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Submitted 9 December 1988. Accepted 24 March 1989.

J. Mamm., 71(1):94–100, 1990

CHROMOSOMES OF FIVE SPECIES OF VESPERTILIONID BATS FROM AFRICA

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Vespertilionidae, with 37 genera, is the second largest chiropteran family (Koopman, 1984*a*); 15 of these genera, comprising approximately 80 species, are represented in Africa (Hayman and Hill, 1971; Nowak and Paradiso, 1983). Until recently, work on African bats has been of a survey and distributional nature (Aggundey and Schlitter, 1984; Harrison, 1961; Hayman and Hill, 1971; Kingdon, 1974; Koopman, 1965, 1975; McLellan, 1986; Schlitter et al., 1982). Based on current morphological studies, presently recognized taxonomic boundaries may not be accurate in several groups (Koopman, 1984*b*; Robbins, 1978; Schlitter and Aggundey, 1986; Schlitter et al., 1980). Although karyological data may shed light on taxonomic relationships, such data are unavailable for most species of African bats; chromosomal studies of vespertilionid taxa mostly are restricted to Holarctic species (Bickham, 1979*a*; McBee et al., 1986, 1987).

Herein, we present karyotypes of five species of African vespertilionids (chromosome terminology follows that of Bickham, 1979*a*, 1979*b*). These include the first reported karyotypes for *Nycticeius schlieffenii*, *Scotophilus nux*, and *Miniopterus inflatus*. Differentially stained karyotypes are presented for *S. dinganii* and *S. nux*, showing lack of interspecific variation. Results of these investigations increase understanding of the patterns of chromosomal evolution within the Vespertilionidae.

Karyotypes from bone marrow were prepared by the methods described by Baker et al. (1982). Sterile-tissue biopsies from *Scotophilus dinganii* and *S. viridis* were taken in the field and placed in medium F-10 (KC Biological DM-322) fortified with 20% fetal bovine serum (Gibco 200-6140AJ), 1% penicillin-streptomycin (10,000 units penicillin G/ml, 10,000 μg streptomycin/ml, in normal saline; Irvine Scientific 9366), and 1% neomycin sulfate (10,000 μg/ml in normal saline; Irvine Scientific 9360). Monolayer cultures were established in the laboratory, harvested, and karyotypes prepared (Baker et al., 1982). G-bands were produced with the technique of Seabright (1971). Q-bands were produced by staining with quinacrine dihydrochloride (1 mg/50 ml distilled deionized water) for 10 min at room temperature, rinsing with distilled deionized water for 5–10 min, and mounting in sucrose mountant (Ellison and Barr, 1972) modified by addition of 3% formaldehyde; this mountant is self sealing and long lasting, with a high refractive index. Slides also were stained in 4′-6-diamidino-2-phenylindole by placing 5–6 drops stain (1 mg stock solution/30 ml absolute ethanol) under a coverslip for 2–5 min (Bickham, 1987). Staining with chromomycin A3 followed Amemiya and Gold (1986). Fluorescence photomicrography was accomplished as described by Bickham (1987). The three fluorochromes used in this study are known to be specific for certain types of DNA (Comings, 1978). The fluorochrome 4′-6-diamidino-2-phenylindole is a nonintercalating compound that binds to sequences rich in adenine and thymine (Schweitzer, 1976). Chromomycin A3 stains DNA sequences rich in guanine and cytosine (Schweitzer, 1976), and nucleolar-organizer regions (Amemiya and Gold, 1986). Quinacrine also stains sequences rich in adenine and thymine but binds to DNA differently than 4′-6-diamidino-2-phenylindole (Comings, 1978; Distèche and Bontemps, 1974). The two fluorochromes that stain sequences rich in adenine and thymine often enhance different chromosomal regions.

