

## The Role of Karyotypes in Phylogenetic Studies of Bats

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There are several reasons for using chromosome form and number (the karyotype) in phylogenetic studies. Since the material of the genotype forms part of the chromosomes, the karyotype is less influenced by external factors than are other morphological and physiological characteristics (John and Lewis, 1966). There are, however, two obvious ways natural selection can act on changes in the karyotype. The first involves the ability of the karyotype to proceed through the mechanics of mitosis or meiosis. If mitosis is blocked the cell line would die. If the rearrangement interferes with proper functioning of meiosis the result is reduced or there is no gamete production. In either case a new karyotype must pass this test, regardless of the advantage it confers to the animal's phenotype. Further, this aspect of natural selection would act before the new karyotype could be inherited.

The second way in which natural selection could favor one karyotype over another would be the degree of phenotypic fitness as the result of different karyotypes. That karyotypic variation can affect the phenotype is well documented (see John & Lewis, 1966, for a review). Thus, from a genetic standpoint the karyotype offers a unique morphological level for study.

Considering the deleterious effects usually associated with changes in chromosomal structure (*i.e.*, inversions and translocations, see Bender & Chu, 1963), and also the role of natural selection in regulation of the karyotype, it would be easy to conclude that very few changes could become established. Thus, one or a few established karyotypic differences would always form an isolating mechanism between populations. However, in some mammalian cases a great number of rearrangements have been established, as in *Thomomys* (Patton & Dingman, 1968; and Thaeler, 1968) and *Sigmodon hispidus* (Zimmerman & Lee, 1968). In some cases a great number of rearrangements do not act as a complete isolating mechanism. In two species of gophers (genus, *Thomomys*) having karyotypes differing in diploid number (76 and 78) and in fundamental number (148 and 96), fertile hybrids were produced (Patton & Dingman, 1968). Therefore, karyotypic differences between allopatric populations do not necessarily signal specific status for the two forms.

Before any morphological characters can be used for inferring phylogenetic relationships, it is necessary to understand the nature and degree of variation of that character within the group under study. For instance,

in rodents, karyotypic variation is frequently found within species (Matthey, 1966, 1968; Matthey & Petter, 1968; Hsu & Arrighi, 1968; Patton & Dingman, 1968; Thaeler, 1968; Baker & Mascarello, 1969a). Speciation within rodent genera is generally accompanied by the establishment of karyotypic changes (see literature cited above).

In bats, very few chromosomal variations have been reported within species (Baker, in press), and the majority of the species of some genera have indistinguishable karyotypes, (*i.e.*, *Myotis*, *Eptesicus*, and *Lasiurus*, Baker & Patton, 1967). From these limited examples it is obvious that the patterns of karyotypic variation in rodents and bats are different at the various taxonomic levels.

Karyotypic stability seems to be characteristic of bats (Baker, 1967 and in press; Baker & Patton, 1967; and Capanna *et al.*, 1968a). There are exceptions to this apparent stability, as in the genera *Pipistrellus* and *Tonatia* and the species *Macrotus waterhousii* and *Rhogeessa tumida*. In the genus *Pipistrellus*, ten species have been examined and the diploid number varies from 26 to 44 and the fundamental number from 44 to 56. In the genus *Tonatia* only two species have been examined and the diploid number varies from 16 to 30 and the fundamental number from 20 to 56 (Baker, in press). No karyotypic similarities are shown between the two species of *Tonatia*. In both *Macrotus* and *Rhogeessa* two chromosomal races are known, and several rearrangements are necessary to explain the different karyotypes found within each species (Nelson-Rees *et al.*, 1968; Baker, in press). Nevertheless, in most cases the karyotype appears to be a slowly evolving or stable character, when compared with other morphological features used to indicate generic relationships in bats.

In some groups of bats adaptation to feeding niches often results in rather extreme modifications of dental, cranial, and other characters which were used in more classical taxonomic studies. These extreme modifications can mask past relationships. By using a combination of characters which would not necessarily be altered by adaptations to specific feeding niches, a better perspective of the true relationships of the taxa under question can be obtained. Karyotype, as well as bacula morphology, protein patterns, and immunology would fit into this category.

Some problems in using karyotypes are that it is presently impossible to determine the direction of evolution or the primitive karyotype for specific groups of bats. Also, when karyotypes look alike it does not necessarily mean the two forms are closely related. Most mammalian karyotypes have from 30-60 chromosomes (Matthey, 1968) and there are a limited number of ways that the chromatin can be distributed on

the centromeres. So the possibility of convergent karyotypes must be considered.

