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# Karyotypic Analysis of Five Rodents and a Marsupial from Belize, Central America

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KARYOTYPIC ANALYSIS OF FIVE RODENTS AND A  
MARSUPIAL FROM BELIZE, CENTRAL AMERICA

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ABSTRACT

Karyotypes and chromosomal banding patterns of six species of small mammals from Belize are presented and discussed in light of relevant data in the literature. Individuals of *Heteromys desmarestianus* had FN values of 72, which differs from previous reports. G-bands were obtained and compared to fluorescent bands (DAPI) and with the location of NOR sites. The karyotypes of *Peromyscus mexicanus*, *Sigmodon hispidus*, *Oryzomys couesi*, and *Otodylomys phyllotis* are identical to the karyotypes for these species described in the literature. Active NORs were located on four pairs of chromosomes in *O. phyllotis* and fluorescent bands obtained with the stain Hoechst 33258. The karyotype of *Marmosa robinsoni* appears similar to that described by Hsu and Benirschke (1971). Autosomal C-bands were located at the centromeres. The X had interstitial C-bands and the Y was entirely heterochromatic. Silver stained NORs were present on five chromosomes corresponding to chromosomal pairs 4, 5, 5, and 6.

INTRODUCTION

The small mammal fauna of Belize has not been extensively surveyed (Hershkovitz, 1951; Laurie, 1954). Kirkpatrick and Cartwright (1975) compiled a list of the mammals known to occur in Belize, but the list must be considered incomplete due to the limited amount of study the fauna has received. During the spring of 1983 the Section of Mammals, The Carnegie Museum of Natural History, conducted a collecting trip to obtain specimens, primarily of bats, rodents, and marsupials. This paper presents the findings of karyotypic analyses of six species of mammals collected throughout Belize.

MATERIALS AND METHODS

All karyotypes were obtained from bone marrow preparations made in the field by the methods described by Patton (1967), Lee (1969), and Baker et al. (1982). Individuals to be karyotyped were usually injected with yeast to increase the mitotic index according to the methods of Lee and Elder (1979). Several slides were prepared in the field, with the remaining cell suspension frozen in liquid nitrogen to permit slide preparation in the lab. C-banding was performed on air dried slides by a modification of the technique of Stefos and Arrighi (1971). G-banding was accomplished using the

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technique of Seabright (1971). Nucleolus organizer regions (NORs) were located by the silver staining method of Gold and Ellison (1983). Fluorescent banding was done with the stains 4,6-diamidino-2-phenylindole (DAPI) and Hoechst 33258 as described by Disteché and Bontemps (1974), and Schweizer (1976), respectively. Fluorescent banding permits the identification of homologous chromosomes and identifies regions with specific DNA base composition. Hoechst 33258 and DAPI are specific for AT (adenine and thymine) rich regions and produce a banding pattern analogous to G-bands.

#### SPECIMENS EXAMINED

All specimens were collected in Belize, Central America, and deposited in The Carnegie Museum of Natural History (CM).

*Heteromys desmarestianus*.—CM 91973, female, Stann Creek District, 7.0 km WNW Quam Bank, Cockscomb Basin; CM 91997, female, Toledo District, 1.8 km NNW Forestry Camp (Salamanca), Columbia Forest Reserve; CM 91988, male, Stann Creek District, 3.4 km WNW Quam Bank, Cockscomb Basin.

*Ototylomys phyllotis*.—CM 92020, male, CM 92014, female, Belize District, Altun Ha ruins; CM 92034, female, Toledo District, 0.7 km NNE Forestry Camp (Salamanca), Columbia Forest Reserve.

*Sigmodon hispidus*.—CM92082, female, Stann Creek District, Quam Bank, Airstrip, Cockscomb Basin.

*Oryzomys couesi*.—CM92005, female, Stann Creek District, Cabbage Haul Ridge, 2.8 km SSE Quam Bank, Cockscomb Basin.

*Peromyscus mexicanus*.—CM 92039, female, and CM 92037, male, Toledo District, Jimmy Cut 2.1 km NW Forestry Camp (Salamanca), Columbia Forest Reserve.

*Marmosa robinsoni*.—CM91638, male, Toledo District, 2.1 km N Forestry Camp (Salamanca), Columbia Forest Reserve.