Specimens examined.—Abbreviations used are CM, Carnegie Museum of Natural History; TCWC, Texas

Cooperative Wildlife Collections (Texas A&M University); TM, Transvaal Museum, Pretoria, Republic of South Africa. *Nycticeius schlieffeni* (n = 1), SOMALIA: SNAI Sugar Plantation; 1.5 km S, 0.5 km E Giohar, 2°46′N, 45°31′E (TCWC). *Pipistrellus nanus* (n = 2), SOMALIA: SNAI Sugar Plantation; 1.5 km S, 0.5 km E Giohar, 2°46′N, 45°31′E (CM); SOUTH AFRICA: Transvaal; Kruger National Park, Mockford's Garden, 22°25′S, 31°18′30″E (CM). *Scotophilus dinganii* (n = 2), SOMALIA: SNAI Sugar Plantation; 1.5 km S, 1 km E Giohar, 2°46′N, 45°31′E (CM); NAMIBIA: South Africa Transvaal; Kruger Nat. Park, Pafuri (TM). *Scotophilus nux* (n = 1), CAMEROUN: 9 km S, 10 km W Yaounde, 3°47′N, 11°25′E (CM). *Scotophilus viridis* (n = 1), NAMIBIA: Farm Zuartmodder, 70 km W Maltahohe, 24°54′S, 16°17′E (TM). *Miniopterus inflatus* (n = 1), SOMALIA: 5.5 km S, 11.5 km W Gesira, El Amba Cave, Raas Boqol iyo Toban Afar, 1°54′N, 45°05′E.

Nycticeius schlieffeni (Peters, 1859) has a diploid number (2n) of 34 and a fundamental number (FN) of 52. The nondifferentially stained karyotype of *N. schlieffeni* (Fig. 1d) consists of 10 pairs of metacentric and six pairs of acrocentric autosomes. The largest pair of acrocentric autosomes possesses a secondary constriction near the centromere. The X chromosome is medium sized and metacentric; the Y is small and acrocentric.

Scotophilus dinganii (A. Smith, 1933) has 2n = 36 and FN = 50. The nondifferentially stained karyotype (Fig. 1a) of *S. dinganii* is composed of eight pairs of metacentric autosomes, the smallest of which shows the presence of a secondary constriction and satellites suggesting the presence of nucleolar-organizer regions. The remaining nine pairs of autosomes are acrocentric. The X chromosome is medium sized and submetacentric; the Y is small and telocentric.

Comparison of the G-banded karyotype of *S. dinganii* (Fig. 2) with the G-band karyotype for *Myotis* (Bickham, 1979*a*, 1979*b*) shows considerable chromosomal homology between the two genera. Although the resolution of G-band preparations in this study was sufficient to identify the larger brachial elements (1–15), the smaller elements are identified only tentatively, and two pairs of autosomes have no identifiable brachial homologies.

Preparations stained with 4′-6-diamidino-2-phenylindole (Fig. 3c) are relatively homogeneously stained, with no regions that appear rich in adenine and thymine. The pericentromeric and telomeric regions of many of the chromosomes appear negatively staining. Staining with chromomycin A3 (Fig. 3d) shows the presence of chromatin rich in guanine and cytosine (bright bands) that correspond to regions that stain negatively with 4′-6-diamidino-2-phenylindole.

Quinacrine staining (which also identifies regions rich in adenine and thymine; Fig. 3e) corresponds well with the 4′-6-diamidino-2-phenylindole pattern, but discriminates additional bands, particularly in the larger chromosomes. In chromosome 19/11 (the first pair in the top row), for example, the long arm is divided into negative-staining pericentromeric and terminal regions by a positive-staining area (Fig. 3e). This was not as evident with 4′-6-diamidino-2-phenylindole.