Preparation of somatic chromosomes is a rather simple process and can be conducted in the field. For the bone marrow-*in vivo* technique given below, live animals are required. When species that die within a few days of capture are studied, field preparation of slides for karyotypic examination is a "must." The following technique is modified after Baker (in press).

1. Inject the live animal intraperitoneally with a 0.025% Vinblastine (Velban of Eli Lilly & Co.) or colchicine solution at 0.01 ml per gram of body weight.

2. Two hours later sacrifice the animal and remove about  $\frac{2}{3}$  of the humerus without damaging the proximal end. Remove the flesh and a chip of bone from the proximal end of the humerus to expose the red bone marrow cavity. Flush the shaft with 3 ml of a 1.0% sodium citrate solution. Pipette vigorously to break up any cell clumps. The sodium citrate solution will support bacterial growth and must be freshly prepared under field conditions.

3. Let the resultant cell-suspension set for 10 minutes.

4. Filter the suspension through two layers of cheesecloth and centrifuge at 500-1500 RPM for four minutes.

5. Discard as much of the supernatant fluid as possible, being careful to leave the button of cells undisturbed. Add 3 ml of freshly prepared Carnoy's fixative (3 parts absolute methanol: 1 part glacial acetic acid). Floating material and lipids may be removed at this stage. Gently disrupt the cell button with a pipette until the cell suspension is homogeneous. Allow cells to fix for 10-12 minutes.

6. Centrifuge for 4 minutes and decant supernate. Re-suspend cells in 1.0 ml of fixative and centrifuge as before. This step is repeated at least three times. After final washing, cells are re-suspended in 1.0 ml of fixative.

7. Place three or four drops of cell suspension on a clean slide and ignite. When the fire extinguishes itself, the residue is promptly slung from the slide. Four slides from each specimen are usually made.

8. Dry slides are stained with Giemsa's stain (1 part Giemsa's stock solution: 8 parts distilled water) for 15 minutes.

9. Pass slides through two baths of acetone, one of acetone and xylol (1:1) and two of xylol, then mount under a 22 x 40 mm coverslip with Permout.

Voucher specimens, with accurate collection data, should be deposited in a reputable museum.

A considerable number of papers dealing with bat chromosomes have

been published. A review of these works is given by Baker (in press). Literature on the chromosomes of the respective bat families is as follows: PTEROPIDAE: Makino, 1948; Manna & Talukdar, 1965; Pathak, 1965b, 1965c, 1966. RHINOPOMIDAE: Ray-Chaudhuri & Pathak, 1966; EMBALLONURIDAE: Ray-Chaudhuri & Pathak, 1966; and Baker (in press), NOCTILIONIDAE: Baker in press; MEGADERMATIDAE: Ray-Chaudhuri & Pathak, 1966; RHINOLOPHIDAE: Makino, 1958; Bovey, 1949; Matthey & Bovey, 1948; Capanna & Civitelli, 1964a and 1964b Capanna *et al.*, 1967; Dulic 1966 and 1967; Baker in press; PHYLLOSTOMATIDAE: Baker, 1967, in press; Kniazeff *et al.*, 1967, Nelson-Rees *et al.*, 1968; Hsu *et al.*, 1968; Gardner & O'Neill, 1969; DESMODONTIDAE: Hsu & Benirschke 1967; Foreman *et al.*, 1968; NATALIDAE: Baker, in press; THYROPTERIDAE Baker, in press; VESPERTILIONIDAE: Matthey & Bovey, 1948; Bovey, 1949; Osborne, 1965; Capanna, 1968, Capanna & Civitelli, 1964b and 1965, 1966 and 1967: Capanna *et al.*, 1967; Capanna *et al.*, 1968; Takayama, 1965; Pathak, 1965a, Manna & Talukdar, 1965; Baker & Patton, 1967; Dulic *et al.*, 1967; Baker & Mascarello, 1969b B; MOLLOSIDAE: Painter, 1925, Kniazeff *et al.*, 1967; Patton & Baker, 1966; Baker, in press.

