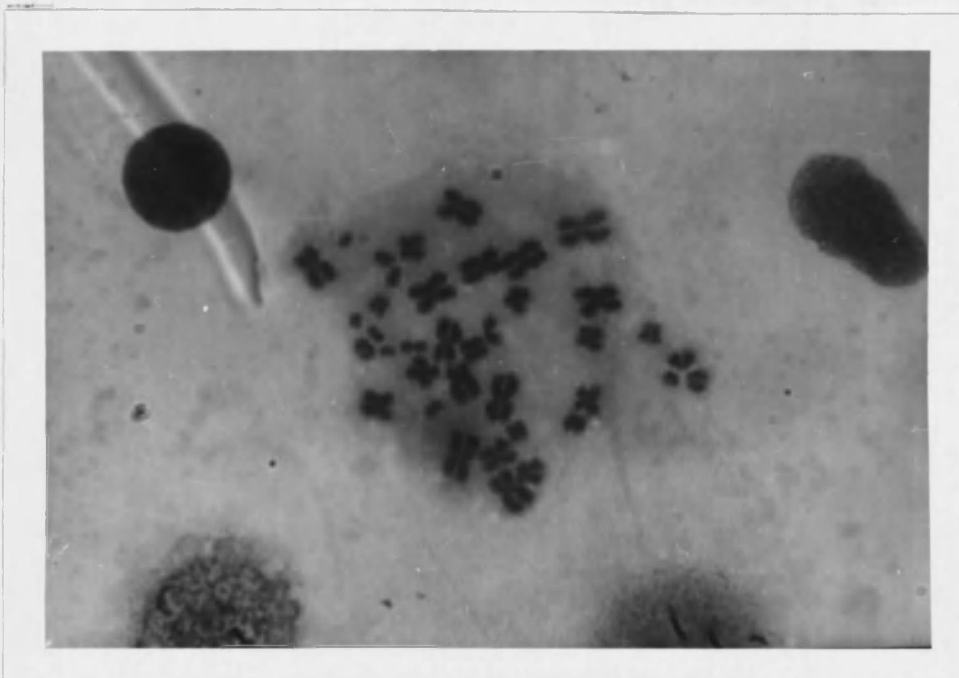

Figure 1. Pteronotus davyi: Photomicrograph of representative chromosome spread (9,600X)

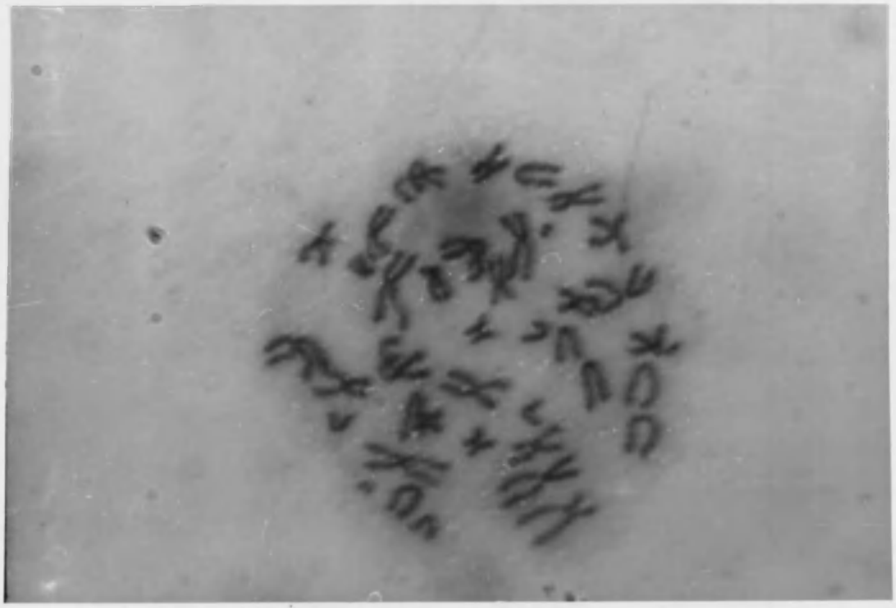

Figure 2. Macrotus waterhousii: Photomicrograph of representative chromosome spread (9,600X)