#### RESULTS AND DISCUSSION

##### *Heteromys desmarestianus* ( $2n = 60$ , $FN = 72$ )

The karyotype presented in Fig. 1a shows chromosomes 1, 2, 4, and 5 are large and subtelocentric and chromosome 3 is large and submetacentric. There are 22 acrocentric pairs that grade in size from large to small, and two pairs of small metacentric autosomes. The X chromosome is medium-sized and metacentric. Fig. 1b and 1c display the G-bands and fluorescent bands obtained with DAPI, respectively. It appears that the centromeric regions are not A-T rich and that dark G-bands correspond to bright regions with DAPI as expected. Fig. 1d shows active NOR sites on at least six pairs of autosomes. Genoways (1973) and Engstrom et al. (in press) report  $2n = 60$ ,  $FN = 82$  and  $2n = 60$ ,  $FN = 66$ , respectively, for *H. desmarestianus*. Thus, variation in fundamental number is extensive within this species.

##### *Ototylomys phyllotis* ( $2n = 48$ , $FN = 78$ )

The big-eared climbing rat is found throughout the Yucatan Peninsula and south through Belize, Guatemala, Honduras, El Salvador, Nicaragua, and the northern half of Costa Rica (Hall, 1981). Lawlor (1969) recognized three subspecies, which are separated altitudinally. *Ototylomys* is most closely related morphologically to *Tylomys* and is allied to *Neotoma* by similarities of the accessory reproductive glands (Lawlor, 1969). The nondifferentially stained karyotype, presented in Fig. 2a, contains one pair of large submetacentric, three pairs of large metacentric, one pair of large acrocentric, one pair of medium-sized submetacentric, one pair of medium-sized acrocentric, five pairs of medium-sized metacentric, six pairs of small metacentric and five pairs of small acrocentric autosomes. The X and Y are large and metacentric. The karyotype of *O. phyllotis* reported here is identical to the nondifferentially stained karyotype originally reported by Hsu and Benirschke (1973). Fig. 2b shows a spread stained with the