The two most commonly reported karyotypic values are diploid number and fundamental number. In Figure 1 the range of diploid numbers reported for each bat family is shown in relationship to the range of values found within the order. In Figure 2 the range of fundamental numbers reported for each bat family is shown in relationship to the range of values found within the order. In the best-studied families a wide range of variation is found in both diploid and fundamental number. Wide ranges of variation are common for diploid numbers found within closely related forms. Such variation is usually explained by the Robertsonian process. In Robertsonian variation the diploid number varies while the fundamental number remains constant. The two families (Phyllostomatidae and Vespertilionidae) having the widest range of diploid-number variation also have the widest range of fundamental-number variation.

From Figs. 1 and 2 it is apparent that considerable chromosomal evolution has occurred within bat families.

Because of the wide range of overlapping variation in chromosomal values between bat families, karyotypic studies will seldom shed any light on relationships between bat families.

In making karyotypic study of a group of bats the logical sequence of investigation is a survey of somatic chromosomes, followed by intensive efforts on specific problems revealed by the survey. Before any character can be used in phylogenetic studies, an understanding must

exist of its variation at various taxonomic levels. Therefore, the benefits of such a survey would be two-fold; first it would establish the nature and degree of karyotypic variation as well as reveal any unique chromosomal features of the group. The occurrence of such features as chromosomal races, polymorphisms, and atypical sex chromosome systems is not predictable. For example, there seems to be no reason to expect chromosomal races in *Macrotus waterhousii*, (Nelson-Rees *et al.*, 1968) and *Lasiurus ega*, (Baker & Patton, 1967), and not to expect such races in such widely distributed species as *Glossophaga soricina*, *Eptesicus fuscus*, *Lasiurus borealis* and *Lasiurus cinereus* (Baker & Patton, 1957 and Baker, 1967). Also it would have been impossible to predict the occurrence of so many unique sex chromosomal systems in the family Phyllostomatidae (Hsu *et al.*, 1968, and Baker, in press). In this family, in addition to the classical XX/XY system, atypical sex chromosomal systems have been found. One system (XX/XY<sub>1</sub>Y<sub>2</sub>) resulted when an autosome was translocated to the X but not to the Y. Another system appears to be XX/XO; however, it is hypothesized that the Y is present but translocated to an autosome (Baker, in press). No variant sex determining systems have been reported for other bat families. Now that these unique situations are known, intensive research can give a better understanding of the species and genetic systems involved.

For example, in *Macrotus* the two chromosomal races are quite different. A study of karyotypes from the zone of contact of the two races would reveal (if the two forms were interbreeding) parapatric or sympatric without any hybrids. Until such data are available the significance of the different karyotypes as isolating mechanisms cannot be understood. It is important that the two chromosomal races occur in one subspecies and presently there is no morphological way to separate the two forms except by karyotype.

In the phylogenetic studies based on karyotypic data that have been published, each situation is different and methods vary in presenting the data. A few specific examples are discussed below.

As stated above, the two families which have the widest range of diploid- and fundamental-number variation are the Vespertilionidae and Phyllostomatidae. Even though they have such widely overlapping ranges in both values (Figs. 1 and 2), when diploid number is plotted against fundamental number the values from the two families separate very well (Fig. 3; Foreman *et al.*, 1968). Since the values for the two species of the Desmodontidae were in close agreement with values of phyllostomatids, these data were used to support the conclusion that the vampire bats (Desmodontidae) were closely related to the Phyllostomatidae (Foreman *et al.*, 1968). This is a case where karyotypic data

