

CHROMOSOMAL STUDIES AND EVOLUTIONARY RELATIONSHIPS
OF AN ENDANGERED SPECIES,
REITHRODONTOMYS RAVIVENTRIS

CRAIG S. HOOD, LYNN W. ROBBINS, ROBERT J. BAKER, AND
HOWARD S. SHELLHAMMER

*Department of Biological Sciences and The Museum, Texas Tech University,
Lubbock, TX 79409 (CSH, LWR, RJB)*

*Department of Biological Sciences, San Jose State University,
San Jose, CA 95192 (HSS)*

*Present address of LWR: Savannah River Ecology Laboratory,
Drawer E, Aiken, SC 29801*

ABSTRACT.—G- and C-banded karyotypes of *Reithrodontomys raviventris* were examined to identify the amount and types of chromosomal change that have occurred in this species. The evolutionary origin and phylogenetic relationships of *R. raviventris* were evaluated by comparing G- and C-band data with six additional species of *Reithrodontomys* (*fulvescens*, *creper*, *mexicanus*, *megalotis*, *montanus*, and *sumichrasti*). Banding homologies indicate that the low diploid number forms (*raviventris*, *montanus*, *megalotis*, and *sumichrasti*) shared a common ancestry and that a major dichotomy exists among these taxa. *R. raviventris* and *R. montanus* share a phylogenetic relationship as indicated by 10 shared-derived chromosomal rearrangement events. *R. megalotis* and *R. sumichrasti* represent a second well-defined clade. These chromosomal banding data suggest an alternative hypothesis to the traditionally accepted view that *R. raviventris* is most closely related to *R. megalotis*.

The salt-marsh harvest mouse, *Reithrodontomys raviventris*, is an endangered species restricted to salt marshes in the San Francisco Bay area. Two subspecies, *R. r. raviventris* (Dixon, 1908) and *R. r. halicoetes* (Dixon, 1909) are recognized, representing southern and northern populations, respectively (Fig. 1). Previous studies have concluded that *R. raviventris* and another locally abundant harvest mouse, *R. megalotis longicaudus*, were derived from a common ancestral stock with *R. megalotis* being the closest living relative of *R. raviventris*. Hooper's (1944) assessment of San Francisco Bay as an isolating mechanism for speciation of small mammals first introduced the notion of a phylogenetic relationship between *R. raviventris* and *megalotis*. Fisler (1965) pursued the idea that *R. raviventris* was a *megalotis* derivative in a detailed study of adaptation and speciation of harvest mice in the San Francisco Bay area.

Chromosomal studies were undertaken by Shellhammer (1967, 1969) to document variability in the three forms of harvest mice from San Francisco Bay. Both subspecies of *R. raviventris* possess a $2n = 38$, $FN = 72$ karyotype, but vary in chromosomal morphology. The standard karyotype of *R. megalotis longicaudus* is similar ($2n = 42$, $FN = 80$), which led Shellhammer (1967) to propose that the karyotype of *R. raviventris* had most likely evolved from one like that found in *R. megalotis*.

Recent studies of chromosomal evolution within and among species of *Reithrodontomys* document that the genus is characterized by a high amount of chromosomal variability (Carleton and Myers, 1979; Robbins and Baker, 1980; Engstrom et al., 1981). On the basis of standard karyotypic data, there appear to be two major groups. One group includes species with high diploid numbers (50–52) and mostly acrocentric elements (*Reithrodontomys fulvescens*, *mexicanus*, *creper*, *gracilis*, *tenuirostris*, and *humilis*). The second assemblage includes species characterized by low diploid number (38–42) and an entirely biarmed karyotype (*R. raviventris*, *montanus*, *megalotis*, and *sumichrasti*). Carleton and Myers (1979) proposed that the high diploid number, mostly acrocentric karyotype was primitive for the genus.

Based on G- and C-band data, Robbins and Baker (1980) proposed a primitive karyotype for the genus that has a $2n = 50$, with entirely acrocentric autosomes as found in *R. fulvescens*.

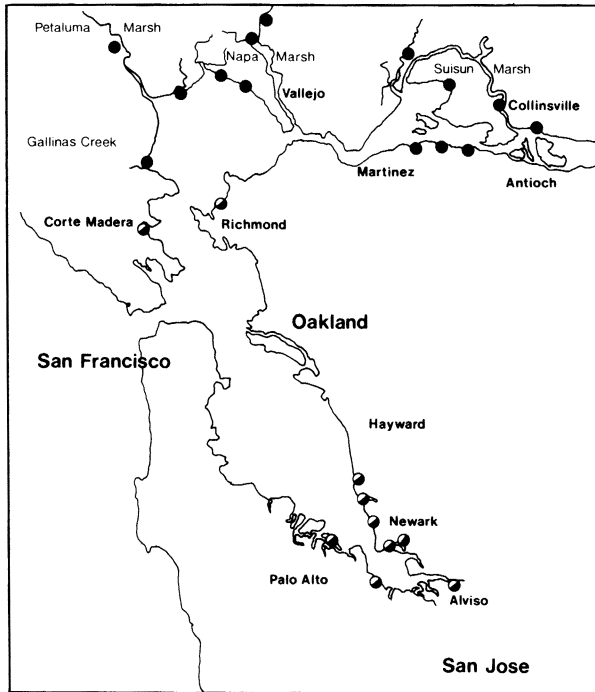


FIG. 1.—Geographic distribution of *Reithrodontomys raviventris*. Closed circles indicate populations of *R. r. halicoetes* and half-closed circles indicate those of *R. r. raviventris*.