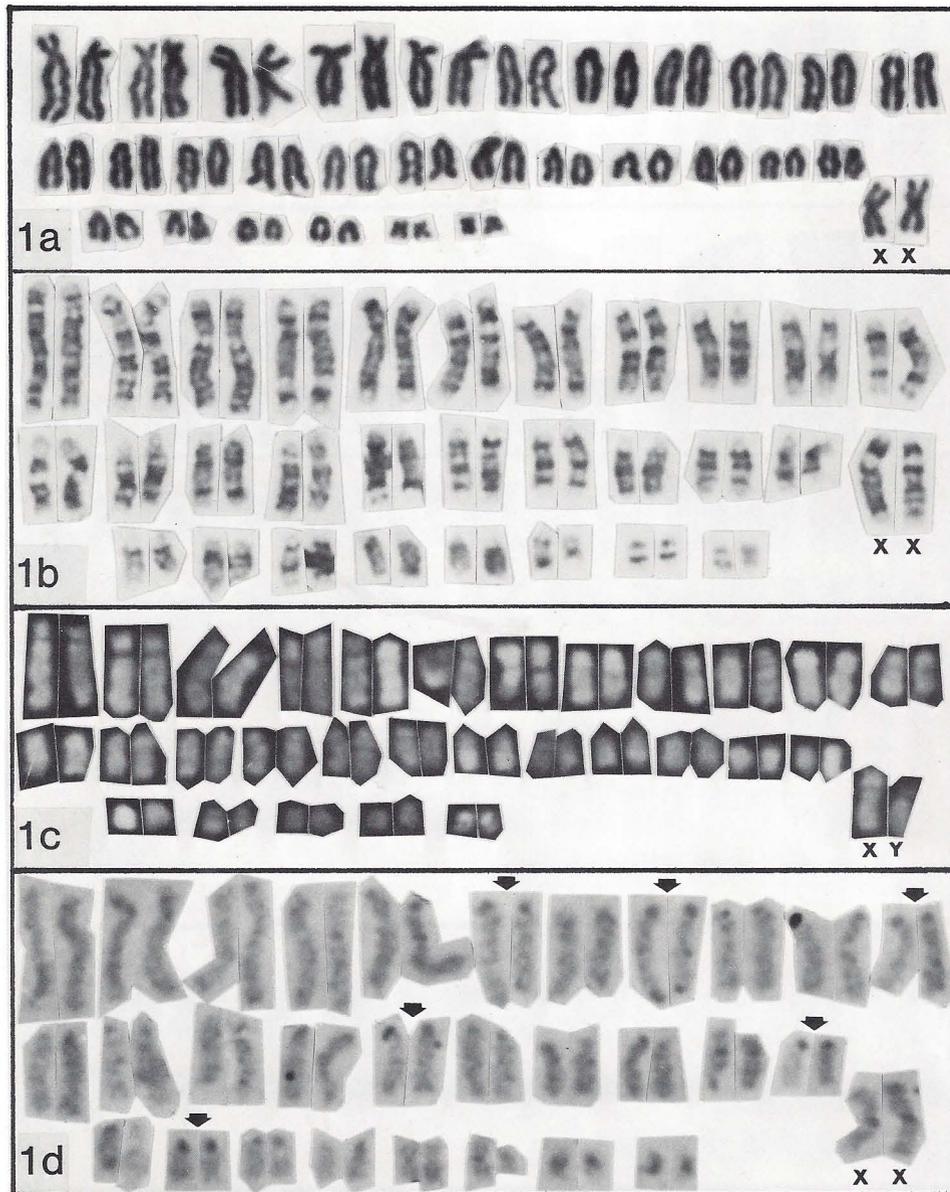


Fig. 1.—a) Nondifferentially stained karyotype of *Heteromys desmarestianus*. b) G-banded karyotype. c) DAPI stained karyotype. d) Silver-stained NOR sites.

fluorescent dye Hoechst 33258. The banding pattern indicates that much of the X chromosome is AT rich but not the centromere. Active NORs (Fig. 2c) are present on one pair of small acrocentric and three pairs of medium-sized meta-centric autosomes.