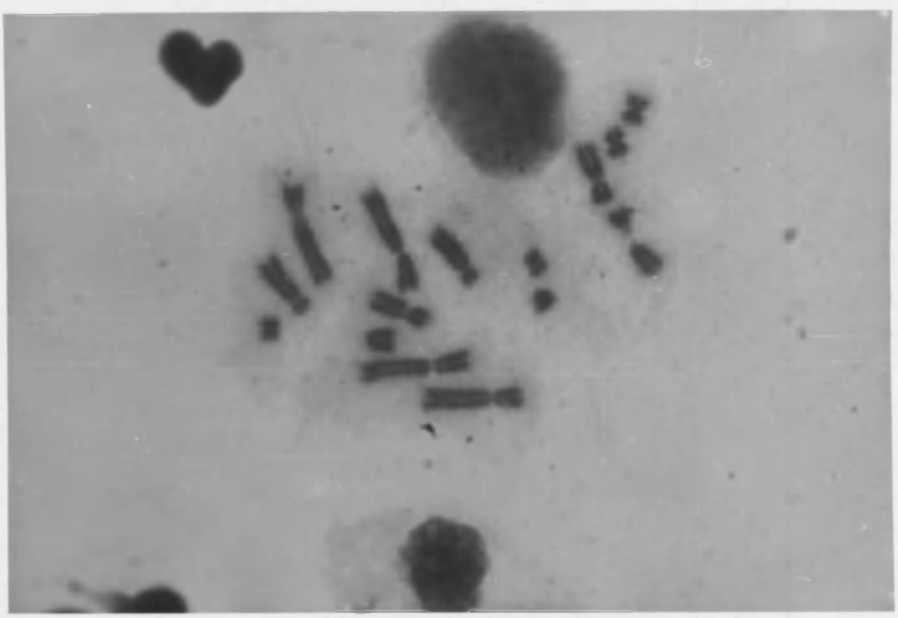

Figure 3. Choeronycteris mexicanus: Photomicrograph of representative chromosome spread (9,600X)

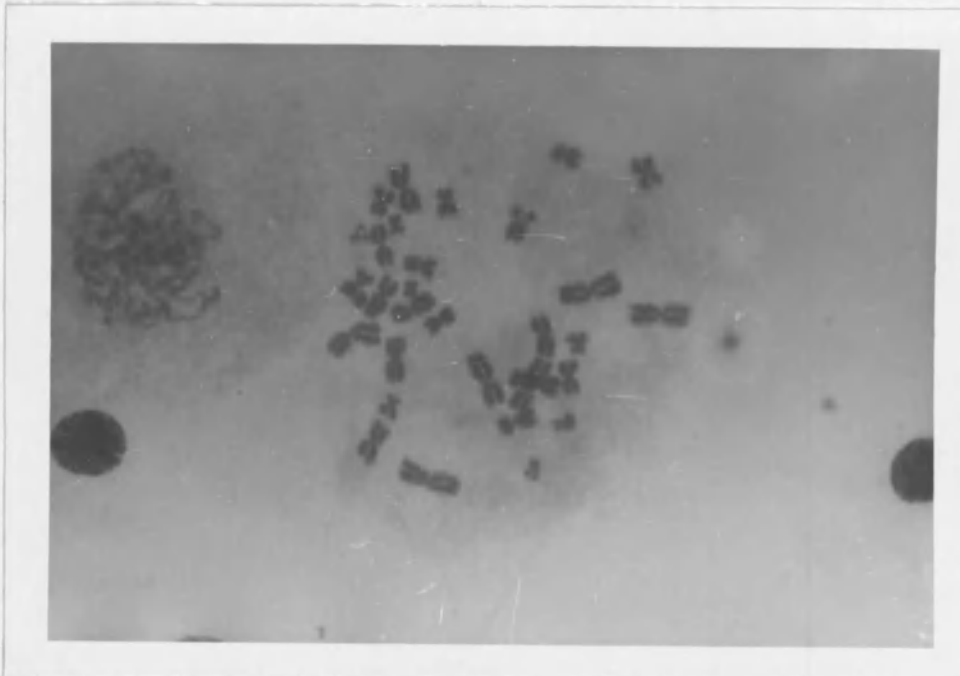

Figure 4. Leptonycteris nivalis: Photomicrograph of representative chromosome spread (9,600X)

