

## Chromosome studies in Edentata

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### Abstract

The karyotypes of three additional species of Edentata are described. The differences in karyotype between *Tolypeutes matacus* ( $2n = 38$ ), and other species of armadillo is remarkable. Three species of Euphractini were compared through G- and C-banding patterns. *Chaetophractus villosus* appears to be much closer to *Zaedyus pichiy* than suggested by taxonomy. On the other hand, *Tamandua tetradactyla* and *Tamandua longicaudata*, two different species of anteater, possess the same karyotypes. The presence of an unusual Y-chromosome in *Choloepus hoffmanni* may be responsible for the peculiar sex-ratio reported in this species.

### Introduction

Cytogenetic studies in Edentata have been undertaken only rarely. Because of unfavourable cytogenetic techniques before 1962, no karyotype was described correctly (Newmann and Patterson, 1910; Painter, 1925a,b). In 1962 and 1964, two papers described the karyotypes of *Dasybus novemcinctus* and *Dasybus hybridus* (Beath *et al.*, 1962; Saez *et al.*, 1964). Next, five species of armadillos were described by Benirschke *et al.* (1969). Further, Meritt *et al.* (1973) briefly described the karyotype of *Zaedyus pichiy*. So far, no banding techniques have been applied for the study of edentate chromosomes.

A detailed cytogenetic study of Hoffmann's sloth (*Choloepus hoffmanni*) yielded unusual findings (Corin-Frédéric, 1969). The diploid number was found to be  $2n = 49$  with the female being XO, the males having Y-material attached to an autosome.

Of the anteaters (*Myrmecophagidae*), only the karyotypes of *Myrmecophaga tri-dactyla* and *Tamandua tetradactyla* (Hsu, 1965) have been ascertained.

It is the purpose of this paper to describe three additional karyotypes in Edentata: *Tolypeutes matacus*, *Choloepus hoffmanni* with  $2n = 50$ , and *Tamandua longicaudata*. Through the G- and C- banding patterns, we compare several of the edentate species in order to further our understanding of taxonomic relationships.

### Materials and methods

The following specimens of skin and muscle come from Lincoln Park Zoological Society, Chicago, U.S.A.: female large hairy armadillo (*Chaetophractus villosus*), male and female La Plata three-banded armadillo (*Tolypeutes matacus*), male Pichiy armadillo (*Zaedyus pichiy*), female long tailed tamandua (*Tamandua longicaudata*) and male and female Hoffmann's sloth (*Choloepus hoffmanni*). The female Hoffmann's sloth was bred and born at Lincoln Park Zoo, Chicago, and had a lineage that corresponds to Cabrera's (1957) taxonomy as follows:

♂ *Choloepus hoffmanni florenciae* × ♀ *Choloepus hoffmanni hoffmanni*  
(Colombian origin) (Panamanian origin)

The male Hoffmann's sloth also represents a cross of Colombian x Panamanian subspecies. The skin and muscle biopsies of *Euphractus sexcinctus* come from Botucatu, Brazil and were identified (D.A.M.) as *Euphractus sexcinctus flavimanus*. The male seven-banded armadillo (*Dasybus hybridus*) was imported from Buenos Aires, Argentina.

Cytogenetic studies were done from skin biopsies that were cultured according to the technique of Basrur *et al.* (1963). Fibroblasts were preserved with glycerol in liquid nitrogen. Giemsa banding was accomplished by two procedures. (a) Air dried slides (about 1-week-old) were exposed to 0.025% trypsin solution for 3–6 min in a Coplin jar, washed with 0.9% NaCl and stained in 25% Giemsa solution diluted in water for 1 min, followed by two washes in water. (b) Air dried slides (about 1-week-old) were incubated in 2 X SSC at 60°C followed by a 3–6 min 0.025% trypsin solution, two washes with 0.9% NaCl and staining in 25% Giemsa solution diluted in water for 1 min, followed by two washes in water. Centromeric heterochromatin was stained by placing the slides (about 1 week old) in 0–2N HCl for 30 min followed by a rinse with water, wash in alkaline SSC for 2 min (1 part 0.7 N NaOH, 6 parts 2 X SSC), 2 min in 2 X SSC, rinse with 70% and 95% alcohol, air drying. The slides were placed on filter paper wetted with 2 X SSC in a Petri dish for 4–6 h at 60°C, then exposed to 70% and 95% alcohol and air dried. Subsequently, they were stained in 4% Giemsa solution made in phosphate buffer for 4–7 min.

## Results and discussion

### Family Dasypodidae (Armadillos)

#### Tribe Euphractini

This tribe is composed of the genera *Euphractus* (*sexcinctus*), *Zaedyus* (*pichiy*) and *Chaetophractus* (*nationi*, *vellerosus*, *villosus*). Most of the species of this tribe live south of Brazil and Bolivia. They have a bicornuate uterus and do not have polyembryony (Benirschke *et al.*, 1969). The phenotypes of *E. sexcinctus*, *Z. pichiy* and *C. villosus* are compared in Table 1, the chromosomes are shown in Figures 1–4 and Table 2 summarizes the cytogenetic findings.

Table 1 Comparative phenotype among the species of the tribe Euphractini

|              | <i>Euphractus sexcinctus</i>             | <i>Zaedyus pichiy</i>                                  | <i>Chaetophractus villosus</i>             |
|--------------|--|--|--|
| Size         | Larger than <i>Z. pichiy</i>             | About the same size or smaller than <i>C. villosus</i> | Same size or smaller than <i>Z. pichiy</i> |
| Body hair    | Small amount                             | Very small amount                                      | Large amount                               |
| Shell-bands  | 6–8                                      | —  | 8  |
| Distribution | Brazil, Bolivia, Argentina, and Paraguay | Argentina, Chile (introduced)                          | Bolivia, Argentina                         |