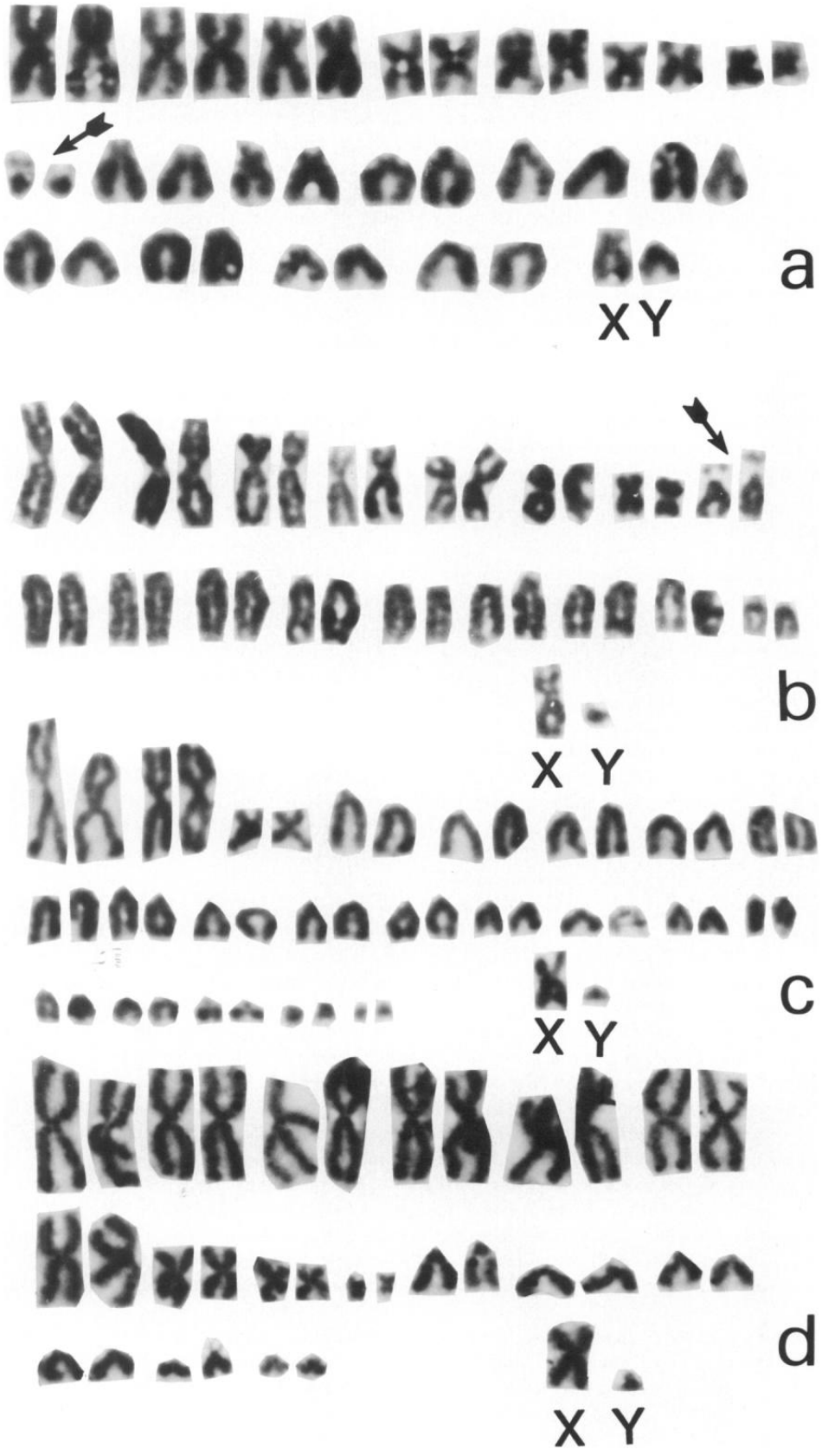
Scotophilus nux Thomas, 1904 has 2n = 36 and FN = 50 (Fig. 1b). The nondifferentially stained karyotype of this species is identical with that of *S. dinganii* (Fig. 1a) except the autosomal pair bearing the secondary constriction is distinctly larger in *S. nux*. In addition, the X chromosome of *S. nux* (Fig. 1b) has short arms substantially larger than those of *S. dinganii* (Fig. 1a); the Y chromosome, however, is smaller.

Scotophilus viridis (Peters, 1852) has 2n = 36 and FN = 50 (Fig. 3a and 3b). The nondifferentially stained karyotype of *S. viridis* (not shown) is identical with that of *S. dinganii*. The secondary constriction (Fig. 3a and 3b) in chromosome 24/21 is not as obvious as in *S. dinganii*. The fluorescence patterns of 4′-6-diamidino-2-phenylindole and chromomycin A3 (Fig. 3) appear identical between the two species.

Miniopterus inflatus Thomas, 1901 has 2n = 46 and FN = 50. The nondifferentially stained karyotype of *M. inflatus* (Fig. 1c) consists of three pairs of metacentric and 19 pairs of acrocentric autosomes. The X chromosome is medium sized and metacentric; the Y is small and acrocentric.

Three patterns of chromosomal variation are described for the family Vespertilionidae (McBee et al., 1986) as follows: conservative genera in which all species have the same or nearly the same karyotype (*Myotis*, 2n = 44, FN = 50, 52; *Eptesicus*, 2n = 50, FN = 48); genera exhibiting a high degree of interspecific variability, including genera such as *Pipistrellus* with 11 different diploid numbers reported in the 15 species examined to date; and species exhibiting a high degree of intraspecific variability, as illustrated by *Rhogeessa tumida*.