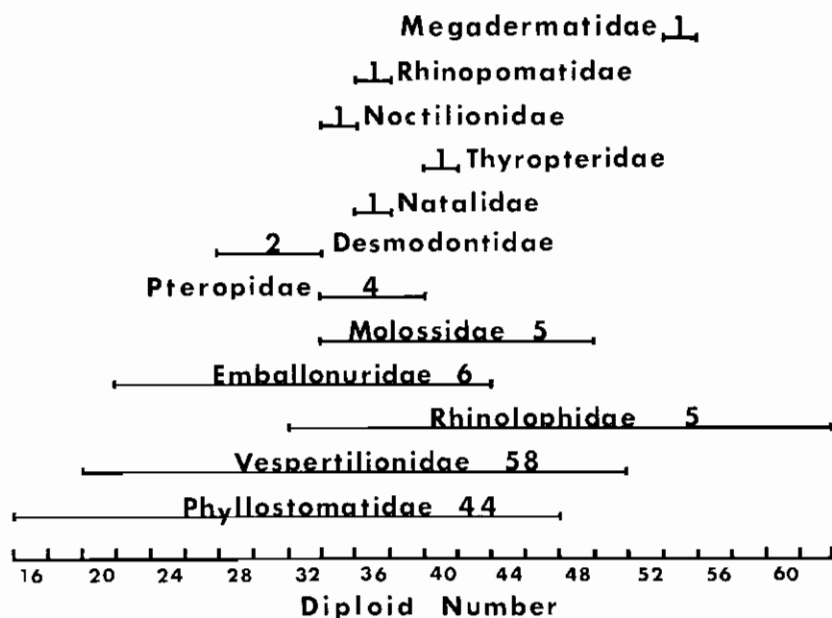


FIG. 1. Range of diploid numbers reported for bat families. Number of species examined in each family is presented by family names.

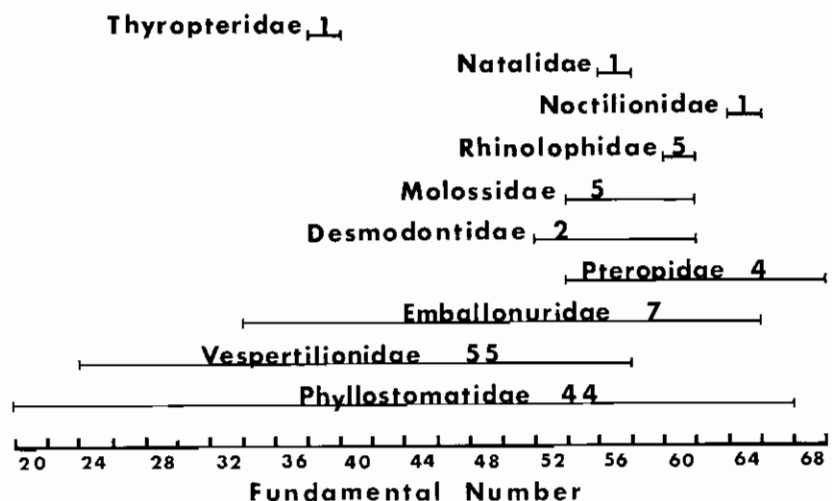


FIG. 2. Range of fundamental numbers reported for bat families. Number of species examined in each family is presented by family names.

supported data from several other characters, including results from immunology and sperm morphology studies (Foreman *et al.*, 1968).

The New World leaf-nosed bats of the family Phyllostomatidae have undergone adaptive radiation in feeding niches, and the relationships of several of the genera and subfamilies are not well understood. In a phylogenetic study based on karyotypes, most of the data supported the existing classification of this family (Baker, 1967). One exception involved the subfamily Glossophaginae which, based on karyotypic studies, represents a diphyletic group. The other two subfamilies involved are the Phyllostomatinae and the Carrollinae. In Figure 4 are the karyotypes of *Carollia*, subfamily Carrollinae; *Choeroniscus* and *Glossophaga*, subfamily Glossophaginae, and *Phyllostomus*, subfamily Phyllostominae. It is obvious that the karyotype of *Glossophaga* is more like that of *Phyllostomus* than like that of *Choeroniscus*; the karyotype of *Choeroniscus* is very similar to that of *Carollia* (two species have been karyotypically studied and they have identical karyotypes). The question then arises, are the two members of the Glossophaginae more closely related to other subfamilies than they are to each other? The following data would support such a hypothesis. *Carollia* and *Choeroniscus godmani* (Fig. 4) both have a low diploid number (20 and 18) and fundamental number (36 and 32), and both have XX/XY<sub>1</sub>Y<sub>2</sub> sex-determining systems. There are also similarities between some of the autosomes. *Glossophaga* and *Phyllostomus* both have a higher diploid number (32) and a fundamental number (60) and both have a XX/XY sex-chromosome system. Their autosomes are also very similar in appearance. The relationship suggested by these data is shown in Fig. 5. That is, the two glossophaginae genera have evolved to the nectar feeding way of life independently: *Glossophaga* and *Phyllostomus* in the same line of evolution, and *Choeroniscus* and *Carollia* in the same line of evolution. If the suggested relationship is true, the subfamily Glossophaginae is diphyletic and is not valid. In Fig. 6 the chromosomes of a specimen of *Choeroniscus intermedius* are shown along with the chromosomes of female *Carollia perspicillata*. The chromosomes of the *Choeroniscus intermedius* are more like those of *Carollia* than like the chromosomes of *Choeroniscus godmani* (Fig. 4). Such data seem to offer strong evidence that the two forms are closely related, and that the karyotype found in *Carollia* and *Choeroniscus intermedius* is like that of their common ancestor. However, several problems arise on closer examination. The *Choeroniscus intermedius* karyotype shown is that of a male, and the sex chromosomes are not XY<sub>1</sub>Y<sub>2</sub> as is found in *Carollia* and *Choeroniscus godmani*. Further, the karyotype shown for *Choeroniscus intermedius* is only one of eight different karyotypes