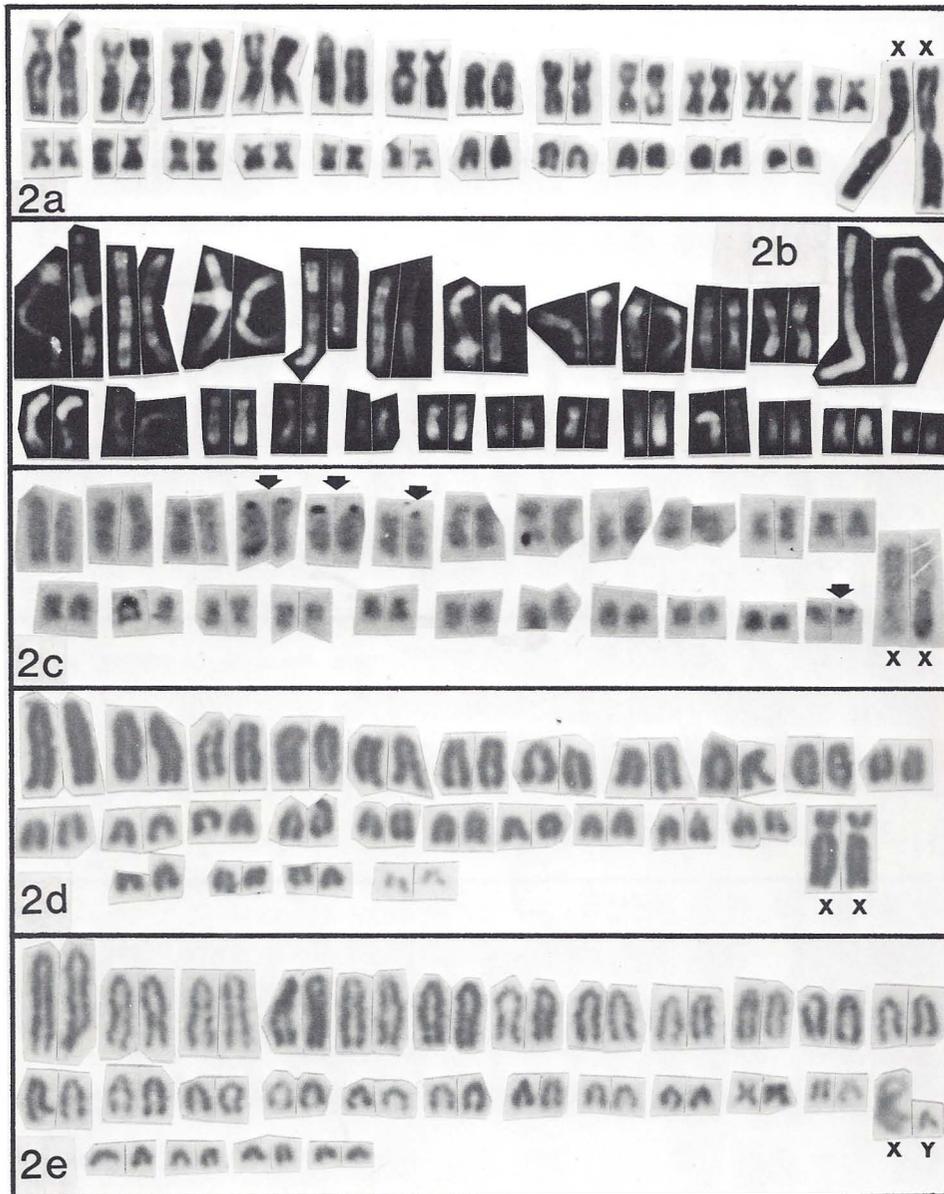


Fig. 2.—a) Nondifferentially stained karyotype of *Ototylomys phyllotis*. b) Karyotype stained with Hoechst 33258. c) Silver stained NOR sites. *O. phyllotis*. d) Nondifferentially stained karyotype of *Sigmodon hispidus*. e) Nondifferentially stained karyotype of *Oryzomys palustris*.

*Sigmodon hispidus* ( $2n = 52$ ,  $FN = 52$ )

The karyotype presented in Fig. 2d contains 48 acrocentric and two very small metacentric autosomes. The X chromosome is large and subtelocentric and the Y small and metacentric. This karyotype is identical to that described by Zim-

merman (1970) and Kibliskey (1969) who stated this karyotype is found from the eastern United States to Venezuela.

Elder (1980) studied chromosomal evolution in the genus *Sigmodon* and found that two morphologically similar but karyotypically different species, *S. arizonae* ( $2n = 22, 24, FN = 38$ ) and *S. mascotensis* ( $2n = 28, FN = 29$ ), share a high degree of G-band homology. Chromosomal evolution in the genus has been toward lower diploid numbers by the rearrangement events of tandem and centric fusion.

*Oryzomys couesi* ( $2n = 56, FN = 56$ )

The karyotype presented in Fig. 2e is identical to that reported by Benson and Gehlbach (1979) and Haiduk et al. (1979) for *O. couesi*. There are 26 pairs of acrocentric and one small pair of metacentric autosomes. The X is large and submetacentric and the Y, as reported by Haiduk et al. (1979), is small and subtelocentric.

Honacki et al. (1982) list over 50 species of *Oryzomys* ranging from South America to the eastern United States making this one of the most complex mammalian genera. Diploid numbers within the genus range from 52 to 80 and possess FNs from 62 to 112 (Gardner and Patton, 1976). Haiduk et al. (1979) observed extensive G-band differences between *O. melanotis* and *O. palustris*, which possess very similar nondifferentially stained karyotypes, and concluded that karyotypic evolution in *Oryzomys* is very rapid compared to other vertebrates. The analysis of G-banding patterns by Baker et al. (1983) found that current models of chromosomal evolution do not adequately explain the variation found within and between the peromyscine-neotomine-oryzomine lineages.

*Peromyscus mexicanus* ( $2n = 48, FN = 58$ )

Chromosomal banding patterns have been studied for 12 species of the *Peromyscus mexicanus* group (Robbins and Baker, 1981; Rogers et al., 1984; Stangl and Baker, 1984; Smith et al., 1986). These species possess a high degree of karyotypic conservatism, which is unusual for the genus. The karyotypes of members of the *P. mexicanus* group are characterized by having  $2n = 48$  and  $FN = 58$  with biarmed chromosomes 1-3, 9, 22, and 23. Seven members of the *P. mexicanus* group have been described as having heterochromatin restricted to the centrometric regions (Huckaby, 1980; Rogers et al., 1984; Stangl and Baker, 1984).