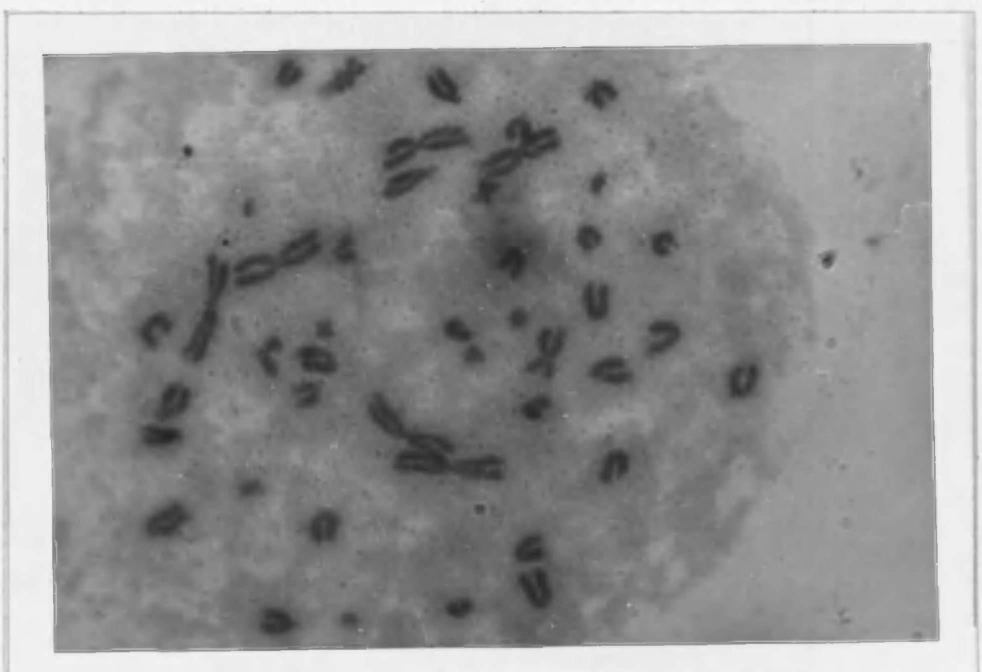

Figure 5. Myotis thysanodes: Photomicrograph of representative chromosome spread (9,600X)

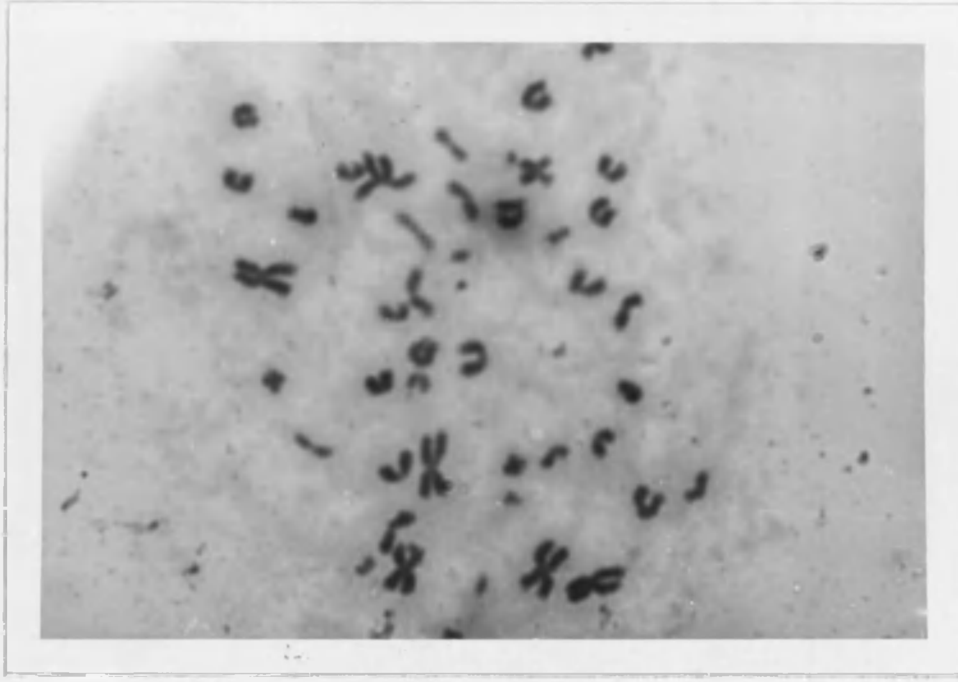

Figure 6. Myotis volans: Photomicrograph of representative chromosome spread (9,600X)