Robbins and Baker (1980) failed to identify G-band homologies between *Reithrodontomys megalotis* and *R. fulvescens* and could not document shared G-band sequences with another low diploid number species, *R. montanus*. Nonetheless, extensive chromosomal rearrangement has occurred in *R. megalotis* and *R. montanus* (and other low diploid number forms as well) relative to the other species of the genus *Reithrodontomys*.

The purpose of this paper is to present G- and C-band data for *Reithrodontomys raviventris*, as well as other *Reithrodontomys*, in order to assess the following questions. (1) What is the nature of the chromosomal variation found in the standard karyotype (Shellhammer, 1967, 1969) that distinguishes the two subspecies of *R. raviventris*? (2) Do G- and C-band homologies document a relationship between *R. raviventris* and *R. megalotis*, or is one of the other low diploid number forms (*R. montanus* or *R. sumichrasti*) the closest relative of *R. raviventris*? (3) What are the evolutionary relationships of the low diploid number forms as indicated by cladistic analysis of G-bands?

A genic analysis of *R. raviventris* that represents a companion study to this work is presented in Nelson et al. (1984).

METHODS AND MATERIALS

All specimens were collected from natural populations. Standard, G-, and C-band preparations were obtained from bone-marrow technique as modified by Lee and Elder (1981). G- and C-banding followed Seabright (1971) and Stefos and Arrighi (1971). A minimum of 10 complete spreads was scored for each specimen in the analysis of G- and C-band preparations. All figures presented in this paper represent the complete chromosomal complement of a single cell.

The numbering system proposed by The Committee for Standardizing the Chromosomes of *Peromyscus* (1977) was used to identify homologies in determining the primitive nature of the *R. fulvescens* karyotype, and is used in the present paper to identify proposed banding homologies among the taxa under study. The

TABLE 1.—Chromosomal data for the genus *Reithrodontomys*. Chromosome morphology for sex elements: A = acrocentric, M = metacentric, SM = submetacentric, ST = subtelocentric. Type of study: 1 = standard, 2 = C-band, 3 = G-band.

Taxa	2N	FN	Autosomes		X	Y	Study	Reference
			Biarm	Acro				
<i>Reithrodontomys</i>								
<i>raviventris</i>	38	72	36	0	SM	ST	1 2, 3	Shellhammer (1967) Present study
<i>montanus</i>	38	72	36	0	SM	ST	1 2, 3	Robbins (1981) Robbins and Baker (1980); present study
<i>sumichrasti</i>	40	76	38	0	M	SM	1 2, 3	Carleton and Myers (1979) Present study
<i>megalotis</i>								
<i>amoles</i>	48	92	46	0	M	ST	1	Engstrom et al. (1981)
<i>dychiei</i>	42	80	40	0	SM	ST	1 2, 3	Hsu and Benirschke (1968) Robbins and Baker (1980); present study
<i>longicaudus</i>	42	80	40	0	SM	ST	1	Shellhammer (1967); Blanks and Shellhammer (1968)
<i>megalotis</i>	42	80	40	0	SM	ST	2, 3 1 2, 3	Present study Hsu and Benirschke (1968) Robbins and Baker (1980); present study
<i>saturatus</i>	40	76	38	0	SM	ST	1	Engstrom et al. (1981)
<i>zacatecae</i>	50	96	48	0	SM	ST	1, 2, 3	Present study
<i>humulis</i>	51	60?	10?	40?	—	—	1	Carleton and Myers (1979); Engstrom et al. (1981)
<i>fulvescens</i>	50	48	0	48	A	A	2 1 2 2, 3	Robbins and Baker (1980) Hsu and Benirschke (1968) Engstrom et al. (1981) Robbins and Baker (1980); present study
<i>creper</i>	52	50	0	50	A	A	1 2, 3	Carleton and Myers (1979) Present study
<i>gracilis</i>	52	50	0	50	A	A	1	Carleton and Myers (1979)
<i>mexicanus</i>	52	50–52	0–2	48–50	A	A	1	Carleton and Myers (1979); Rogers et al. (1983)
<i>tenuirostris</i>	52	50	0	50	A	A	2, 3 1	Present study Rogers et al. (1983)

number and distinct patterning of G-bands in the largest 14 pairs of chromosomes allowed identification of homologous elements to the *Reithrodontomys fulvescens* standard. However, many of the smaller chromosomes lacked a sufficient degree of G-band resolution to allow identification of homologous segments between species.

In addition to bone marrow preparations, heart, kidney, and liver tissues were saved for electrophoretic studies (Nelson et al., 1984). Ear biopsies were taken from representative specimens of *Reithrodontomys raviventris* and are maintained as living cell lines suspended by freezing in the Collection of Living Tissues, The Museum, Texas Tech University. Voucher specimens are deposited at The Museum, Texas Tech University, except for the series of *R. raviventris* that are housed at the Museum of Birds and Mammals, San Jose State University.