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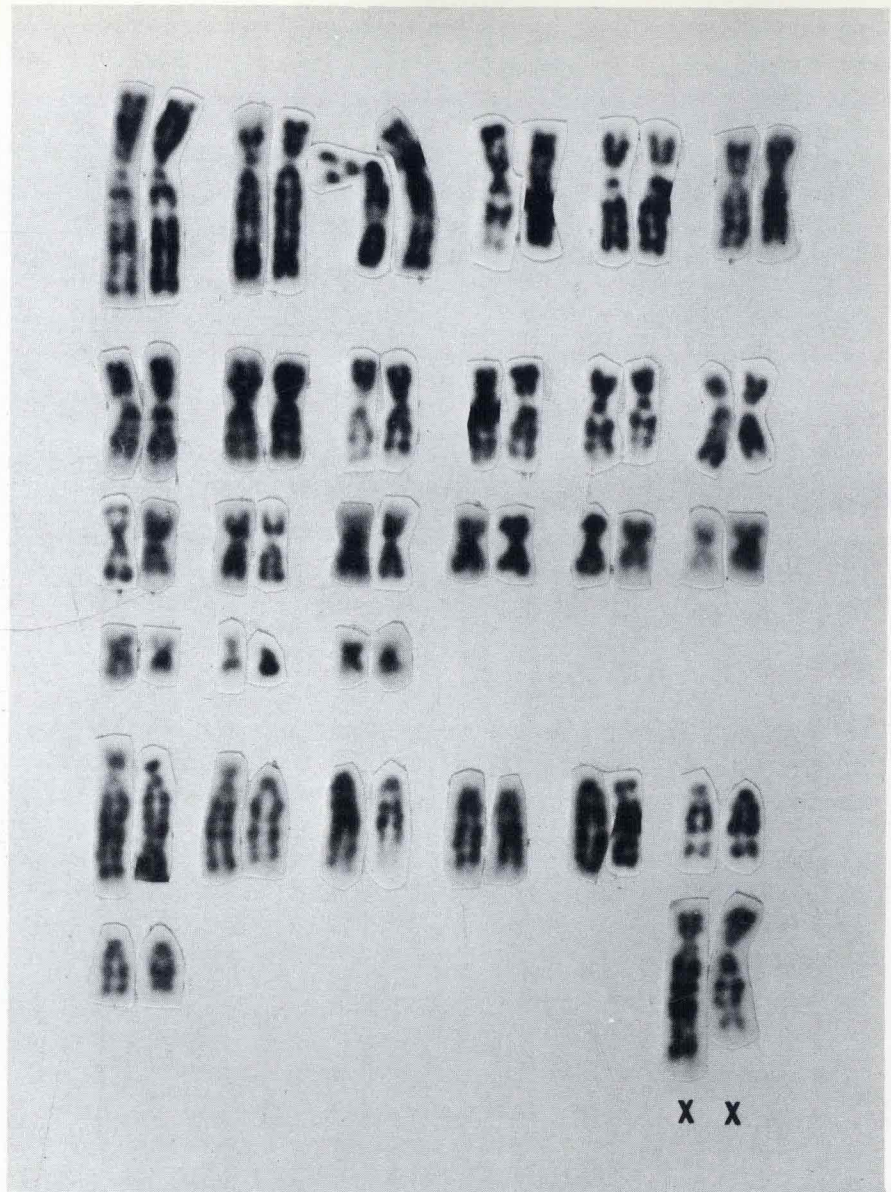


Figure 1 G-banding of female *Euphractus sexcinctus flavimanus*.  $2n = 58$ . Presumptive X-chromosomes last.

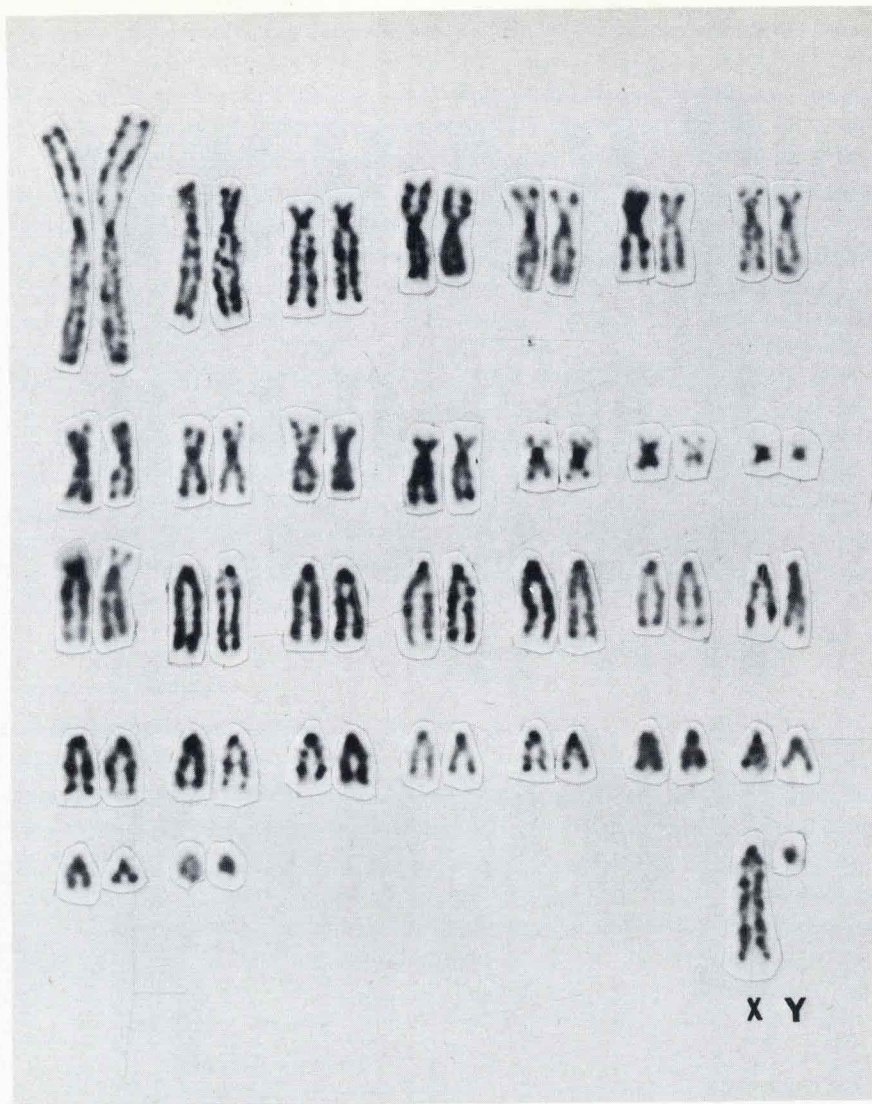
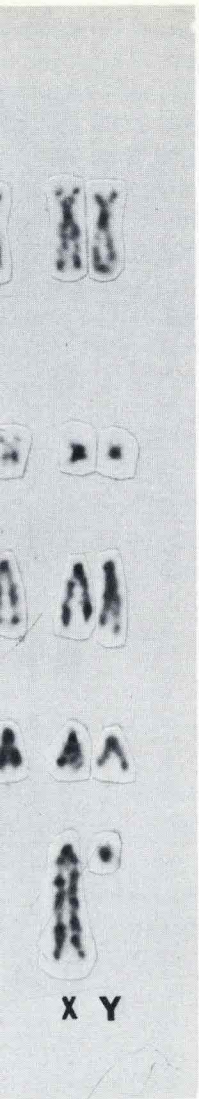


Figure 2 Karyotype of male *Zaedyus pichiy*. The X-chromosome is largest acrocentric.  $2n = 62$ .

Figure 3 (opposite) G-banding of female *Chaetophractus villosus*. The X-chromosomes are the largest acrocentrics, also demonstrated in C-banding preparations (Figure 4).  $2n = 60$ .