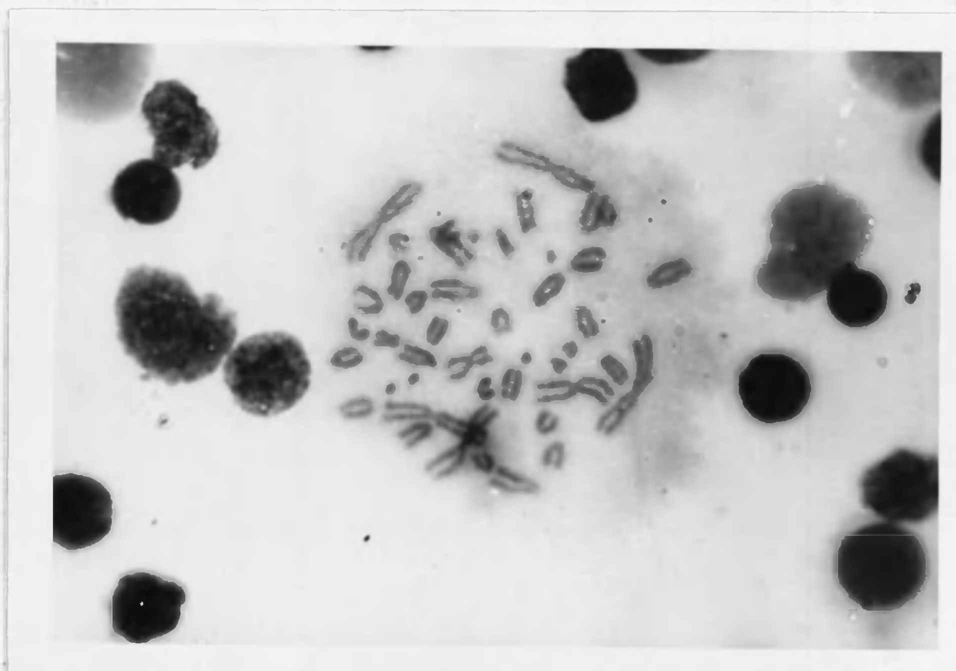

Figure 7. Myotis fortidens: Photomicrograph of representative chromosome spread (9,600X)