Smith et al. (1986) state that there is a considerable amount of variation in the morphology of the X chromosome within the *P. mexicanus* group. They list seven species with submetacentric and two species with nearly acrocentric X chromosomes, and state that the differences are caused by heterochromatic short arm variations. The Y chromosome in members of the *P. mexicanus* group are either biarmed or acrocentric (Smith et al., 1986). Rogers et al. (1984) and Smith et al. (1986) conclude that the *P. mexicanus* karyotype does not represent the primitive autosomal karyotype for the genus, but is an intermediate form that has stabilized relative to more highly derived karyotypes, possibly due to selective forces.

The karyotype of *P. mexicanus* presented in Fig. 3a has the autosomal complement typical of *P. mexicanus*, but the Y chromosome seems to be considerably smaller. The G-band pattern (Fig. 3b) of the autosomal complement is generally similar to that of other members of *P. mexicanus*. This includes biarmed autosomes 1-3, 9, 22, and 23 and acrocentric autosomes 4-8 and 10-21. The G-band

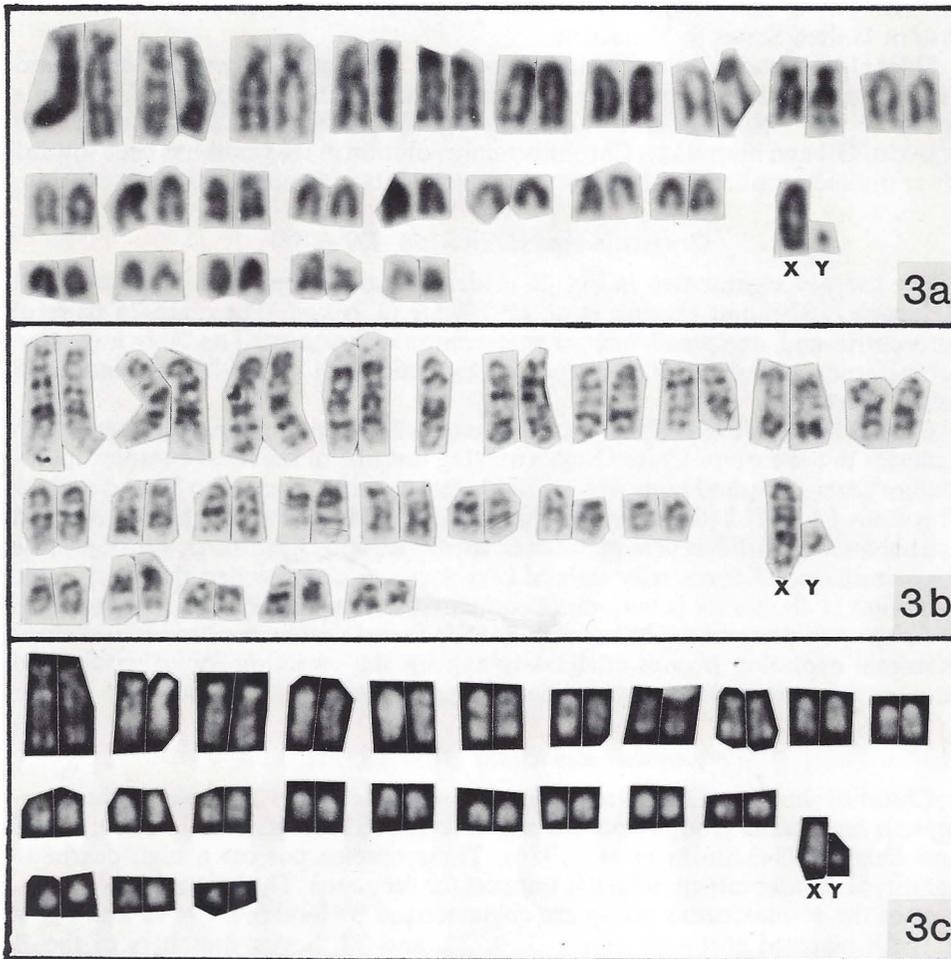


Fig. 3.—a) Nondifferentially stained karyotype of *Peromyscus mexicanus*. b) G-banded karyotype of *P. mexicanus*, homolog to chromosome five missing. c) DAPI stained karyotype of *P. mexicanus*.

chromosomes (Fig. 3b) are placed in numerical order (decreasing size) and identified according to the nomenclature of Committee (1977).