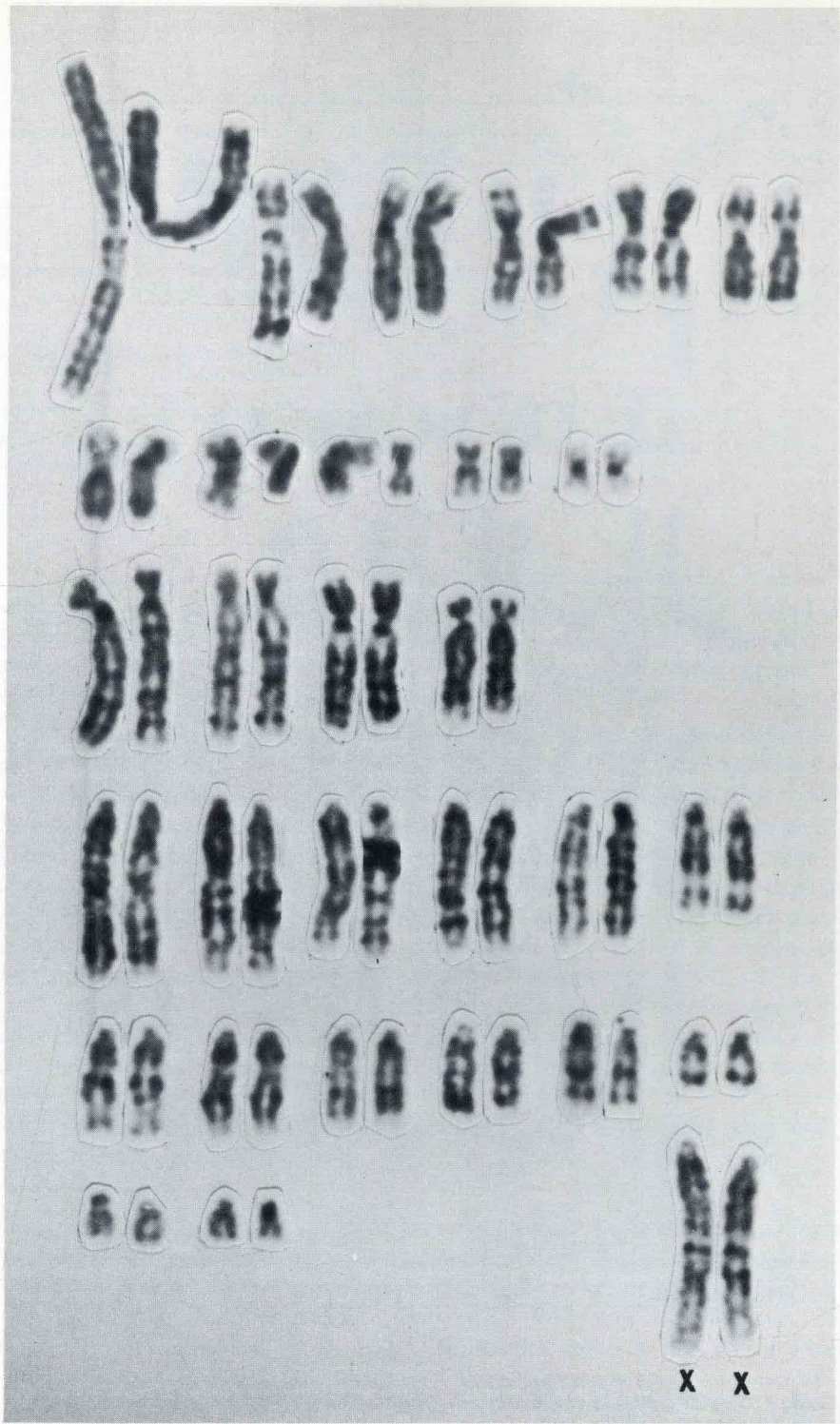


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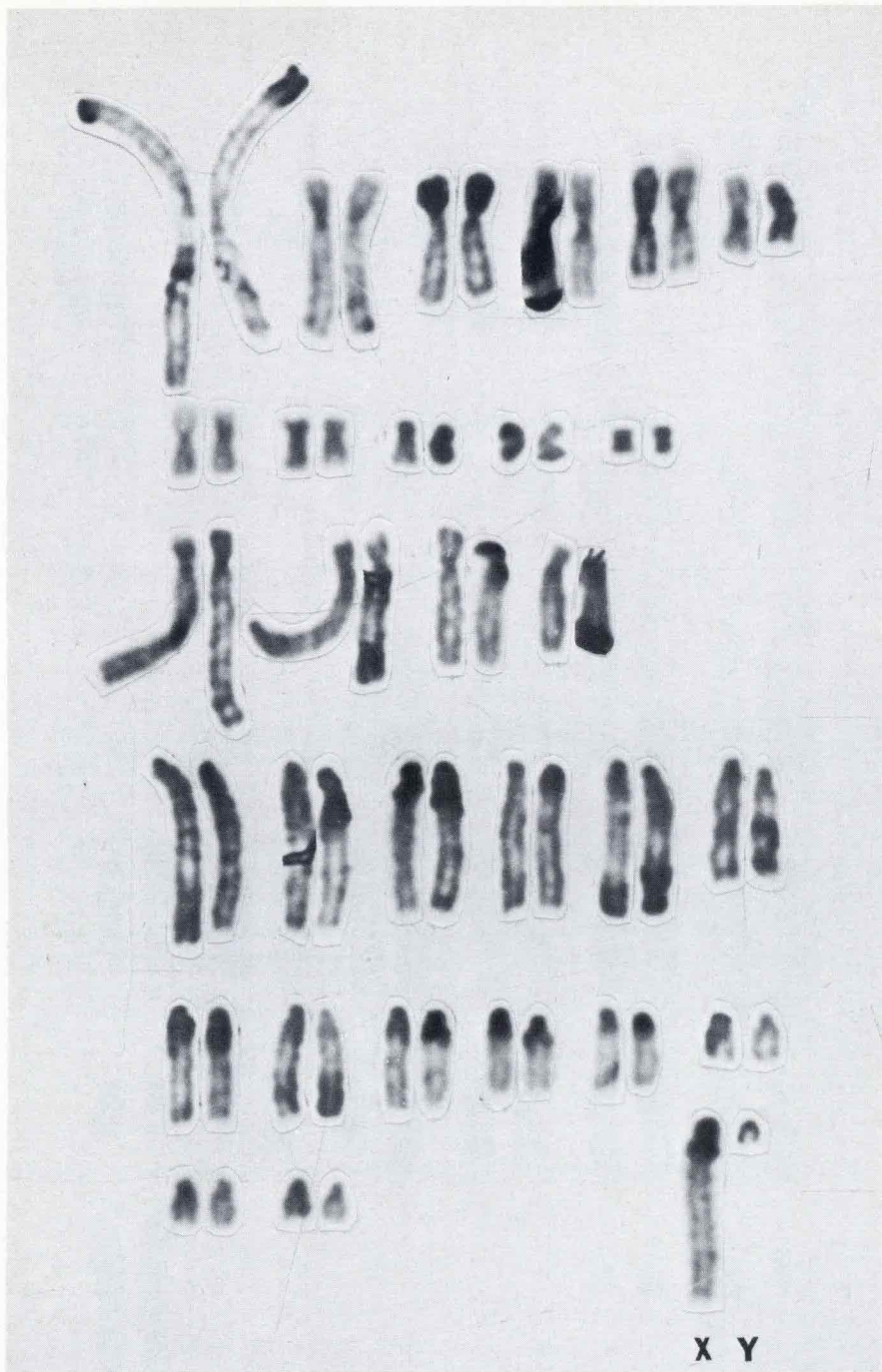


Figure 4 C-banding of male *Chaetophractus villosus*. The X-chromosome has a prominent paracentromeric C-band.  $2n = 60$ .