The second pattern of chromosomal variation, that of interspecific variability, is exemplified in this study by *Nycticeius*. Baker and Patton (1967) and Bickham (1979*a*) reported 2n = 46, FN = 48 for *Nycticeius humeralis*, a species distributed throughout eastern North America (Hall, 1981). The other species in the genus are the African *N. schlieffeni*, reported herein to have 2n = 34 and FN = 52, and four Australian species: *N. balstoni*, *N. greyii*, *N. inflatus*, and *N. rupellii* (Honacki et al., 1982) for which no chromosomal data are available. Our karyotype for *N. schlieffeni* differs from the American species in both diploid number


a
X Y
b
X Y
c
X Y
d
X Y

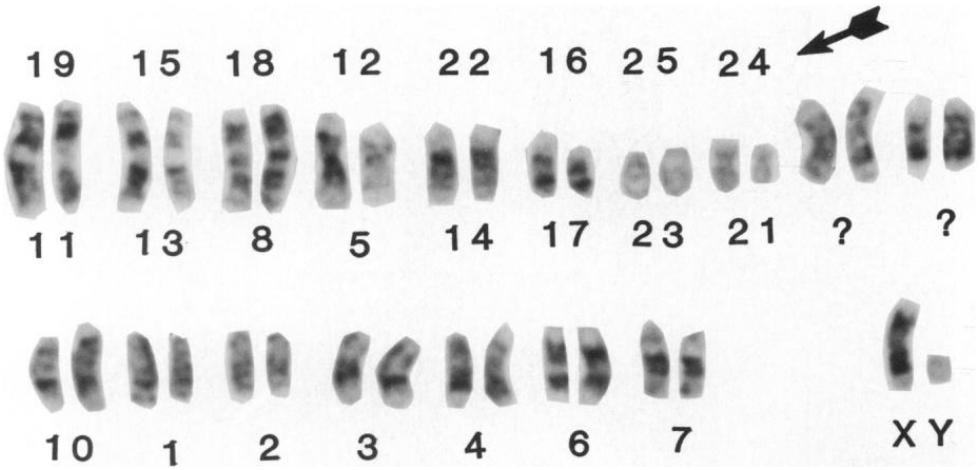


FIG. 2.—Brachial homologies between *S. dinganii* (shown) and the vespertilionid standard, *Myotis* (Bickham, 1979*a*, 1979*b*). The numbers above and below each pair of chromosomes correspond to the chromosome arms of the standard. Thus, all biarmed chromosomes of *Scotophilus* share brachial homologies with *Myotis*. The chromosome bearing the nucleolar-organizer region (indicated by an arrow) is presumed to be metacentric autosome "24/21," which does not stain positively in this preparation. Only two chromosomes, those acrocentric autosomes with question marks beneath them, were not found to share identifiable homologies with the *Myotis* standard.

and fundamental number. This represents one of the largest intrageneric differences in diploid number within the Vespertilionidae. Such disparity in diploid numbers (with the caveat that the *Nycticeius* chromosomes presented herein are unbanded) suggests that the African and American species of *Nycticeius* may not be congeneric. Hill and Harrison (1987) recently elevated *Nycticeius schlieffeni* to full generic status, under the name *Nycticeinops*, based on morphology of the baculum. The chromosomal data presented herein are further justification for recognition of *Nycticeinops schlieffeni*. The Australian forms of *Nycticeius* have been referred to *Scotoenax* and *Scotorepens* (Hill and Harrison, 1987). Additional chromosomal investigations of these taxa should better resolve their phylogenetic relationships.

The karyotype of *Miniopterus inflatus* is identical in diploid and fundamental number to those reported for the other species in this genus (*M. australis*, *M. magnater*, and *M. schreibersi*—Baker et al., 1974; Bickham, 1979*a*; Bickham and Hafner, 1978; Harada, 1973; Harada and Kobayashi, 1980; Matthey and Bovey, 1948; McBee et al., 1986), suggesting this genus is karyotypically conservative.