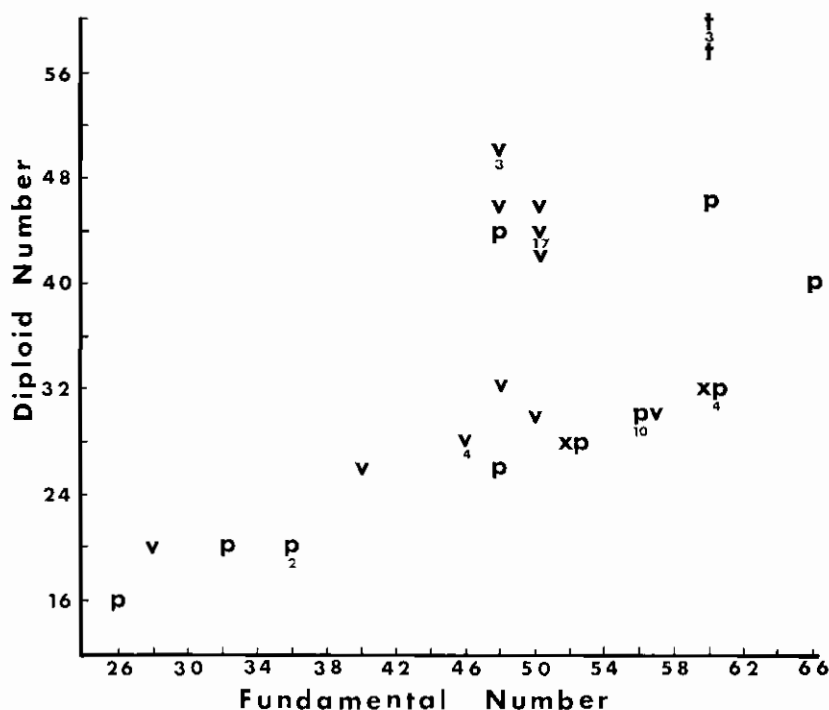


FIG. 3. Chromosomal values (diploid number plotted against fundamental number) for species of four families of bats: Vespertilionidae (V), Phyllostomatidae (P), Desmodontidae (X), and Rhinolophidae (T). Numbers below symbols indicate number of species at that coordinate. (Modified after Forman *et al.* 1968).

found for that species on the island of Trinidad (Hsu & Baker, unpubl. data). Karyotypic variation in *Choeroniscus intermedius* is so great that it is impossible to determine the sex chromosomes from a comparison of the somatic karyotypes of the five males and five females examined.

Nevertheless, based on karyotypic data, several hypotheses can be made concerning the subfamily Glossophaginae: (1) There is a major evolutionary division in the glossophagines; (2) *Glossophaga* and related genera such as *Leptonycteris* may have evolved from the phyllostomine line of evolution; (3) *Choeroniscus* may have evolved from the carolline line of evolution.

For the sake of simplicity very few genera were involved in the above discussion. The situation is further complicated by karyotypic data from the glossophagine genus *Anoura* which do not suggest a close relationship of *Anoura* to either of the two hypothesized lines of evolu-