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Tribe Tolypeutes

This tribe is *cus* inhabits *T. tricinctus* locally, they are the only *T. tricinctus* distinguishing

**Table 2** Edentata, Dasypodidae

| Sub-family     | Genus                        | Species             | 2n               | NF  | M  | A  | X  | Y  |
|----------------|------------------------------|---------------------|------------------|-----|----|----|----|----|
| Dasypodinae    | <i>Dasypus</i> (? 7 sp)      | <i>novemcinctus</i> | 64               | 82  | 16 | 46 | M  | A  |
|                |                              | <i>hybridus</i>     | 64               | 82  | 16 | 46 | M  | A  |
|                | <i>Tolypeutes</i> (2 sp)     | <i>matacus</i>      | 38               | 76  | 36 | 0  | M  | A  |
|                |                              | <i>Priodontes</i>   | <i>giganteus</i> | 50  | 80 | 26 | 24 | M  |
|                | <i>Cabassus</i> (5 sp)       | <i>centralis</i>    | 62               | 78  | 14 | 46 | M  | M  |
| Euphractinae   | <i>Euphractus</i>            | <i>sexcinctus</i>   | 58               | 102 | 42 | 14 | M  | SA |
|                | <i>Chaetophractus</i> (5 sp) | <i>villosus</i>     | 60               | 90  | 30 | 28 | A  | A  |
|                |                              | <i>Zaedyus</i>      | <i>pichiy</i>    | 62  | 94 | 30 | 30 | M  |
| Chlamyphorinae | <i>Burmeisteria</i>          | <i>retusa</i>       |                  |     |    |    |    |    |
|                | <i>Chlamyphorus</i>          | <i>truncatus</i>    |                  |     |    |    |    |    |

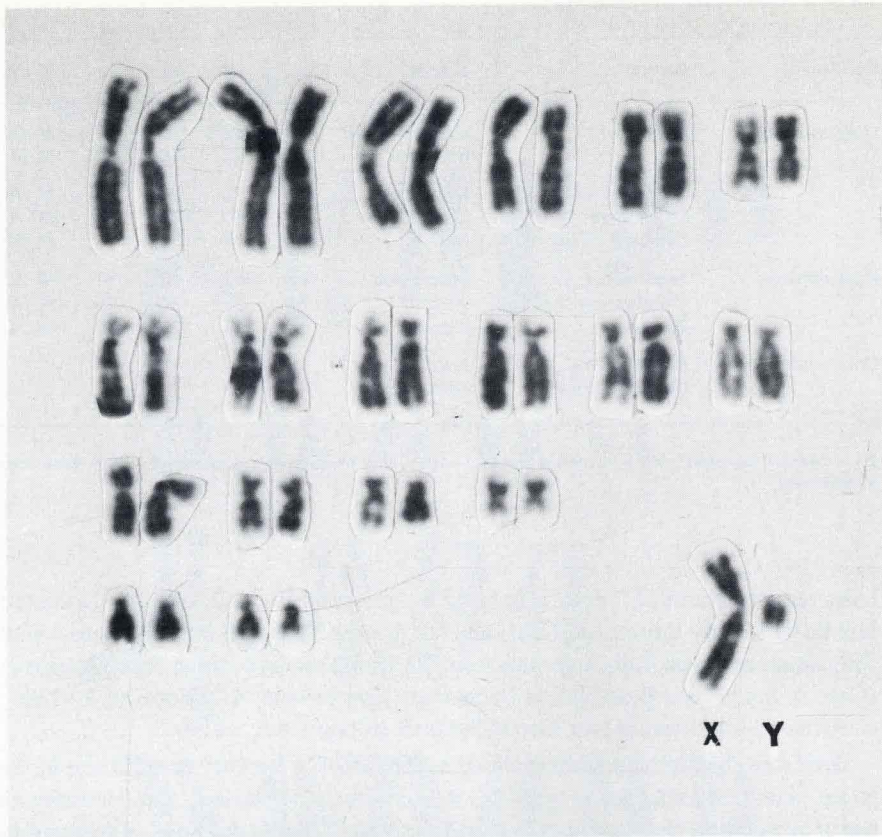
2n = Diploid number. NF = Nombre fundamental. M = Metacentric autosomes. A = Actocentric autosomes.

body size and about 15% heavier in body weight. Additionally, they have a characteristic dark body (armor) colouration. This is near black in San Juan animals while *Tolypeutes matacus* from Argentina near the Rio Pilcomayo, those from Paraguay (Chaco), Bolivia and Brazil (Mato Grosso) are light brown. Additionally, the hair covering the abdomen in San Juan individuals is longer and coarser.

Although the diploid numbers are close (58, 60, 62), karyotype evolution in this group cannot be explained only by the Robertsonian mechanism. The excessive number of acrocentric chromosomes in *C. villosus* and *Z. pichiy* does not correspond to the number of metacentric and submetacentric chromosomes in *E. sexcinctus* which is reflected in differing NF values. Pericentric inversions must have participated in karyotype evolution. According to G- and C-banding patterns, some relationships can be demonstrated. Chromosome pairs numbers 2 to 6 and the large arm of pair number 1 are believed to have approximately the same banding pattern in *E. sexcinctus* and *C. villosus*. Chromosome pair number 1 seems to be conservative in *C. villosus* and *Z. pichiy*. The large arm of metacentric and submetacentric chromosomes of *E. sexcinctus* and *C. villosus* can also be compared. The acrocentric pairs are believed to be more similar between *C. villosus* and *Z. pichiy*. On the other hand, the X-chromosome is acrocentric in *C. villosus* and *Z. pichiy* and submetacentric in *E. sexcinctus*. These findings suggest that *C. villosus* is more closely related to *Z. pichiy* than to *E. sexcinctus*, at least karyologically.

#### Tribe Tolypeutini

This tribe is composed of two species, *Tolypeutes matacus* and *T. tricinctus*. *T. matacus* inhabits Bolivia, Brazil (Mato Grosso), Paraguay (Chaco) and northern Argentina. *T. tricinctus* is an endangered species which lives in north eastern Brazil. Phenotypically, they are very similar with usually three, rarely two or four movable bands. They are the only armadillos that can completely enclose themselves by rolling up in a ball. *T. tricinctus* has five claws on the forefoot, *T. matacus* has only four, the principal distinguishing feature.



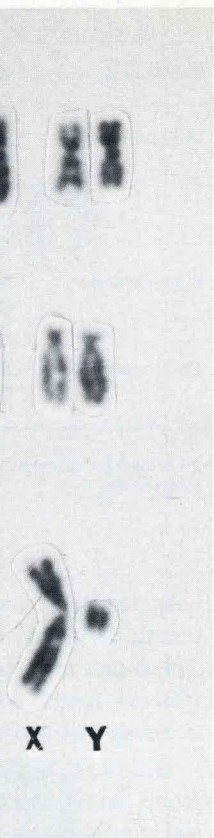
**Figure 5** Karyotype of *Tolypeutes matacus*.  $2n = 38$ . The X-chromosome is the third meta-centric in size and Y is the smallest element. The two smallest autosomal pairs are nearly acrocentric but a short arm is present in these elements as well.

Two males and two females of *T. matacus* were studied. The differences in karyotypes between *T. matacus* and the other species of armadillos are remarkable in that there are no truly acrocentric autosomes and the chromosome number ( $2n = 38$ ) is the lowest in edentates examined so far (Figure 5). The submetacentric X is the third in size and Y is the smallest and acrocentric element. Except for the *Priodontes giganteus* with  $2n = 50$  (Benirschke and Wurster, 1969), all other armadillo species described to date have had chromosome numbers between 58 and 64.