Scotophilus dinganii, *S. nux*, and *S. viridis* all have 2n = 36, FN = 50 which differs from the previously reported FN = 52 for *S. dinganii* and FN = 54 for *S. viridis* (Schlitter et al.,.1980). In *S. dinganii*, Schlitter et al. (1980) reported four pairs of metacentric autosomes (to our eight), five pairs of submetacentric autosomes (to our none), and eight pairs of acrocentric autosomes (to our six). In *S. viridis*, the chromosomal complement was shown to be composed of five pairs of metacentric autosomes, four pairs of submetacentric autosomes, one pair of subtelocentric autosomes, and seven pairs of telocentric autosomes. The discrepancy between this study and that of Schlitter et al. (1980:232, 239) appears to be more than just a matter of interpretation, and indicates the existence of at least two cytotypes in each of the two *Scotophilus* species examined, suggesting the presence of a pattern of chromosomal variation similar to that found in bats of the genus *Rhogeessa*.

The fundamental number in *S. viridis* (FN = 50) also is identical to that of *S. dinganii* reported herein and differs from the FN = 54 reported for *S. viridis* by Schlitter et al. (1980). This discrepancy raises the

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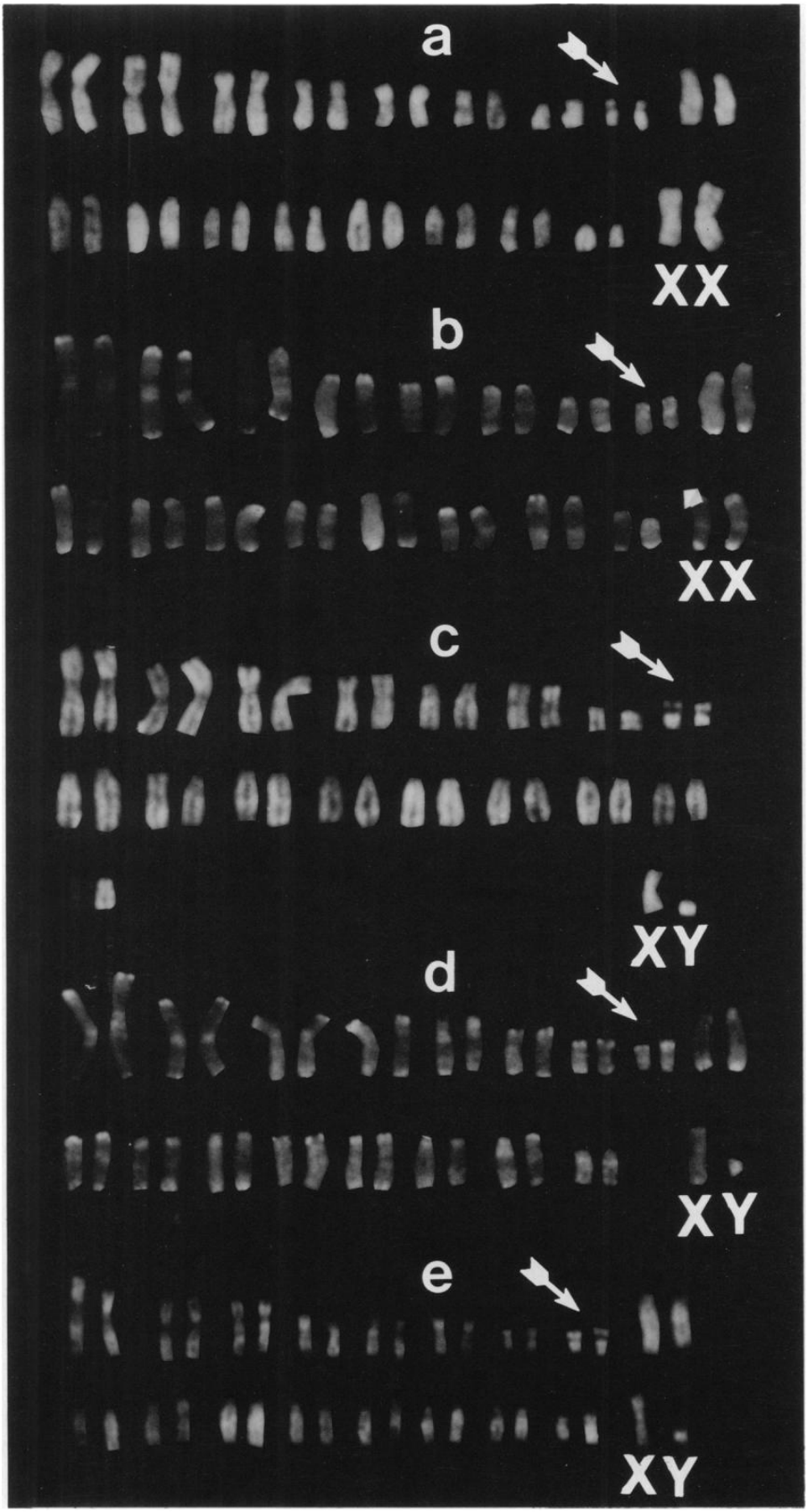
FIG. 1.—Nondifferentially stained karyotypes of: a, *Scotophilus dinganii*; b, *S. nux*; c, *Miniopterus inflatus*; and d, *Nycticeinops* (=*Nycticeius*) *schlieffeni*. Arrows indicate chromosomes bearing nucleolar-organizer regions used as marker chromosomes in *Scotophilus*.