Specimens examined.—Standard, G- and C-banded karyotypes were examined for all specimens, except where noted. ***Reithrodontomys creper*.**—Costa Rica: San Jose Prov., 2.2 km E La Trinidad de Dota, 2,600 m (1 ♂). ***Reithrodontomys fulvescens*.**—Oklahoma: McIntosh Co., 2 mi E Raiford (1 ♂, 1 ♀), Texas: Jeff Davis Co., 10 mi N Fort Davis (2 ♂♂). 2 ♂♂, 1 ♀ G- and C-banded. ***Reithrodontomys megalotis dychiei*.**—Kansas: Ellis Co., 1 mi W Hays (4 ♂♂). ***Reithrodontomys megalotis longicaudus*.**—California: San Mateo Co.; 10 mi SE Pescadero (2 ♂♂). ***Reithrodontomys megalotis megalotis*.**—Texas: Jeff Davis Co.; 10 mi N

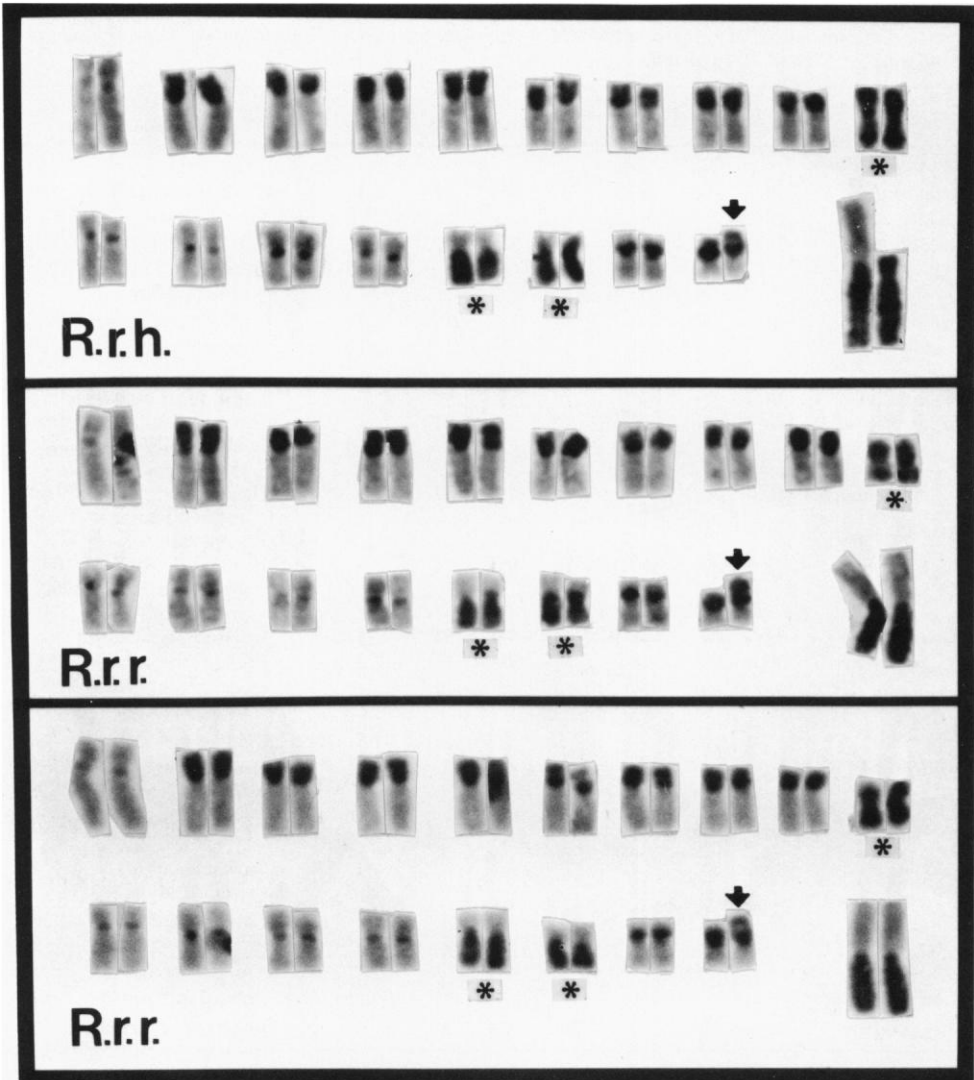


FIG. 2.—C-banded karyotypes of *Reithrodontomys raviventris*. Top: male *R. r. halicoetes*, middle and bottom: two females of *R. r. raviventris*. Asterisks indicate three chromosomal pairs with variable amounts of heterochromatin added. Arrows indicate a heterochromatic polymorphism.

Fort Davis (1 ♂, 1 ♀). *Reithrodontomys megalotis zacatecae*.—Mexico: Durango, 12.4 mi W El Salto (1 ♂). *Reithrodontomys montanus*.—Texas: Culberson Co., 6 mi W Kent (1 ♂), Garza Co.; Southland (1 ♀). *Reithrodontomys raviventris halicoetes*.—California: Sonoma Co., mouth of Tolay Creek (2 ♂♂, 1 ♀). *Reithrodontomys raviventris raviventris*.—California: Alameda Co., Newark slough, 2.5 mi N Newark (1 ♂, 3 ♀♀). *Reithrodontomys sumichrasti*.—Costa Rica. San Jose Prov., 2.2 km E La Trinidad de la Dota, 2,600 m (1 ♂).

RESULTS

A summary of chromosomal data for all species of *Reithrodontomys* that have been examined is presented in Table 1 (adapted after Carleton and Myers, 1979).