It is interesting to note that there are phenotypic differences between examples of *T. matacus* from northwestern Argentina (Provincia San Juan) and this same species from other parts of its range. The San Juan individuals are about 10% larger in overall

**Figure 6** (opposite) Karyotype of nine-banded armadillo from Texas (*Dasypus novemcinctus*).  $2n = 64$ , Giemsa banding



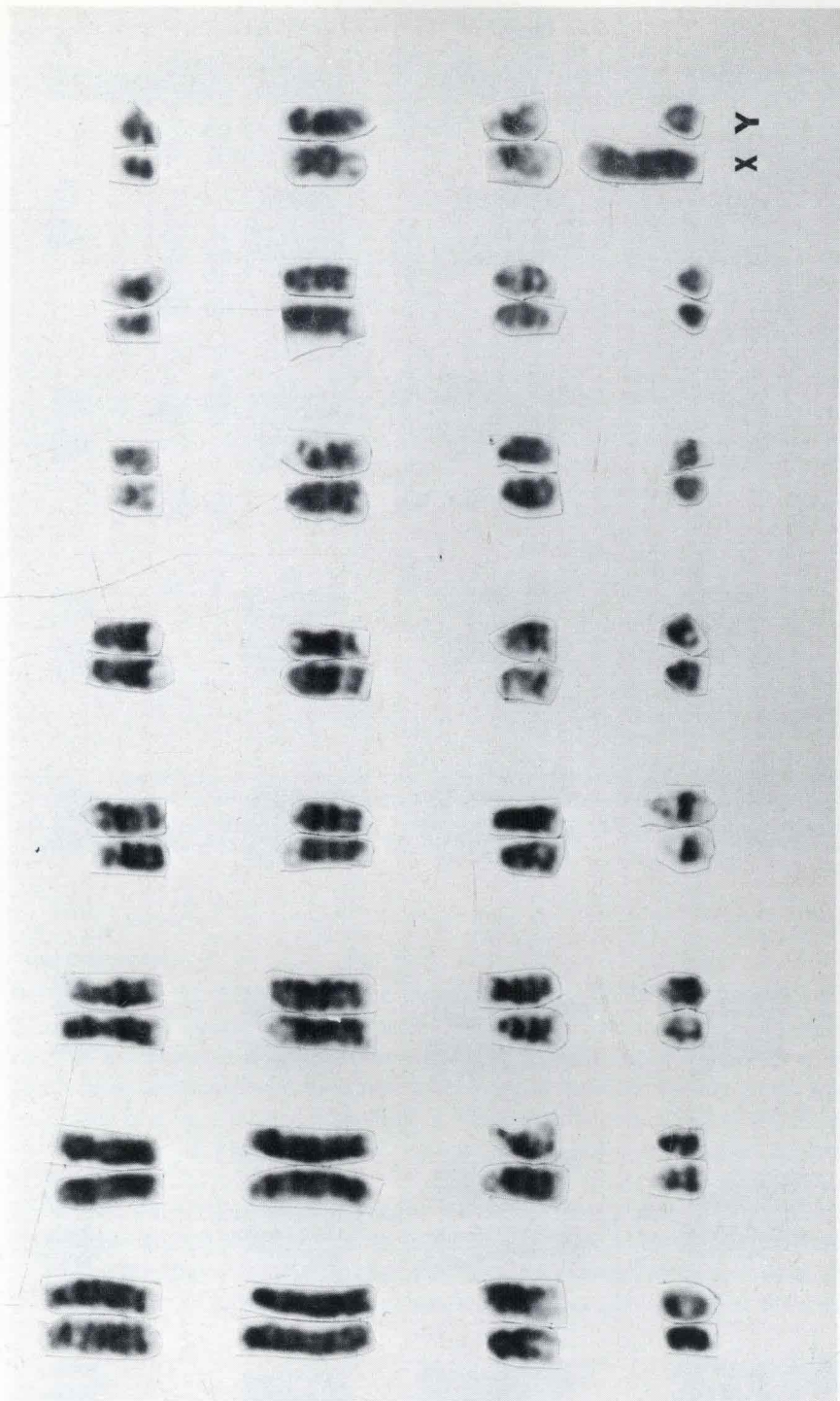


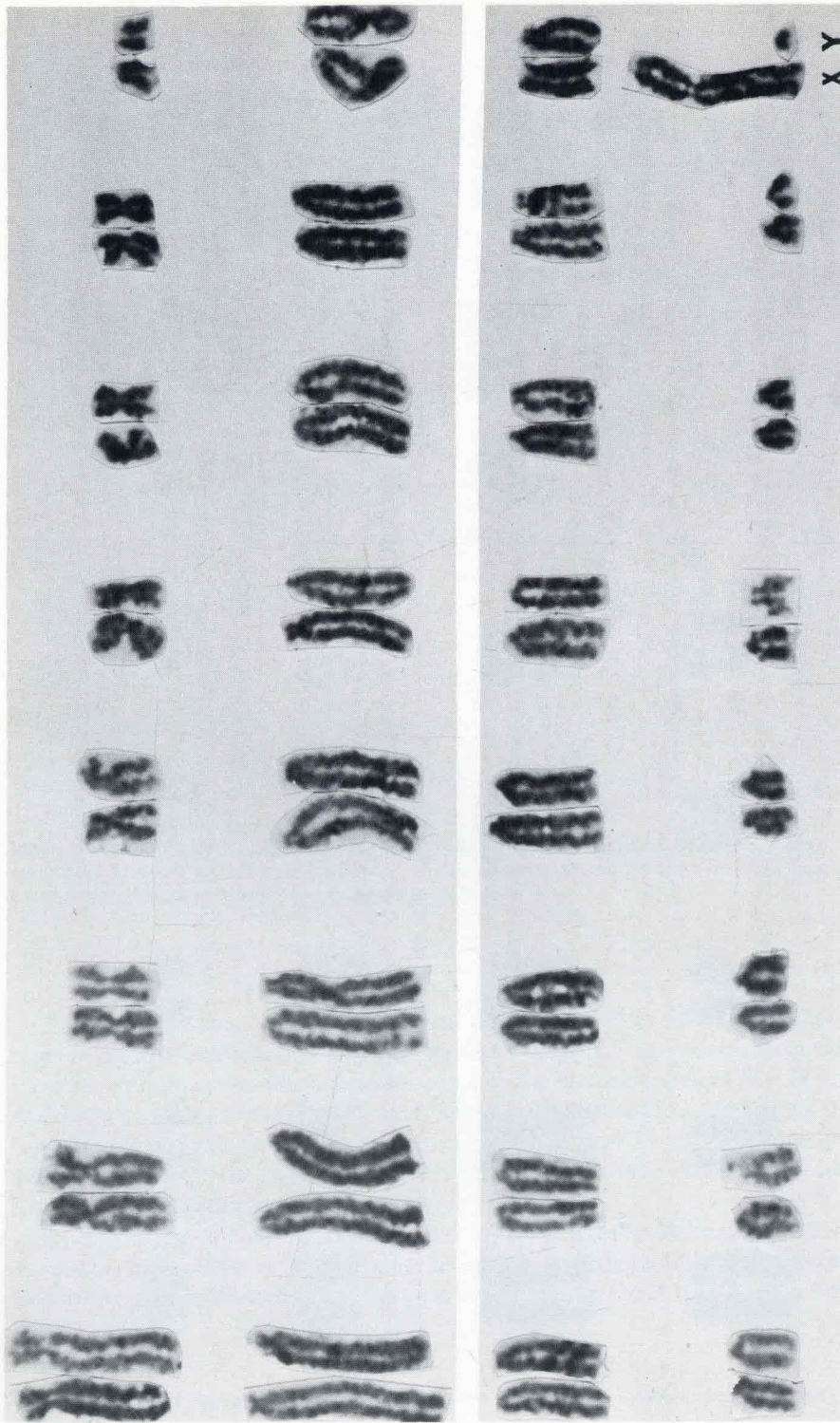
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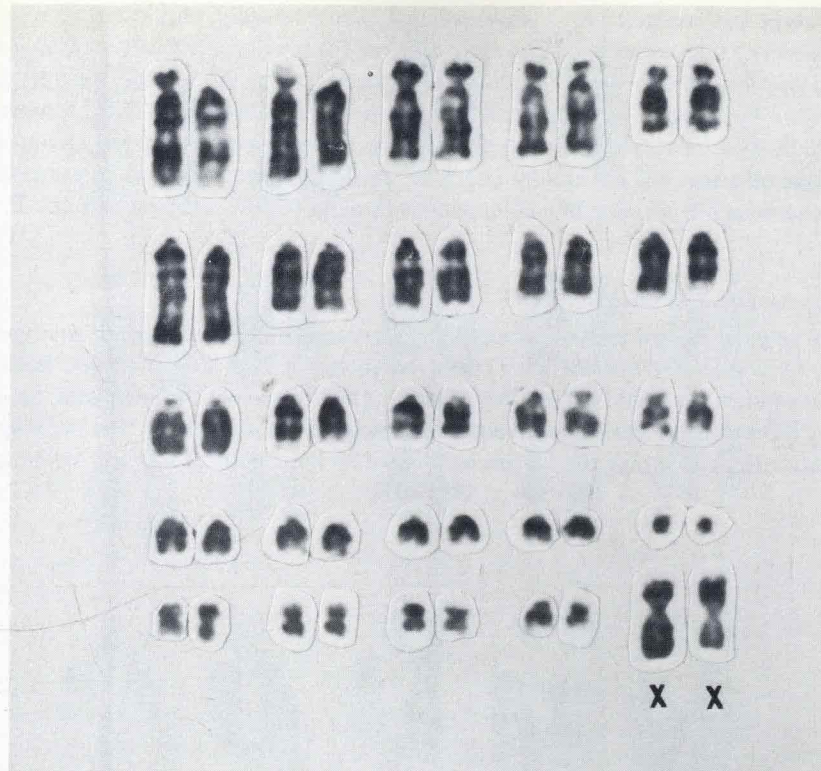
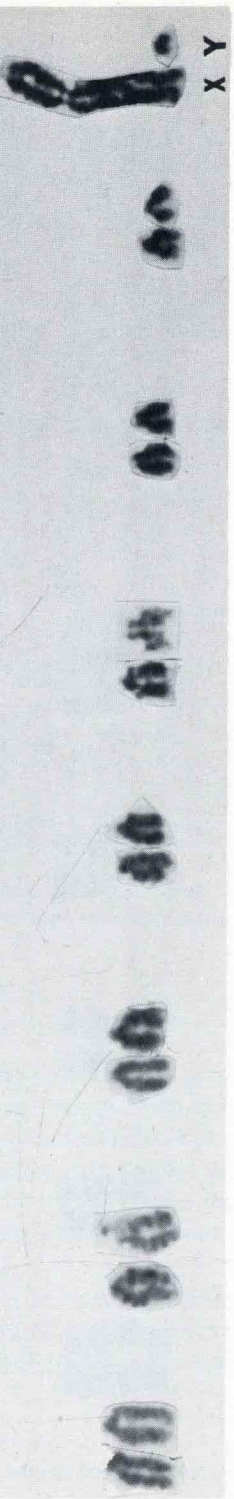




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**Figure 8** G-banding of female offspring from *C. hoffmanni florencae* × *C. hoffmanni hoffmanni*. Note the different G-banding pattern in the X-chromosomes. The differences exhibited between the autosomal homologues (e.g. numbers 1 and 2) may be the result of this specimen being a cross between different subspecies.  $2n = 50$ .

#### Tribe Dasypodini

Best studied in this tribe which is the only group with variable polyembryony and contains several species and/or subspecies of *Dasypus*, is the nine-banded armadillo, *Dasypus noveminctus*. The phenotype of this widely distributed species varies widely with consequent subspecies, even separate species designation. Mainly North American specimens have been studied from Florida, Texas and Louisiana. All have had  $2n = 64$  and a characteristic karyotype of a Texas specimen is shown in Figure 6. Benirschke *et al.