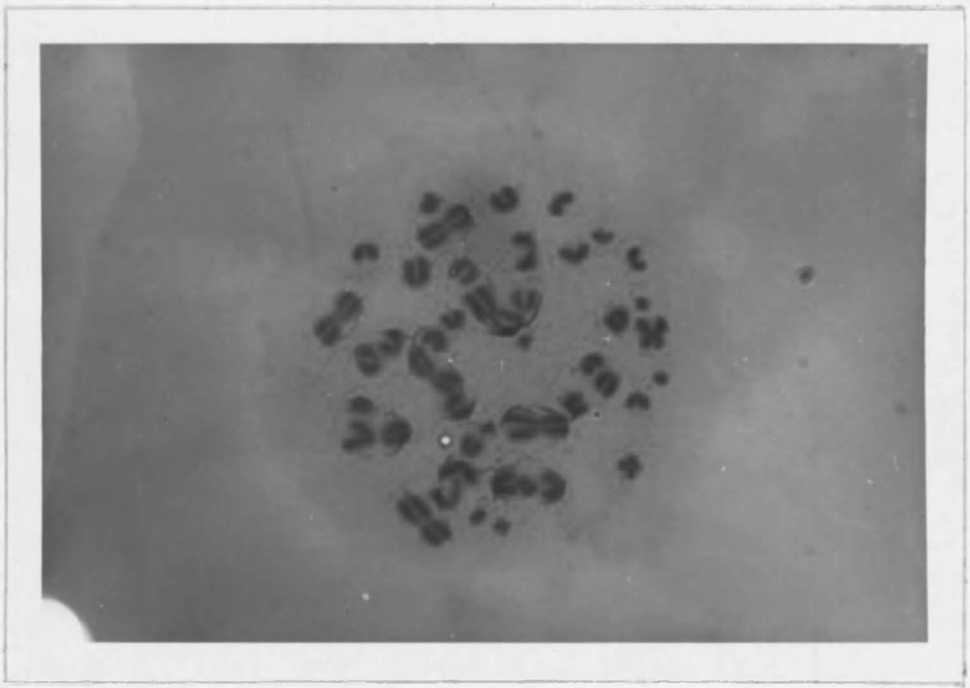

Figure 8. Pizonyx vivesi: Photomicrograph of representative chromosome spread (9,600X)

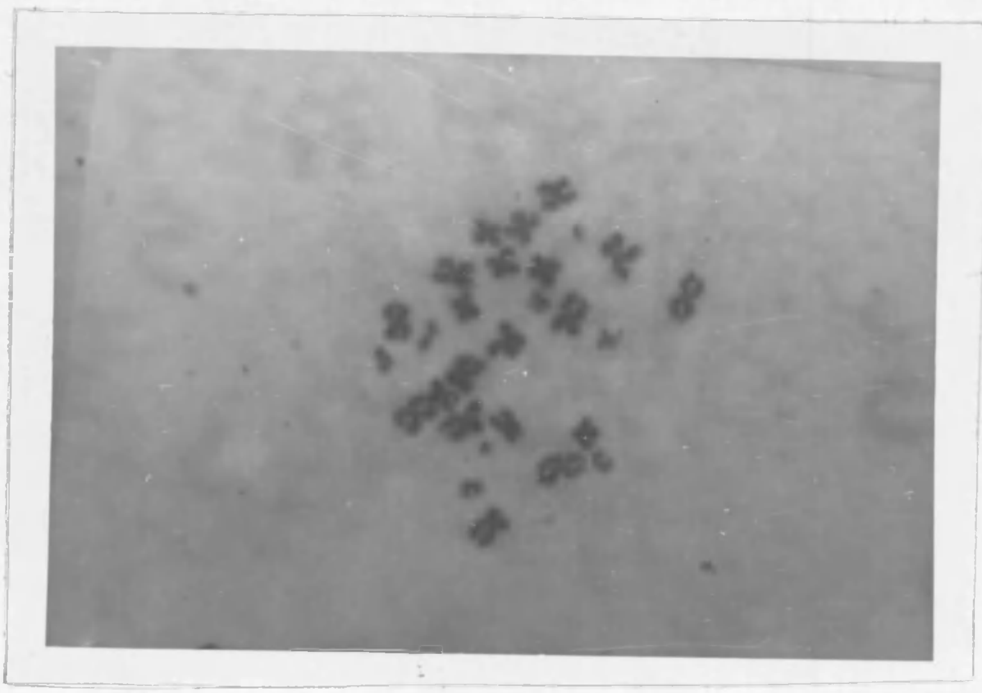

Figure 9. Pipistrellus hesperus: Photomicrograph of representative chromosome spread (9,600X)