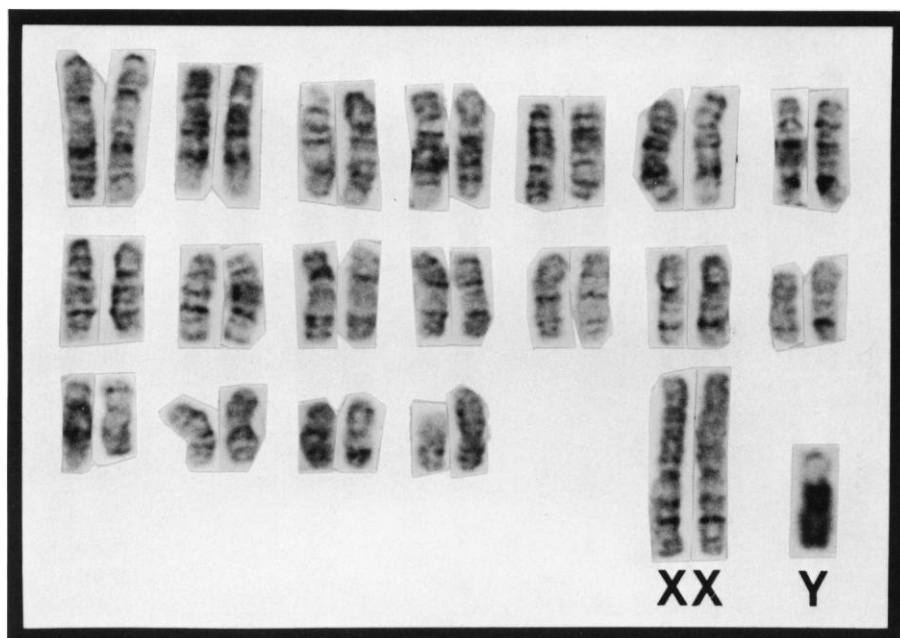


FIG. 3.—G-banded karyotype of a female *Reithrodontomys r. raviventris*. Y chromosome from another individual is also illustrated.

Reithrodontomys raviventris has a $2n = 38$, entirely biarmed karyotype that characterizes both subspecies, *R. r. halicoetes* and *R. r. raviventris*. However, intraspecific variation in chromosomal morphology was documented in an extensive standard karyotypic study (Shellhammer, 1967), with variation in relative arm lengths involving a minimum of three chromosomal pairs. Our examination of G- and C-banded chromosomes confirms these observations and provides further data on variation within the species. The C-banded karyotypes of *R. r. halicoetes* and *R. r. raviventris* are identical with the exception of three chromosomal pairs (Fig. 2). These preparations contain extensive amounts of C-band positive material, presumed to be heterochromatic regions, including the short arms of 10 chromosomal pairs, long arms of two pairs, one entirely heterochromatic pair, and within the sex elements. Variation between the two subspecies appears to involve the amount of heterochromatin in the three chromosomal pairs that possess the greatest amount of C-positive material (see asterisks, Fig. 2). In addition, two specimens of *R. r. halicoetes* and three of *R. r. raviventris* appear to possess a polymorphic heterochromatic addition or deletion in the smallest chromosomal pair (Fig. 2).

G-band comparisons of the two subspecies of *R. raviventris* revealed no variation in banding patterns, although homologies involving the smaller chromosomes are not well resolved (Fig. 3). Comparisons with the G-banded *Reithrodontomys fulvescens* standard (see below) and with C-banded and standard preparations suggest that intraspecific variation in *R. raviventris* is limited to the addition or deletion of heterochromatic material to three pairs of chromosomes.

Standard, G-, and C-banded data for *Reithrodontomys montanus* were presented in Robbins and Baker (1980) and Robbins (1981). The C-banded karyotype possesses extensive amounts of heterochromatin with C-positive material comprising the short arms of seven chromosomal pairs. In addition, the long arms of four biarmed chromosomes are entirely heterochromatic. Comparisons of G-band homologies between *R. montanus* and other *Reithrodontomys* are discussed below.

Reithrodontomys sumichrasti has a $2n = 40$, $FN = 76$ standard karyotype that appears to resemble that of *R. raviventris* and *R. montanus*. However, C-band data show that *R. sumi-*

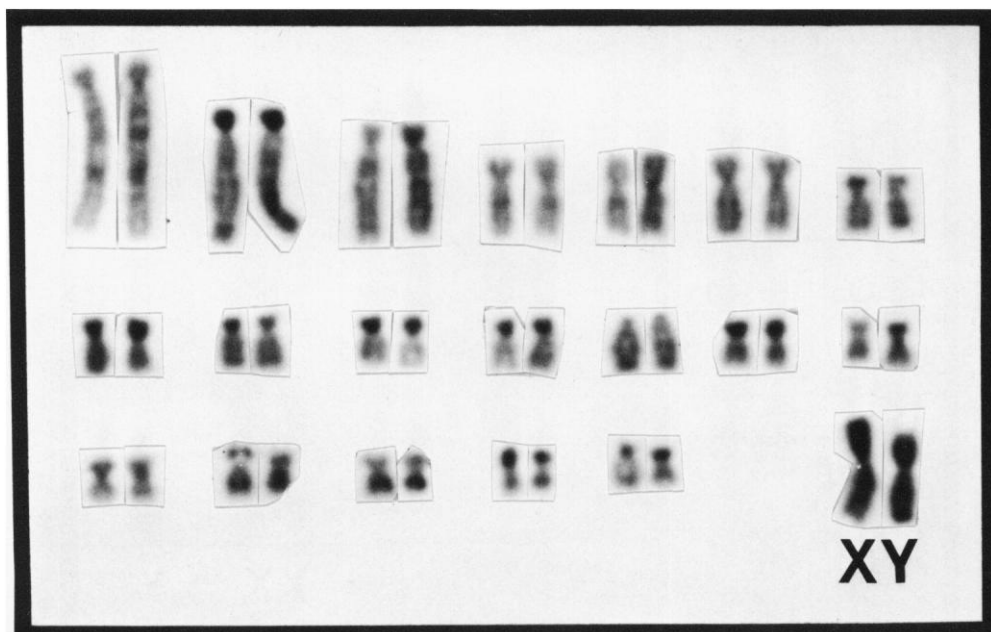


FIG. 4.—C-banded karyotype of *Reithrodontomys sumichrasti*.

chrasti possesses a very different pattern of C-band positive areas. Whereas *R. raviventris* and *R. montanus* have three and four pairs of chromosomes, respectively, that are nearly entirely heterochromatic, heterochromatic material in *R. sumichrasti* is restricted to the short arms of 10 chromosomal pairs (Fig. 4). The G-banded karyotype of *sumichrasti* is presented in Fig. 5.