* (1969) found minor structural differences in specimens collected from Columbia. These need verification since cytogenetic techniques have much improved since then. Perhaps the striking phenotypic differences can be related to chromosome structure.

The mulita (*Dasypus hybridus*) has previously been described by Saez *et al.* (1964) from bone marrow cells and only very minor differences were identified when the

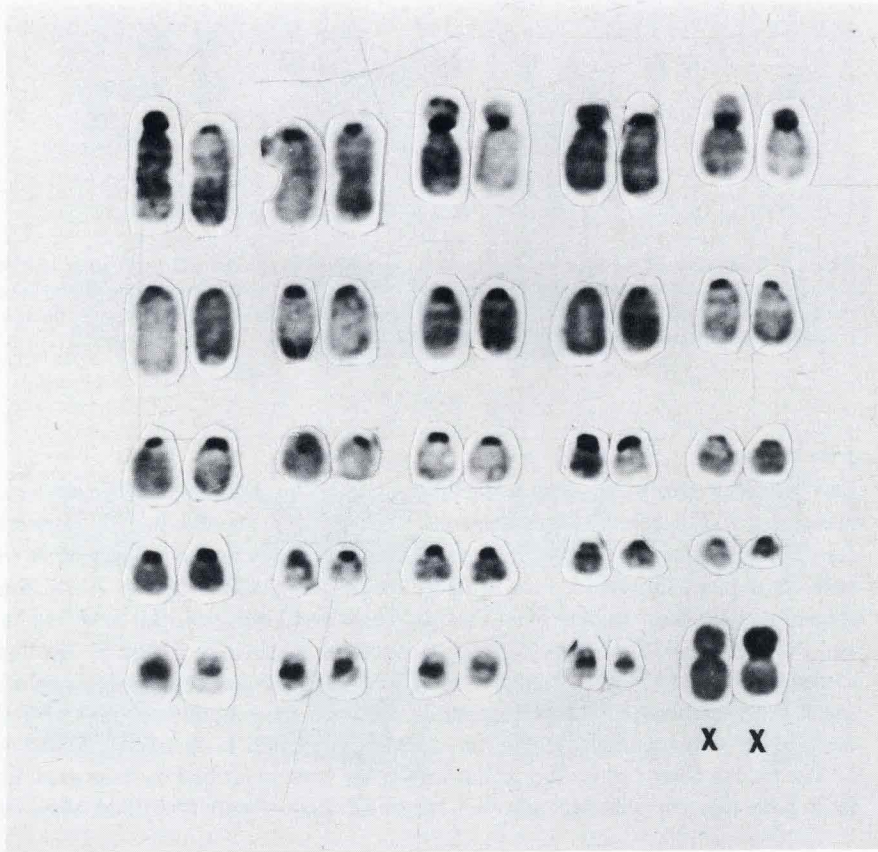
**Figure 7** (opposite) Karyotype of male seven-banded armadillo from Argentina (*Dasypus hybridus*). This karyotype is comparable to that of the nine-banded, Figure 6.

karyotype was compared to *D. novemcinctus*. Unfortunately, only drawings were published of that karyotype. The chromosomes from a fibroblast culture of *D. hybridus* from Argentina are shown in Figure 7. They are set out as those of the nine-banded specimen in Figure 6. No noticeable differences are identified but careful comparison of banding pattern is needed. This is underway but hampered by the sluggish growth of these cells that will not readily attach to plastic dishes and suffer in trypsinization.

