

## NOTES

KARYOTYPES OF THREE SPECIES OF HARVEST MICE (*REITHRODONTOMYS*)

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**ABSTRACT**—We report the karyotypes of *Reithrodontomys hirsutus* and *R. mexicanus* from Oaxaca and Puebla, Mexico, and *R. sumichrasti* from Puebla and Veracruz, Mexico; the first is described for the first time and the latter 2 represent karyotypic variants from localities different from those previously reported. Our findings confirm the complex chromosomal evolution that has occurred within the genus *Reithrodontomys* and support molecular data indicating that an undescribed species inhabits the highlands of the Sierra Madre Oriental in Mexico. We also document chromosomal variation within *R. sumichrasti* congruent with phylogenetic patterns reported previously.

**RESUMEN**—Reportamos los cariotipos de *Reithrodontomys hirsutus* y *R. mexicanus* de Oaxaca y Puebla, México, y *R. sumichrasti* de Puebla y Veracruz, México; el primero es descrito por primera vez y los últimos dos representan variaciones cariotípicas provenientes de localidades diferentes a las reportadas previamente. Nuestros resultados confirman la compleja evolución cromosómica que ha ocurrido dentro del género *Reithrodontomys* y apoyan datos moleculares que indican que una especie aún no descrita habita las tierras altas de la Sierra Madre Oriental en México. También documentamos variación cromosómica dentro de *R. sumichrasti* congruente con los patrones filogenéticos reportados previamente.

Harvest mice of the genus *Reithrodontomys* are represented by 21 species in 2 subgenera (*Aporodon* and *Reithrodontomys*). Karyotypes of 12 species have been described, and these studies reported extensive intraspecific and interspecific variation for chromosome number and structure (Carleton and Myers, 1979; Robbins and Baker, 1980; Engstrom et al., 1981; Rogers et al., 1983; Hood et al., 1984). Diploid numbers (2n) range from 38 to 52. In the species belonging to the subgenus *Aporodon*, the karyotypes are mainly composed of acrocentric chromosomes, while species in the subgenus *Reithrodontomys* show a biarmed composition. This variation in 2n and fundamental number (FN) is considered to be the result of karyotypic megaevolution within the

subgenus *Reithrodontomys* through rearrangements such as pericentric inversions and heterochromatic additions (Hood, 1984; Bell et al., 2001). In this paper, we describe karyotypes for *R. mexicanus* and *R. sumichrasti* that differ from those previously published and present the first report of the karyotype of the Mexican endemic *R. hirsutus*. We discuss these results in the context of the evolutionary history of the genus.

Specimens were collected from natural populations. Somatic metaphases were prepared in the field following (Patton, 1967) or processed in the laboratory of Evolutionary Genetics at UAMI according to (Baker et al., 2003).

The 2n and FN of each species was determined by examining an average of 50 mitotic meta-

phases from each specimen acquired with a digital camera, zoom 7.5X and a microscope objective 100X. Karyotypes were arranged following (Carleton and Myers, 1979) and described as suggested by (Patton, 1967). Voucher specimens were deposited at the Monte L. Bean Museum Brigham Young University (BYU) or at the Mammal Collection (CMC) of the Centro de Educacion Ambiental e Investigacion Sierra de Huautla of the Universidad Autonoma del Estado de Morelos.

A summary of chromosomal data is presented in Table 1. For comparative purposes, this table also includes karyotypic information from previous reports for *R. mexicanus* and *R. sumichrasti*. Specimens examined are documented in Appendix I.

*Reithrodontomys mexicanus*—The specimens of this species from the localities in Oaxaca and Puebla, Mexico, had the same karyotype,  $2n = 50$  and  $FN = 48$ . This karyotype was composed exclusively of acrocentric chromosomes; we inferred the morphology of the sex chromosomes inasmuch as there were no clear matches with the hypothesized X or Y chromosomes (Fig. 1A).