Standard karyotypes of *Reithrodontomys megalotis* include a $2n = 42$, $FN = 80$ condition in *R. megalotis dychei*, *R. m. longicaudus*, and *R. m. megalotis*, and a highly modified $2n = 50$, $FN = 96$ form in *R. m. zacatecae*. C-band comparisons of all four taxa document that, as in *R. raviventris* and *R. montanus*, many of the short arms of biamed chromosomes are heterochromatic (for example, see fig. 3 of Robbins and Baker, 1980). G-banded karyotypes of the subspecies *dychei*, *longicaudus*, and *megalotis* show extensive homology of the 14 largest chromosomes. Although a number of chromosomal rearrangement events have likely occurred within the smaller chromosomes, there is certainly not a great deal of chromosomal variation among these three taxa. In contrast, *R. megalotis zacatecae* has undergone a considerable amount of chromosomal change; on the basis of standard karyotypic data alone, there must have been a minimum of eight events to account for variation in diploid number. G-band data for *R. m. zacatecae* illustrate that this form shares only seven identifiable chromosomes with *R. megalotis longicaudus*, and of these seven, four pairs are further modified (Figs. 5, 7).

All members of the subgenus *Aporodon* that have been examined (*creper*, *gracilis*, *mexicanus*, *tenuirostris*) have a $2n = 52$, $FN = 50$ karyotype (*R. mexicanus* has $FN = 50-52$ —Rogers et al., 1983). No G- or C-band data have been reported for the subgenus. G- and C-banded karyotypes of *R. creper* and *R. mexicanus* examined in this study showed that, except for the addition of a small pair of chromosomes, these taxa are identical to *R. fulvescens*. Heterochromatic material is restricted to centromeric regions and no rearrangement of G-band patterns was observed. The primitive karyotype for the genus is therefore a mostly acrocentric autosomal complement with G-band sequences as in *R. fulvescens* (Robbins and Baker, 1980). If the primitive diploid number for the genus is 50, then the increase in diploid number to 52 in the subgenus *Aporodon* represents an autapomorphy for the subgenus. Comparisons of G-banded