A summary of all armadillo chromosome findings to date is shown in Table 2.

**Family Bradypodidae (Sloths)**

*Choloepus hoffmanni* is one of about six species and twelve subspecies of *Bradypodidae*. Only *Choloepus hoffmanni* (Peters) has cytogenetically been studied so far. Both males and females described by Corin-Frédéric (1967) have 49 chromosomes, females being XO and the males having Y-material attached to an autosome. The taxonomic classification concerning the subspecies studied by these authors was not determined.



**Figure 9** C-banding pattern of the same female shown in Figure 8. Note the different C-banding pattern of the X-chromosomes.

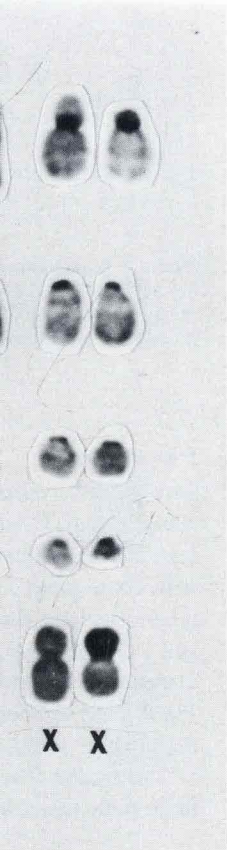


**Figure 10** C-banded Y-chromosome

Both male and female of different subspecies (*Choloepus hoffmanni*), from the same diploid number and chromosome patterns (Figure 9) are of the same size and shape, but the pair has different C-banding patterns. The male is of unusual size and has a very dark C-banded Y-chromosome in the sex chromosome.

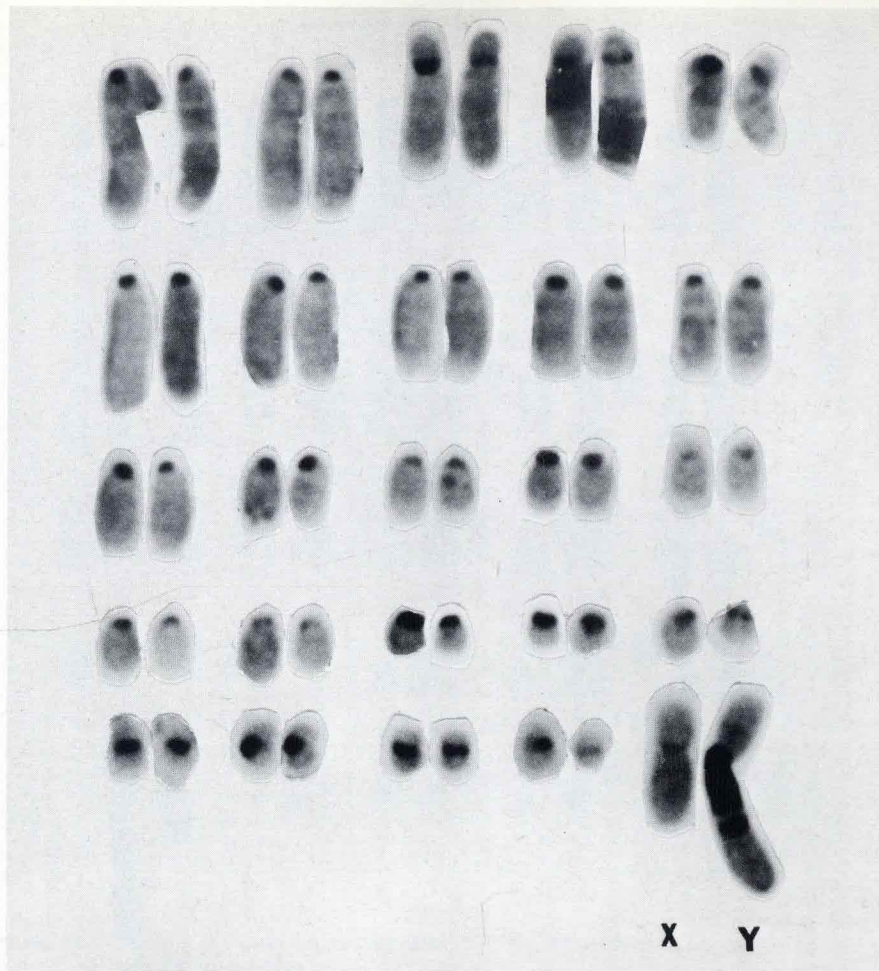
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**Figure 10** C-banding pattern of male *C. hoffmanni*. Observe the typical C-banding in the presumed Y-chromosome.  $2n = 50$ .

Both male and female specimens studied by us come from a cross between two different subspecies ( $\delta$  *Choloepus hoffmanni florenciae* x  $\eta$  *Choloepus hoffmanni hoffmanni*), from Colombia and Panama respectively. Both had 50 chromosomes as diploid number. The X-pair in the female is easily distinguished by G- and C- banding patterns (Figures 8 and 9). One of the homologues of the X-pair has approximately the same size and morphology of Corin-Frédéric's specimens. The other is smaller, slightly more metacentric and it is heavily stained by the C-banding technique. Also, the pair has different patterns of G-banding. The presumed Y-chromosome of our male is of unusual size. It is about 50% larger than the first autosome and has two very dark C-bands of different size (Figure 10). The G- and C-banding of the X-chromosome in the male (Figures 10 and 11) shows that his X is the first element shown in the sex chromosomes in karyotypes of the females (Figures 8 and 9).