*Reithrodontomys hirsutus*—The karyotype of the single specimen examined was  $2n = 46$  and  $FN = 44$ . The autosomes were all acrocentric. The X chromosome was a large metacentric, and the Y likely was a small acrocentric (Fig. 1B).

*Reithrodontomys sumichrasti*—The specimens from Puebla, Mexico, had a  $2n = 42$  and  $FN = 80$  (Fig. 1C), whereas those from Veracruz, Mexico, had a  $2n = 40$  and  $FN = 72$  (Fig. 1D). The X chromosome was a large subtelocentric, and the Y was a large submetacentric in both populations. Sex chromosome morphology was inferred based on standard karyotypes of *R. sumichrasti* reported by (Carleton and Myers, 1979) and (Engstrom et al., 1981), as well as differentially stained karyotypes described by (Hood et al., 1984).

Based on morphology, (Hooper, 1952) proposed that *R. hirsutus*, together with *R. fulvescens*, occupied an intermediate phyletic position between members of the subgenera *Aporodon* and *Reithrodontomys*, whereas (Arellano et al., 2005) found that these 2 species were basal to all other members of the subgenus *Reithrodontomys*. Our results support these interpretations inasmuch as the karyotype of the single specimen of *R. hirsutus* examined had a relatively low  $2n$  and a biarmed X chromosome, as commonly occurs among species of the subgenus *Reithrodontomys*, but possessed acrocentric autosomes and a small acrocentric Y, as observed among the species of *Aporodon*. Given the small geographic distribution of *R. hirsutus*, it is likely that the karyotype described herein was representative of this species.

TABLE 1—Karyotypes of 3 species of *Reithrodontomys* and comparisons with previous descriptions.

Species	Locality	2n	FN	Autosomes		Sex chromosomes		Reference
				Bi-armed	Acrocentric	X	Y	
<i>R. hirsutus</i>	Ameca, Jalisco, Mexico	46	44	–	44	M	A	Present study
<i>R. mexicanus</i>	Puerto de la Soledad, Oaxaca, Mexico	50	48	0	48	A	A	Present study
	Cerro Mozotal, Chiapas, Mexico	52	50	0	50	A	A	Rogers et al., 1983
	Guatemala and Ecuador	52	52	2	48	A	A	Carleton and Myers, 1979
<i>R. sumichrasti</i>	Ameca, Jalisco, Mexico	42	80	40	0	?	?	Engstrom et al., 1981
	Cerro Chignautla, Puebla, Mexico	42	80	40	0	ST	SM	Present study
	Costa Rica	40	76	38	0	M <sup>1</sup>	SM <sup>1</sup>	Carleton and Myers, 1979; Hood et al., 1984
	Puerto del Aire and Mesa Yerba, Veracruz, Mexico	40	72	34	4	ST	SM	Present study

<sup>1</sup> = Described by (Hood et al., 1984).