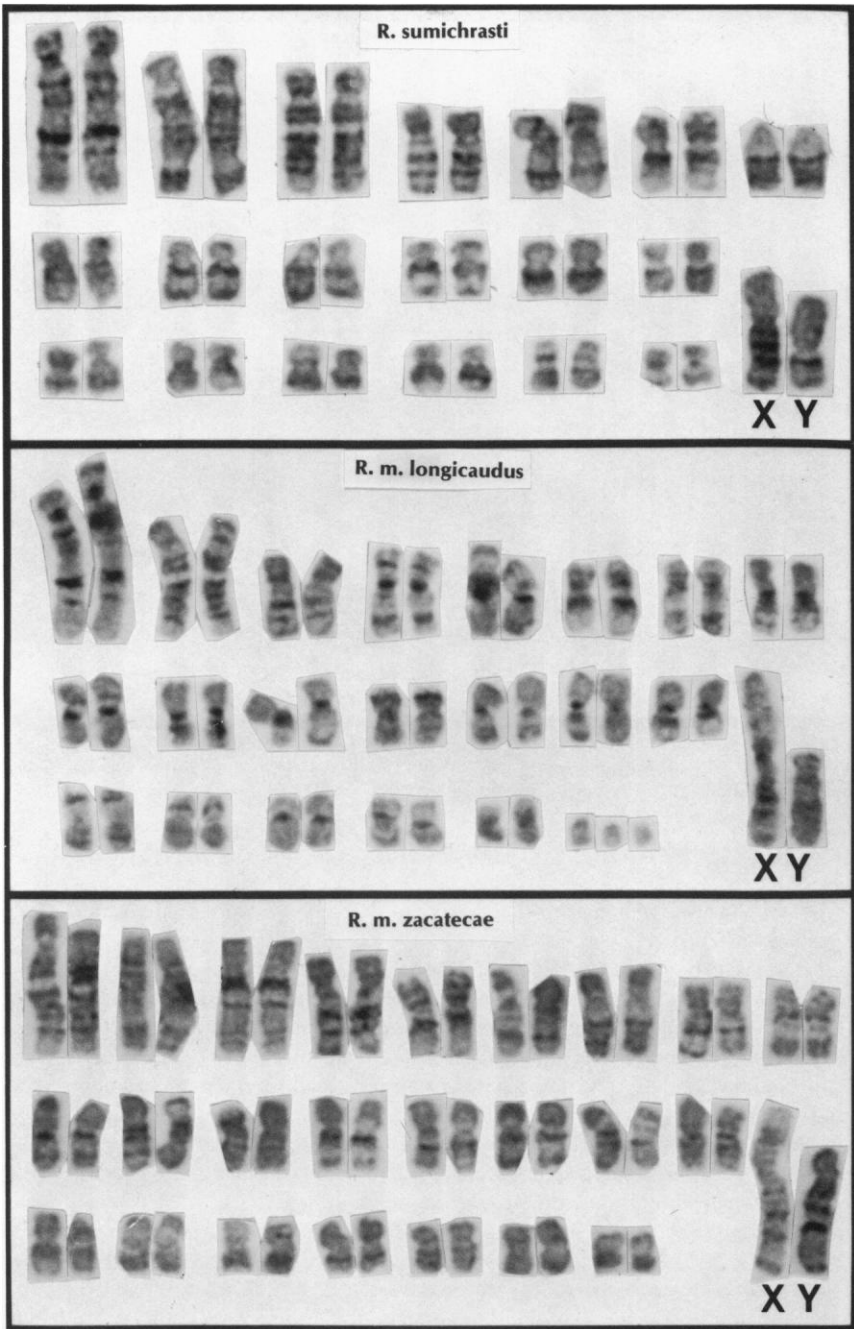


FIG. 5.—G-banded karyotypes of *Reithrodontomys sumichrasti* and *R. megalotis*. The specimen of *R. m. longicaudus* illustrated here had three supernumerary chromosomes.



FIG. 6.—G-band comparisons of haploid complements of *Reithrodontomys fulvescens* (f), *R. montanus* (m), and *R. raviventris* (r). Numbering system follows that for *Peromyscus* (Committee, 1977). Chromosomes A, B, and C do not correspond to any primitive chromosomal arms.

karyotypes among species of *Reithrodontomys* presented below were made using *R. fulvescens* as the primitive standard.

Banding homologies among species of *Reithrodontomys*.—G-band comparisons of taxa of *Reithrodontomys* examined in this study with the *R. fulvescens* standard allowed us to identify specific chromosomes and to hypothesize the type of rearrangement events that have modified chromosomal morphology. Taxa that possess low diploid numbers (*R. raviventris*, *montanus*, *sumichrasti*, and *megalotis*), do not share any completely unrearranged chromosomes with the proposed primitive G-banded karyotype. However, primitive chromosomal arms could be identified in all of these species. Chromosome 3 of the *R. fulvescens* standard was observed to have a euchromatic addition (rearrangement) in all of the low diploid number forms, except *R. raviventris* in which this chromosome could not be identified.

Reithrodontomys raviventris has retained primitive chromosomal arms corresponding to chromosomes 1, 2, 4, 5, 6, 7, 8, 9, and 20 of the *R. fulvescens* standard (Fig. 6). Heterochromatic addition of short arms has occurred in chromosomes 1, 2, 4, 5, 7, 8, and 9 and the addition of euchromatic material was observed in chromosome 20. Chromosome 6 appears to have undergone a pericentric inversion.

Reithrodontomys montanus has maintained these same primitive chromosomal arms (*R. montanus* has a heterochromatic short-arm addition to chromosome 3). As in *R. raviventris*, the primitive chromosomes have been modified by addition of euchromatic or heterochromatic material. *Reithrodontomys montanus* shares with *R. raviventris* heterochromatic short-arm additions to chromosomes 1, 2, 4, 5, 7, 8, and 9 and an euchromatic addition to chromosome 20 (Fig. 6). The amount of heterochromatin added to chromosome 2 in these two species is

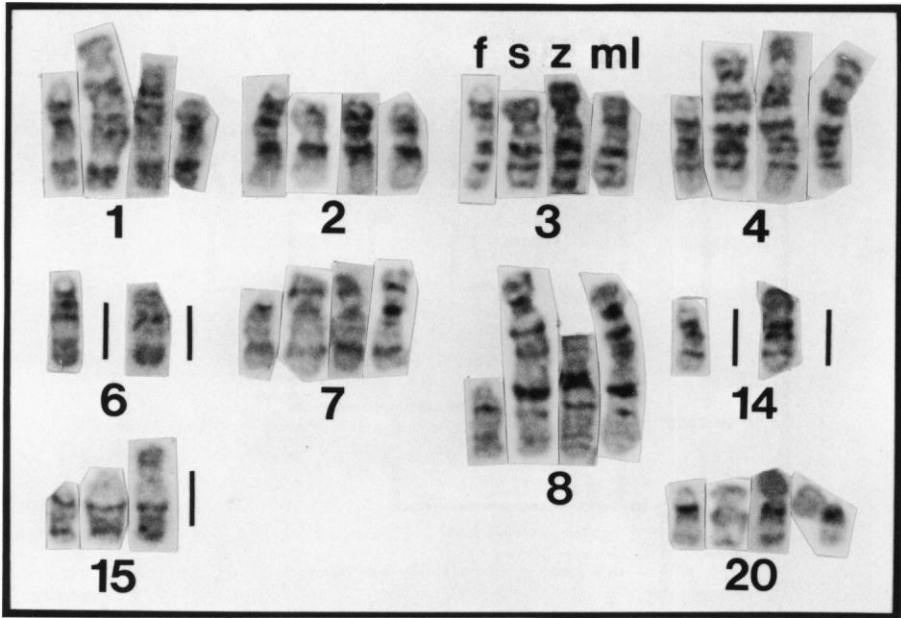


FIG. 7.—G-band comparisons of haploid complements of *Reithrodontomys fulvescens* (f), *R. sumichrasti* (s), *R. megalotis zacatecae* (z), and *R. m. longicaudus* (ml).

significantly different; *R. raviventris* has more material than does *R. montanus* (Fig. 6). Chromosome 6 has undergone two different types of chromosomal rearrangement in *R. raviventris* and *montanus*. In *R. raviventris*, chromosome 6 has been modified by a presumed pericentric inversion, whereas in *R. montanus* it differs from the primitive acrocentric condition by an addition of heterochromatic short-arms. Heterochromatic short-arms occur in four additional pairs of the larger *R. montanus* chromosomes (see Robbins and Baker, 1980: fig. 4), however, we could not positively identify them by number. Three entirely euchromatic chromosomes were found to be identical in *R. raviventris* and *R. montanus* that do not correspond to any primitive chromosomal arms. These chromosomes, labelled A, B, and C in Fig. 6, must have undergone several unique chromosomal rearrangement events and thus represent synapomorphies for these two taxa. Our G-band data indicate that, except for the retained primitive chromosomal arms and the euchromatic addition to chromosome 3, *R. montanus* and *R. raviventris* share no uniquely derived chromosomes with any of the other low diploid number taxa of *Reithrodontomys*.