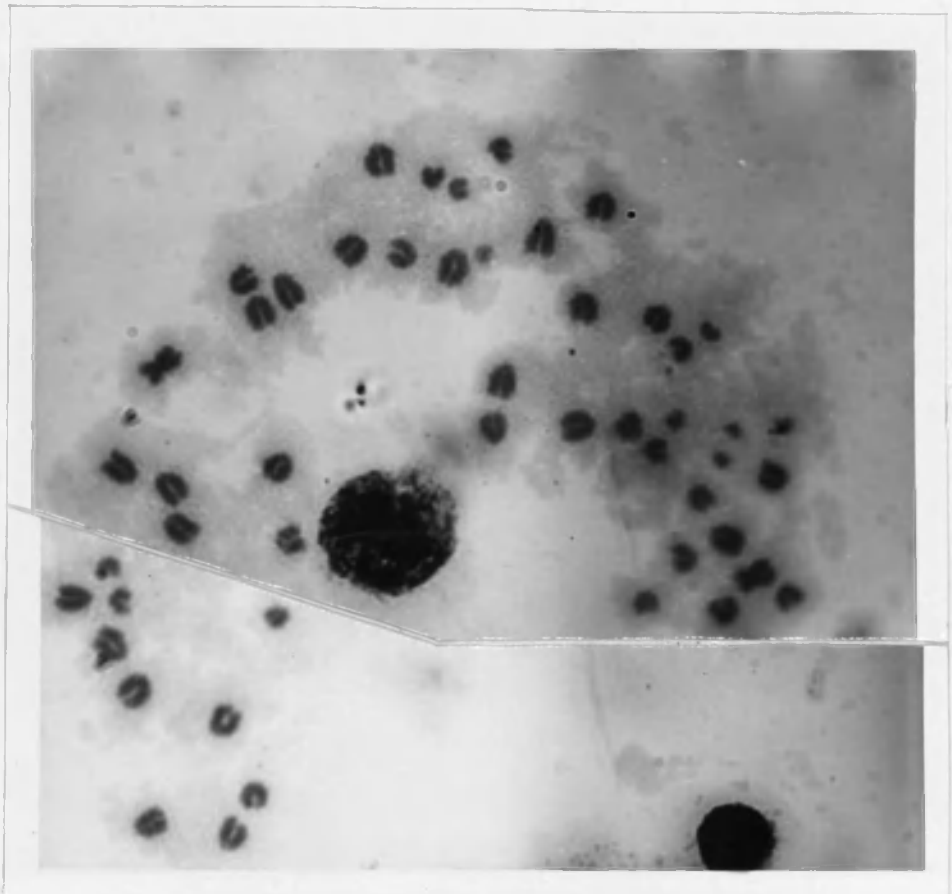

Figure 10. Eptesicus fuscus: Photomicrograph of representative chromosome spread (9,600X)

Figure 11. Lasiurus cinereus: Photomicrograph of representative chromosome spread (9,600X)

Figure 12. Lasionycteris noctivagans: Photomicrograph of representative chromosome spread (9,600X)

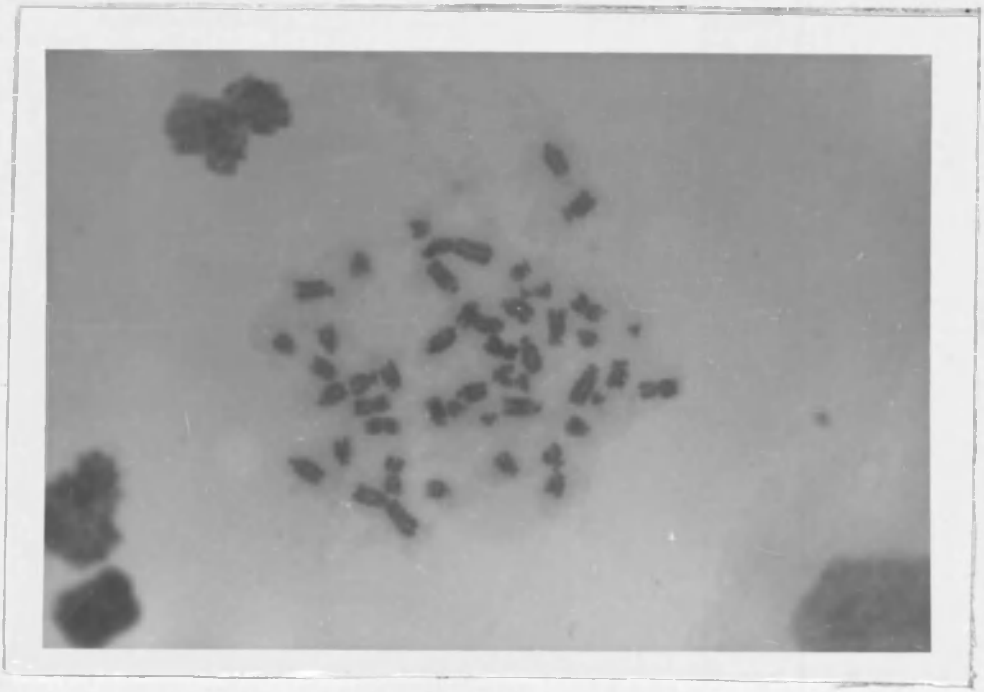

Figure 13. Tadarida brasiliensis: Photomicrograph of representative chromosome spread (9,600X)