The banding pattern obtained with the fluorescent stain Hoechst 33258 is shown in Fig. 3c. This stain indicates that the centromeric heterochromatin in *P. mexicanus* is not particularly AT rich. Chromosome 1 contains bright regions at the end of both the long and short arms and in the area directly below the centromeric region. Chromosomes 2 and 3 also contain bright interstitial bands. Chromosomes 4 through 12 often have bright regions at the ends of the long arms and may also have bright centromeric bands. The small acrocentric chromosomes of *P. mexicanus* appear to be bright primarily in their centromeric regions, and dark distally.

*Marmosa robinsoni* ( $2n = 14$ ,  $FN = 24$ )

There is considerable information on the nonbanded karyotypes of marsupials (see Hayman's 1977 review), however, there are only a few papers (Curcuro-

Giordano et al., 1974; Fernandes-Donoso et al., 1979; Merry et al., 1983; Seluja et al., 1984; Sinha and Kakati, 1976; Sinha et al., 1972; Yonenaga-Yassuda et al., 1982) that present banded karyotypes. The reason that more species have not been examined is that it is difficult to obtain quality preparations with marsupial chromosomes (Seluja et al., 1984). Marsupials have a slow rate of karyotypic evolution. Previous studies have revealed only three different diploid numbers,  $2n = 14, 18, 22$  (Hayman, 1977). Sharman (1961) hypothesized that the evolution of the Australian marsupials may have been associated with reduction in the number of chromosomes. This hypothesis also has been considered to apply to the evolution of American marsupials (Sharman, 1973, 1974; Reig et al., 1977).

The karyotypes of the  $2n = 14$  American didelphids are all very similar with only minor differences between species representing different subfamilies (Reig et al., 1977). Hayman (1977) felt that the possibility existed that the standard non-banded karyotype would not separate some species and that banding techniques would be required for karyotypic identification.

The murine opossums (*Marmosa*) are the most diverse Neotropical marsupials, but of the 37 recognized species almost nothing is known about them karyotypically (Reig, 1970). There are only three "mouse" opossums known to occur in the country of Belize (Kirkpatrick and Cartwright, 1975), these are *Marmosa alstoni*, *M. robinsoni*, and *M. mexicanus*. Curcuru-Giordano et al. (1974) obtained Q-bands on *M. mitis*, a junior synonym for *M. robinsoni* (O'Connell, 1983) and were able to identify every homologous pair of chromosomes. Fernandes-Donoso et al. (1979) reported the location of NORs in *M. elegans*; no other member of the genus *Marmosa* has been studied for banded karyotypes.

Nondifferentially stained karyotypes have been obtained from eight species of *Marmosa* and three species of *Caluromys* (Reig, 1968; Reig et al., 1977; Biggers et al., 1965; Hsu and Benirschke, 1971). All were found to have  $2n = 14$  and  $FN = 24$ . Nearly all have the same proportions (1:3:2) of metacentric, submetacentric, and subtelocentric chromosomes, with the only two exceptions being *M. elegans* with only one subtelocentric and a single acrocentric, and *M. fuscata* with four submetacentric and no subtelocentric chromosomes (Reig et al., 1977). Reig (1970) pointed out that the similarities are noteworthy because *Marmosa* and *Caluromys* are considered to be in different subfamilies of the didelphids. He stated that the differences between the karyotypes of other genera in the Didelphinae and *Marmosa* are greater than the differences between *Caluromys* and *Marmosa* and that the first four pairs of autosomes of *M. fuscata*, *M. robinsoni* and *C. derbianus* are indistinguishable.

The morphology of the X chromosome ranges from acrocentric in *C. derbianus*, *C. lanatus*, and *M. alstoni*, to subtelocentric in *C. philander*, *M. elegans*, and *M. robinsoni*, to metacentric in *M. murina*, *M. pusilla*, and *M. cinerea*. The Y chromosome is acrocentric in all these species (Reig et al., 1977).