Taxa of *Reithrodontomys megalotis* (four subspecies) and *R. sumichrasti* share several unique chromosomal modifications that indicate that they be considered together in a G-band analysis (Fig. 7). *Reithrodontomys megalotis longicaudus* has retained primitive chromosomal arms in chromosomes 1, 2, 3, 4, 7, 8, and 20. Addition of chromosomal material in chromosome 20 is heterochromatic, whereas all other chromosomes listed above have been modified from the primitive condition by euchromatic rearrangements. Chromosome 1 has an apparent pericentric inversion; each of the others has a large amount of euchromatic material added that probably was involved in several rearrangement events.

Primitive chromosomal arms identified in *R. megalotis zacatecae* include chromosomes 1, 2, 3, 4, 6, 7, 8, 14, 15, and 20 (Fig. 7). *R. m. zacatecae* shares with *R. m. longicaudus* euchromatic additions to chromosomes 2, 3, 4, 7, and 8 and a heterochromatic addition to chromosome 20. A comparison of *R. m. zacatecae* with *R. sumichrasti* reveals a shared euchromatic addition to chromosome 1 and heterochromatic short-arm additions to chromosomes 15 and 20 (differing

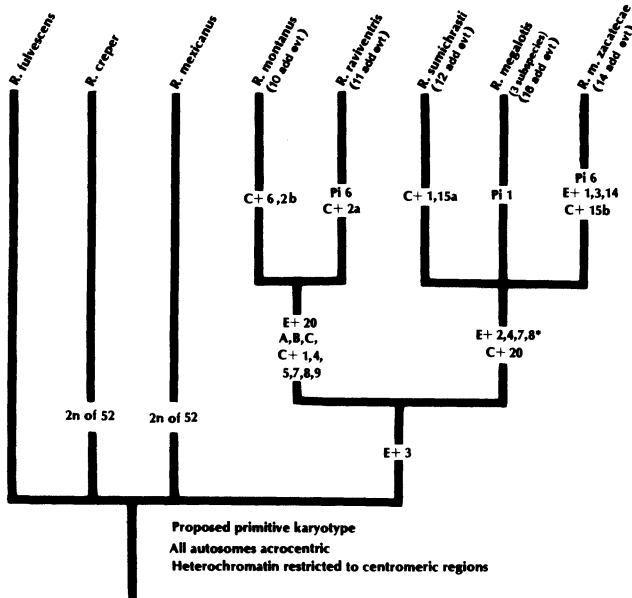


FIG. 8.—Cladogram of *Reithrodontomys* species based on G- and C-banded chromosomal data. Chromosomal rearrangements include heterochromatic additions (C+), euchromatic additions (E+), pericentric inversions (Pi), and the presence of derived chromosomes A, B, and C. Numbering system follows that for *Peromyscus*. Chromosome 8 (asterisk) has additional rearrangements within the *megalotis* group (see Results). Chromosomes 2 and 15 contain variation in amount of heterochromatin found in some species (indicated as a and b). The minimum number of additional chromosomal events needed to explain variation in the karyotype of low diploid number species from the primitive is given in parentheses.

amounts of heterochromatin have been incorporated into chromosome 15). *R. m. zacatecae* possesses numerous uniquely derived rearrangements including additional euchromatic material in chromosomes 3 and 14, a presumed pericentric inversion in 6, and additional heterochromatic material in chromosome 15. The banding patterns in chromosome 8 can be interpreted in two ways. Chromosome 8 of *R. m. longicaudus* and *R. sumichrasti* may represent the shared derived condition for the entire *megalotis* group (including *R. m. zacatecae*) that is further modified in *R. m. zacatecae* as an autapomorphic loss of euchromatic material (Fig. 7). Alternatively, chromosome 8 of *R. m. zacatecae* may represent the shared derived condition for the *megalotis* group, with a synapomorphic euchromatic addition to that chromosome in *R. m. longicaudus* and *R. sumichrasti*. In both interpretations, there is a shared derived condition that unites the species *R. megalotis* and *R. sumichrasti*. In addition, the first interpretation of chromosome 8 identifies an autapomorphy for *R. m. zacatecae*. In contrast, the second alternative predicts a closer evolutionary relationship between *R. m. longicaudus* and *R. sumichrasti*. Parsimony does not indicate which alternative is more likely, however, we note that in both cases *R. m. zacatecae* is markedly different from other taxa of *R. megalotis*.

DISCUSSION

The intraspecific chromosomal variation found in the karyotype of *Reithrodontomys raviventris* was identified by G- and C-band data. No variation was found in the G-banding pattern of the euchromatic segments. The variation in centromere placement that distinguishes the subspecies *halicoetes* from *raviventris* (Shellhammer, 1967) is the result of additional heterochromatic material being present in three chromosomal pairs of *R. r. halicoetes*. The polymor-

phism in the amount of heterochromatin in the smallest pair of autosomes was found within our sample from both subspecies.