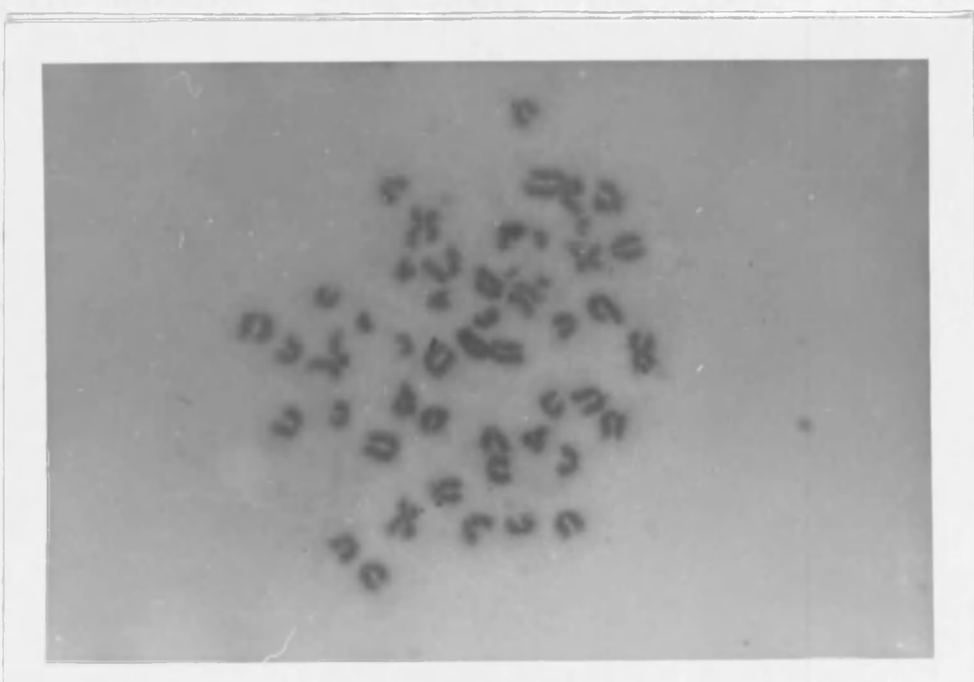

Figure 14. Tadarida femorosacca: Photomicrograph of representative chromosome spread (9,600X)

Appendix C

Pteronotus davyi: Two specimens examined; (251), Cueve de la Tigre, Sonora, Mexico; UA 13420, Minos Armolillo, Sonora, Mexico.

Macrotus waterhousii: Three specimens examined; UA 13412, UA 13415, UA 13416, Cueve de la Tigre, Sonora, Mexico.

Choeronycteris mexicanus: One specimen examined; UA 11530, Madera Canyon, Pima Co., Arizona.

Leptonycteris nivalis: Three specimens examined; UA 13421, Minos Armolillo, Sonora, Mexico; (57), (58), Colossal Cave, Pima Co., Arizona.

Myotis thysanodes: Three specimens examined; UA 11536, UA 11537, (225), Madera Canyon, Pima Co., Arizona.

Myotis volans: Two specimens examined; UA 11519, UA 11522, Santa Catalina Mts., Pima Co., Arizona.

Myotis fortidens: One specimen examined; UA 11538, Colima, Mexico.

Pizonyx vivesi: Two specimens examined; UA 13425, UA 13426, San Carlos Bay, Sonora, Mexico.

Pipistrellus hesperus: One specimen examined; UA 13422, Minos Armolillo, Sonora, Mexico.

Eptesicus fuscus: Two specimens examined; UA 13424, Pima Co., Arizona; (164), Tucson, Pima Co., Arizona.

Lasiurus cinereus: Two specimens examined; UA 11517, UA 11520, Santa Catalina Mts., Pima Co., Arizona.

Lasionycteris noctivagans: Two specimens examined; UA 11518, UA 13423, Santa Catalina Mts., Pima Co., Arizona.

Tadarida brasiliensis: Two specimens examined; UA 13418, (252), Cueve de la Tigre, Sonora, Mexico.

Tadarida femorosacca: One specimen examined; UA 13419, Minos Armolillo, Sonora, Mexico.

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