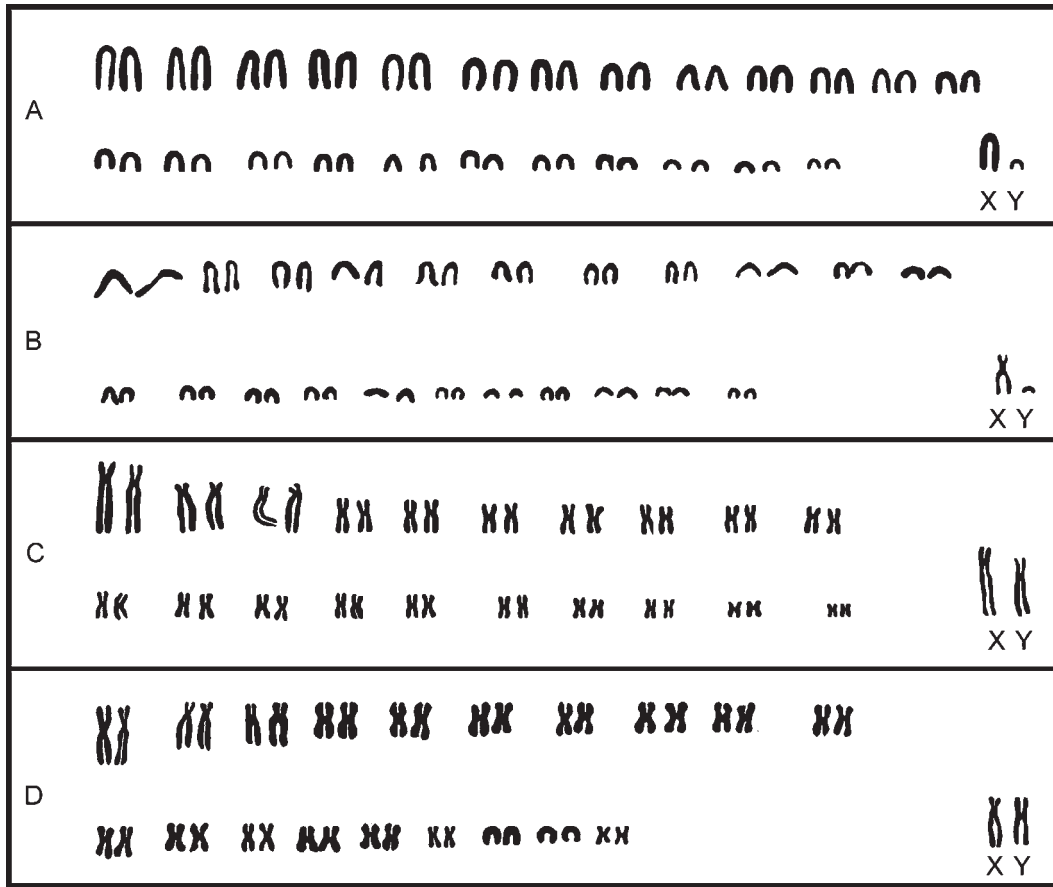


FIG. 1.—A) Karyotype of a male *Reithrodontomys mexicanus* from Puerto de la Soledad, Oaxaca, Mexico ( $2n = 50$ ,  $FN = 48$ ; BYU 16250). B) Karyotype of a male *R. hirsutus* from Ameca, Jalisco, Mexico ( $2n = 46$ ,  $FN = 44$ ; BYU 16238). C) Karyotype of a male *R. sumichrasti* from Cerro Chignautla, Puebla, Mexico ( $2n = 42$ ,  $FN = 80$ ; CMC 1093). D) Karyotype of a male *R. sumichrasti* from Puerto del Aire, Veracruz, Mexico ( $2n = 40$ ,  $FN = 72$ ; CMC 1404).

The karyotype of *R. mexicanus* reported previously was  $2n = 52$ . Populations from Guatemala and Ecuador (Carleton and Myers, 1979) had a  $FN = 52$ , and a sample from Chiapas, Mexico (Rogers et al., 1983), had a  $FN = 50$ . The karyotypes of our specimens have one less pair of chromosomes and are all acrocentric. (Arellano et al., 2003, 2005) found high levels of genetic differentiation (fixed alternate alleles at 6 presumptive genetic loci and approximately 12.5% genetic distance based on cytochrome-*b*) for certain individuals from the Sierra Madre Oriental of Mexico (including specimens examined here) previously regarded as *R. mexicanus* compared to other samples of *R. mexicanus* from localities in eastern Mexico and in Central and

South America. (Arellano et al., 2003, 2005) suggested that these genetically divergent samples of *R. mexicanus* from the Sierra Madre Oriental represent an undescribed species that occurs in sympatry with *R. mexicanus* in 2 locations in northern Oaxaca, Mexico. Our chromosomal data are consistent with this hypothesis. It is also noteworthy that the karyotypic configuration of this undescribed species is similar to the hypothesized ancestral karyotype of the genus (Carleton and Myers, 1979; Robbins and Baker, 1980; Engstrom et al., 1981; Rogers, 1983) and that, according to (Arellano et al., 2005), this undescribed species is ancestral to all other members of the subgenus *Aporodon*.