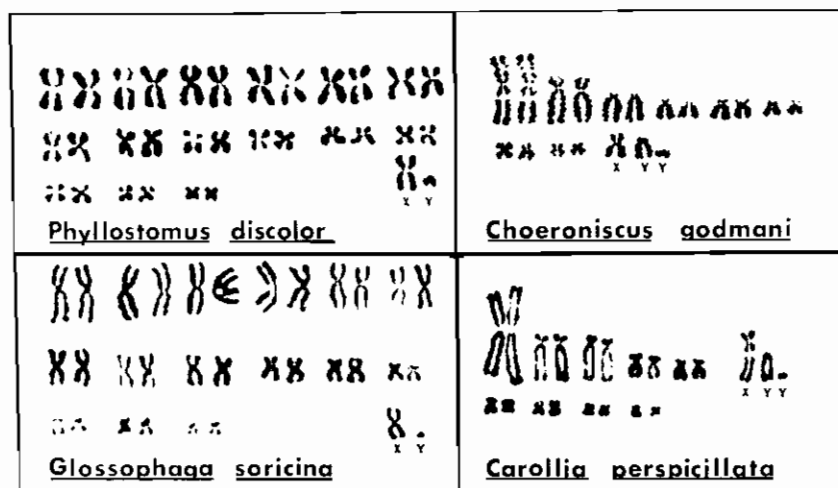


FIG. 4. Karyotypes of four species of phyllostomatid bats. Note the similarities of the karyotypes of *Phyllostomus* and *Glossophaga* and the similarities of the karyotypes of *Choeroniscus* and *Carollia*. See text for implications of these data. The smallest pair of autosomes of *Glossophaga* have been retouched.

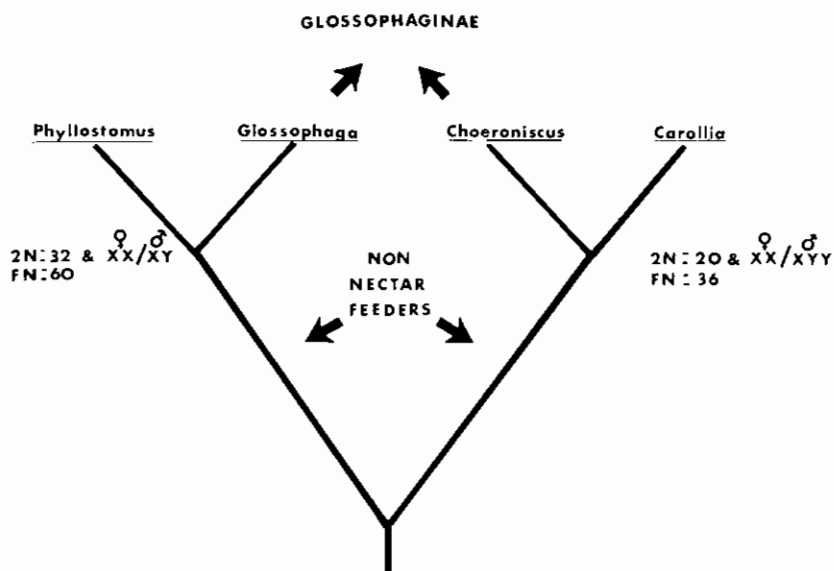


FIG. 5. Possible Phylogenetic relationships of four genera of Phyllostomidae based on karyotypic data. If indicated relationships are true then the subfamily Glossophaginae is diphyletic.

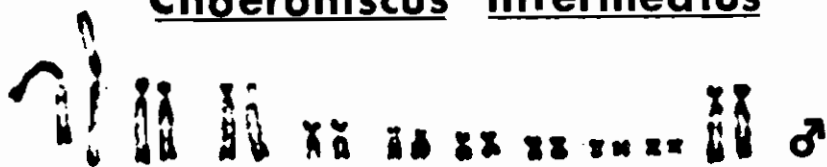
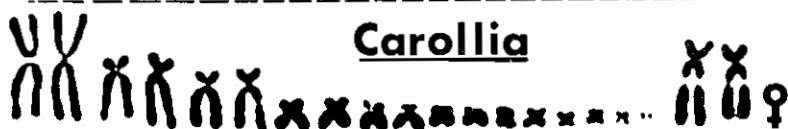
Choeroniscus intermediusCarollia

FIG. 6. Karyotype of a male *Choeroniscus intermedius* from Trinidad and a female *Carollia subrufa* from Mexico.

tion. Also, the great amount of karyotype variation found in the ten specimens of *Choeroniscus intermedius* warns of the problem of drawing conclusions on a small sample-size, and it further suggests that a number of karyotypic changes can occur and become established in a short time-period.

In conclusion, approximately one seventh of the bat species have been karyotypically studied. From these limited data it is suggested that the karyotype will be a useful tool in the study of relationships of bats at the intrafamilial level. The karyotype offers a unique morphological feature for study, and thus similarities of karyotype deserve serious consideration as indicators of a phylogenetic relationship. The karyotype must be evaluated in perspective with results from all other phylogenetic studies.

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