a
XX
b
XX
c
XY
d
XY
e
XY

possibility of chromosomal polymorphisms in a geographically widespread species. Another possibility, however, is that the quality of the preparations for this study was better, and these preparations represent a more reliable determination of the fundamental number.

Fluorochrome-banded karyotypes appeared identical in the two species of *Scotophilus* examined. Considering the similarity we found between *S. dinganii, S. nux,* and *S. viridis*, it appears that chromosomes will be a limited tool in elucidation of phylogenetic relationships within this genus.

Volleth (1987) suggested that nucleolar-organizer regions may be used to distinguish among chromosomally conservative genera. Variation in the morphology of chromosomes bearing nucleolar-organizer regions was observed in each of the *Scotophilus* species examined in this study. In *Scotophilus dinganii,* chromosomes bearing nucleolar-organizer regions are barely larger than the Y chromosome. In contrast, chromosomes with nucleolar-organizer regions in *S. nux* are the same size as the X chromosome, and those of *S. viridis* are intermediate in size between those of *S. dinganii* and *S. nux*. These data indicate the possibility of phylogenetically informative variation in location of nucleolar-organizer regions among these three species and should encourage expanded studies of these regions coupled with G-band analysis. The data do not, however, clarify the taxonomic status of *Scotophilus*. For example, Meester et al. (1986) relegated *S. viridis* to a subspecies of *S. borbonicus,* as did Koopman (1984*b*), but Robbins et al. (1985) considered it a valid species and placed part of *S. borbonicus* as a subspecies of *S. leucogaster* and part as a subspecies of *S. dinganii*.

The genus *Scotophilus* is included in the *Eptesicus*-like group (Bickham, 1979*a*). This group includes chromosomally derived taxa and is composed of *Eptesicus, Rhogeessa, Nycticeius, Antrozous,* and now *Scotophilus*. The primitive chromosomal condition of Vespertilionidae is represented by the *Myotis*-like group in which chromosome arms "1/2, 3/4, and 5/6" form three large metacentric autosomes (Bickham, 1979*a*:352). In the *Eptesicus*-like group, these are present either as acrocentric autosomes or have been rearranged (Bickham, 1979*a*). Therefore, all genera in this group are thought to have evolved from an ancestor with an all-acrocentric karyotype like that of *Eptesicus*. In *Scotophilus*, arms 1–4 and 6 are present as acrocentric autosomes, and 5 is fused with 12 to form a metacentric autosome. Thus, the three chromosomal evolutionary trends described by McBee et al. (1986) are found independently in each of the chromosomal groups of Bickham (1979*a*). This suggests that chromosomal evolution in vespertilionids may be more complex than previously thought.

We thank R. J. Baker, K. Koopman, and D. H. Wurster-Hill for reviewing earlier versions of this manuscript, and I. L. Rautenbach for invaluable assistance during various portions of this study. This research was partially funded by Texas Agricultural Experiment Station Program Development and Expanded Research Area funds.

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Fig. 3.—*Scotophilus viridis* karyotypes stained with: a, 4′-6-diamidino-2-phenylindole, and b, chromomycin A3. The morphology of autosomes bearing nucleolar-organizer regions (indicated by arrows) differs strikingly between this species and *S. dinganii*. *Scotophilus dinganii* karyotypes stained with: c, 4′-6-diamidino-2-phenylindole; d, chromomycin A3; and e, quinacrine mustard.

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Submitted 7 November 1988. Accepted 8 May 1989.