The *R. sumichrasti* from Puebla, Mexico, had the same karyotype as described by (Engstrom et al., 1981) for specimens from Jalisco, Mexico. However, this species shows chromosomal variation over its geographic distribution, given that the karyotype of a population from Costa Rica consists of one less pair of chromosomes ( $2n = 40$ ,  $FN = 76$ ; Carleton and Myers, 1979; Hood, 1984). The karyotypic form from Veracruz also has 40 chromosomes, but 2 pairs of autosomes are acrocentric. (Arellano, 1999) found high levels of genetic divergence among several geographic samples of *R. sumichrasti*. The deepest phylogenetic split documented by (Arellano, 1999) separated populations from the northern part of the distribution from those found in southern Mexico and Central America. This pattern is consistent with the phylogeography of *R. sumichrasti* estimated by (Sullivan et al., 2000). These findings also support the studies of (Hooper, 1952) from which he inferred morphological differentiation between populations of *R. sumichrasti* on either side of the Isthmus of Tehuantepec.

In summary, karyotypic and molecular data support the phyletic position of *R. hirsutus* as intermediate between the subgenera *Aporodon* and the majority of species in the subgenus *Reithrodontomys*. Our observations of additional chromosomal polymorphism within the geographically widespread (and likely composite) *R. sumichrasti* also were consistent with reports of extensive genetic partitioning within this taxon. Finally, the chromosomal complement described for the sample of *R. mexicanus* from Oaxaca, Mexico, fits the hypothesized ancestral karyotype for the genus as first proposed by (Carleton and Meyers, 1979) based on non-differentially stained chromosomes. We conclude that, although recent systematic studies typically rely on molecular data, traditional cytogenetic analyses are still a viable and convincing criterion to assess affinities among taxa. This is particularly true for a group, such as the genus *Reithrodontomys*, in which speciation events seem to be concordant with chromosomal rearrangements.

We thank S. Haymond, R. Mercado, R. Sherwin, and A. L. Villalba for assistance in the field. Permits for fieldwork were issued by the Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). Funding was provided by the Theodore Roosevelt Memorial Fund of the American Museum of Natural History (to E. Arellano), the Consejo Nacional de Ciencia y

Tecnología (grant 132880N to F. X. González-Cózatl), Professional Development Grants, Departments of Zoology and Integrative Biology and the M. L. Bean Life Science Museum, Brigham Young University (to D. S. Rogers), and a Professional Development Grant from the Universidad Autónoma Metropolitana Iztapalapa (to M. A. Aguilar) through an agreement with Universidad Autónoma del Estado de Morelos.

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- Submitted 27 August 2005. Accepted 30 March 2006.  
Associate Editor was Philip Sudman.
- APPENDIX I—*Specimens Examined*—*Reithrodontomys hirsutus*—Jalisco: 29 km SE Ameca, 1,470 m (1 male; BYU 16238); *R. mexicanus*—Oaxaca: 1.5 km S Puerto de la Soledad, Municipio Teotitlán de Flores Magón, 18°09.742'N, 96°59.852'W, 2,600 m (3 male, 1 female; BYU 16250–16253); Puebla: Cerro Chignautla, 3 km W Chignautla, Municipio Atempan, 19°40.062'N, 97°24.952'W, 2,176 m (1 female; CMC 1091); *R. sumichrasti*—Puebla: Cerro Chignautla, 3 km W Chignautla, Municipio Atempan, 19°40.062'N, 97°24.952'W, 2,176 m (1 male; CMC 1093). Veracruz: 2.9 km E. Puerto del Aire, Municipio Acultzingo, 18°40.686'N, 97°19.613'W, 2,440 m (1 male, 2 female; CMC 1403–1405); Mesa de la Yerba, 3.4 km SW deviation to Mazatepec (Xalapa–Perote road). Municipio Acajete, 19°33.558'N, 97°01.110'W, 2,040 m (2 male, 2 female; CMC 1395–1398).