In this paper we report on the Ag-NOR sites and C-bands of *M. robinsoni*. The nondifferentially stained karyotype of our specimen is the same as that described by Hsu and Benirschke (1971). C-bands are presented in Fig. 4a and show heterochromatin in the autosomes to be confined to the centromeric regions. The Y chromosome is entirely heterochromatic, and the X appears to have two interstitial C-bands. NORs are shown in Fig. 4b and are present on chromosomes 4, 5, and 6.

Centromeric heterochromatin has also been found in the autosomes of *Philander opossum*, *Didelphis albiventris* (Yonenaga-Yassuda et al., 1982; Seluja et al., 1984), and *Monodelphis domestica* (Merry et al., 1983). The X chromosome of *P.*

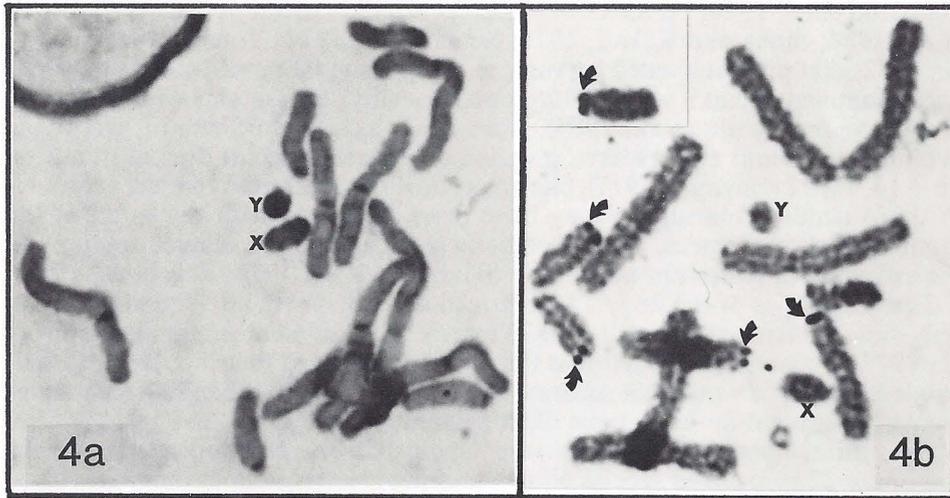


Fig. 4.—a) C-banded spread of *Marmosa robinsoni*. b) Silver stained NOR sites (arrows).

*opossum* has a single band in the long arm and a completely heterochromatic Y chromosome. In *Didelphis albiventris* the X chromosome has a centromeric band. Both *D. marsupialis* and *Lutreolina crassicaudata* lack centromeric heterochromatin on the autosomes but possess C-band positive material on the X chromosomes. The Y chromosomes of both these species are totally heterochromatic (Seluja et al., 1984).

Nucleolar organizer regions (NORs) have been studied in only one other member of the genus *Marmosa*. In *M. elegans* NORs were located at secondary constrictions on the short arm of a single autosomal pair (Fernandes-Donoso et al., 1979). A few studies on the distribution of NORs have been published for several other species of marsupials. Seluja et al. (1984) found NORs located on chromosome pairs 4, 5, and 6 in *Didelphis albiventris* and on the short arm of chromosome pair 5 and the long arm of chromosome pair 7 in *Lutreolina crassicaudata*. In *D. marsupialis* the NORs were terminally located in the long arms of two pairs and in the short arm of one other pair. The NOR bearing chromosomes could not be specifically identified, but there were specimens with up to eight active NORs and as few as four (Yonenaga-Yassuda et al., 1982). In *P. opossum* the NORs were located terminally in the short arm of pair 5 and the long arm of pair 7 (Yonenaga-Yassuda et al., 1982). NOR bearing sex chromosomes have been found in *Potorous tridactylis* (Goodpasture and Bloom, 1975), *Lagorchestes conspicillatus* (Hayman and Sharp, 1981), *Trichosurus vulpecula* (Murray, 1977), and in *Monodelphis domestica* (Merry et al., 1983).

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