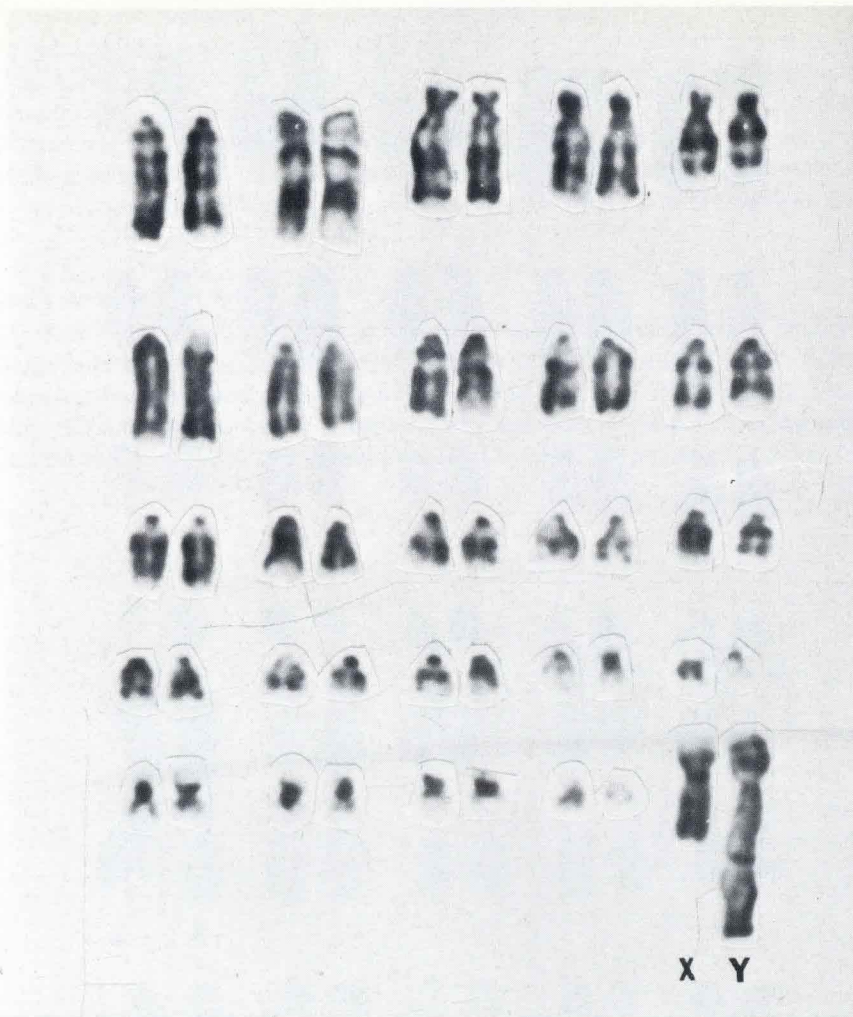


Figure 11 G-banding pattern of male *C. hoffmanni* as in Figure 10.

It is interesting then to inquire of Zoological Gardens about the sex ratio of *C. hoffmanni* in captivity. Of nineteen specimens held currently in U.S. zoos there were nine males, eight females and two of unknown sex. On the other hand, data presented by Dennis Meritt (1976) indicate that three known collections of *Choloepus hoffmanni* (totalling 72 animals) captured in Panama had on the average a 93.1% female to 6.9% male ratio. The presence of the unusual Y-chromosome may determine lethal gametes in meiosis segregation. A translocation of Y-chromosome material onto an autosome cannot be ruled out. Further studies are important in order to determine the participation of this large chromosome in sex determination. In particular, meiosis, sex chromatin and thymidine late labelling should be undertaken when material is available.



Figure 12 G-b Identification

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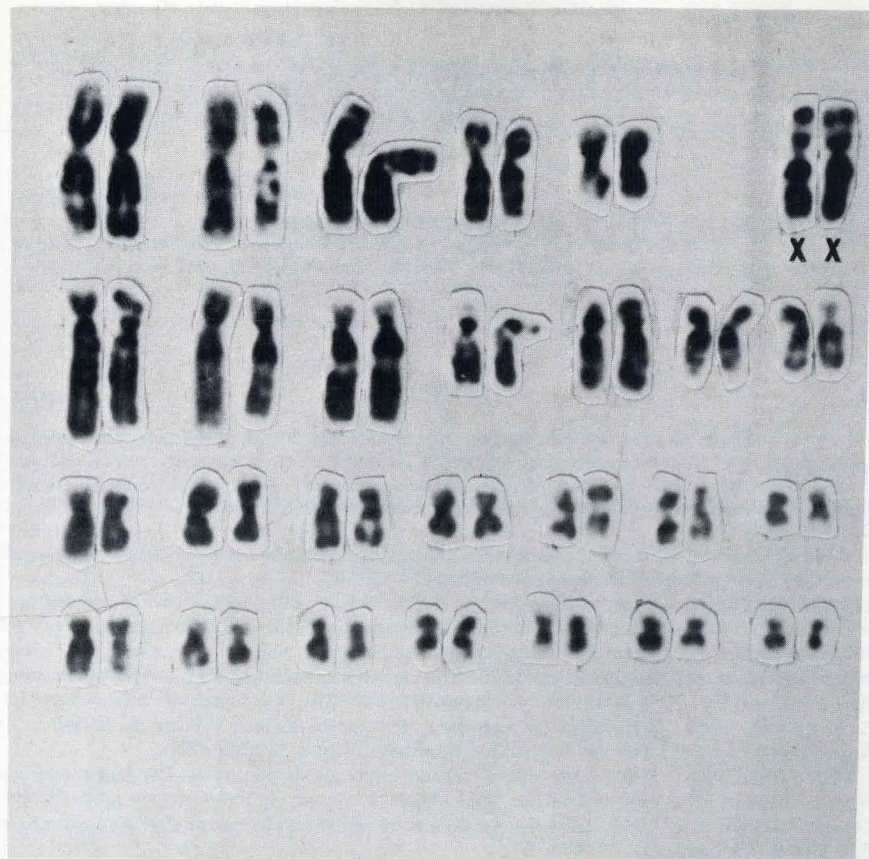


Figure 12 G-banding of *Tamandua longicaudata*.  $2n = 54$ . Most elements are readily paired. Identification of X is speculative.

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**Family Myrmecophagidae (Anteaters)**

Of the three genera and four species of *Myrmecophagidae*, two have been studied to date: *Myrmecophagidae tridactylus* (giant anteater) with 60 chromosomes, and *Tamandua tetradactyla* with 54 chromosomes. According to the description of Hsu (1965), (no illustration given), the giant anteater has six pairs of medium and small-sized acrocentrics, different from *M. tridactyla* and *T. longicaudata* (present paper).

The karyotype of the female *Tamandua longicaudata* with  $2n = 54$  is shown in Figure 12. All chromosomes are metacentric, as are those of *T. tetradactyla*. Although difficult to pair before banding techniques, G-banding allows pairing of almost all elements. The sex chromosomes are identified only tentatively. Although the chromosomes of these two species are apparently very similar, adults at Lincoln Park Zoo have significant phenotypic differences. In particular, body size and weight of *T. longicaudata* always considerably exceeded those of *T. tetradactyla*.

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## Interaction of movement (zip) 3 Theoretical simulation of

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## Abstract

A theoretical analytical model of the interaction of microtubule pulling forces (Bajer, 1973a,b) is presented. It is shown that the stage of mitosis in which the 'zip' (called 'zips') between microtubules is exceeded. The energy requirement for zip are not met. In the spindle can yield sufficient energy of the triphosphate by the model is co

## Introduction

Although much of the spindle at electron microscope resolution is certainly involved in the role in the transition and then to the Bajer and Molè-F. the arguments giving to the formation. Therefore, only the purpose of the 'hypothesis' (Bajer) that the geometrical arrangement of microtubules to microtubule chromosome transition results in chromosomal zip.

This idea received support in 1975; Lambert and exist in the spindle