The evolutionary origin and phylogenetic relationship of *R. raviventris* to other *Reithrodontomys* has been investigated by Hooper (1944, 1952), Fisler (1965), and Shellhammer (1967). These workers proposed that *R. raviventris* was most closely related to *R. megalotis*. In this study, we have presented G- and C-band data that clearly provide an alternative hypothesis: namely, that *R. raviventris* shares a more recent common ancestor with *R. montanus*. A cladistical analysis of the banding data for the genus *Reithrodontomys* illustrates several major points (Fig. 8).

(1) The dichotomy between low and high diploid number taxa is documented with a chromosomal synapomorphy (euchromatic addition to chromosome 3, Fig. 8). This event indicates a common ancestry for the low diploid number forms and presents the first resolution of phylogenetic relationships within that group (Robbins and Baker, 1980; Arnold et al., 1983).

(2) Within the low diploid number forms, two monophyletic groups are recognized on the basis of euchromatic and heterochromatic rearrangement events. *R. raviventris* and *R. montanus* form one group supported by 10 synapomorphous chromosomal rearrangement events (four euchromatic and six heterochromatic). A second group consists of the species *R. sumichrasti* and *R. megalotis* (four subspecies examined), which share five synapomorphies. The distinct nature of these two clades is further illustrated by the types of rearrangements that characterize them. In the *montanus-raviventris* clade, chromosomes 1, 2, 4, 5, 7, 8, and 9 have undergone heterochromatic additions, whereas, in the *sumichrasti-megalotis* group, chromosomes 2, 4, 7, and 8 have been modified by euchromatic rearrangements. Likewise, chromosome 20 has a euchromatic rearrangement in the *montanus-raviventris* group, but has had a heterochromatic addition in *sumichrasti-megalotis*.

(3) Two cases of convergence are found within the banding data, a heterochromatic addition to chromosome 1 (in *R. montanus*, *raviventris*, and *sumichrasti*) and a presumed pericentric inversion in 6 (*R. raviventris* and *R. m. zacatecae*). Multiple inversions in chromosome 6 have been noted in *Peromyscus* (Robbins and Baker, 1981; Rogers, 1983). With the resolution of G-bands available to us it is impossible to distinguish the inversion in chromosome 6 in *Reithrodontomys* from that found in some species of *Peromyscus*.

(4) It was surprising to find that *R. raviventris* has a well-supported phylogenetic relationship with *R. montanus* and not with *R. megalotis* given that earlier studies had correlated morphological, physiological, and behavioral characteristics in proposing a *raviventris-megalotis* relationship (Hooper, 1944; Fisler, 1965). However, a re-examination of these studies reveals that *R. raviventris* has not been consistently compared with species of *Reithrodontomys* occurring outside of the San Francisco Bay area. Furthermore, many of the species of *Reithrodontomys* are not easily distinguished from one another on morphological characteristics. For example, in his classic revision of taxa occurring in Latin America, Hooper (1959:49) summarized his comparisons of *R. megalotis* by stating, "Within the subgenus *Reithrodontomys*, *raviventris* and *montanus* are perhaps most similar to *megalotis* . . . I find no absolute characters that will distinguish *megalotis* and *montanus*."

The evolutionary scenario of adaptation and speciation of *R. raviventris* from a *megalotis*-like ancestor seems to be plausible only if comparisons are restricted to harvest mice inhabiting the San Francisco Bay area. Our G- and C-band results present evidence that strongly suggests that the evolutionary relationship of *R. raviventris* be reconsidered. We propose that these new data support a *raviventris-montanus* relationship and that future studies involve a comparison of these taxa.

(5) A considerable amount of chromosomal variation has been reported for geographic samples of *R. megalotis* (Blanks and Shellhammer, 1968; Shellhammer, 1969; Engstrom et al., 1981). Three of the subspecies of *R. megalotis* examined in this study (*R. m. dychei*, *R. m. longicaudus*, *R. m. megalotis*), had identical G- and C-banded karyotypes, whereas *R. m. zacatecae* possessed highly modified G- and C-banded karyotypes. All *R. megalotis* examined share the five syna-

pomorphous rearrangements with *R. sumichrasti* that distinguish the *sumichrasti-megalotis* clade. However, no synapomorphies characterize the species *R. megalotis* when *R. m. zacatecae* is included. On the basis of our banding data, five unique (autapomorphic) chromosomal rearrangements have occurred in *R. m. zacatecae* compared with other *R. megalotis*. Chromosome 8 may represent either an additional autapomorphic character for *R. m. zacatecae*, or a synapomorphy for *R. sumichrasti* and *R. m. longicaudus* (see Results). In addition, to account for differences in diploid number between *R. m. zacatecae* and other *R. megalotis*, an additional eight rearrangements involving the smaller chromosomes must have occurred. On the basis of morphological data, Hooper (1952) argued that the evolutionary relationship of *R. m. zacatecae* to other forms of *R. megalotis* was enigmatic, and that with additional data, *R. m. zacatecae* may well represent a species separate and distinct from *R. megalotis*. We interpret our chromosomal data as supporting Hooper's suggestion that *zacatecae* probably deserves specific status.

The species *R. megalotis* contains 16 named subspecies, many of which have restricted geographic distributions (Hooper, 1952; Hall, 1981). Hooper (1952) noted that several of these are possibly specifically distinct forms (e.g., *R. m. zacatecae*) and that future studies should investigate this problem. G- and C-band studies on geographic samples of *R. megalotis* promise to be an important source of data for studies on the evolution and systematic relationships of these harvest mice.

(6) Previous G-band studies (Robbins and Baker, 1980) failed to find banding homologies between the low diploid number species *R. montanus* and *R. megalotis* and the proposed primitive karyotype for the genus, leading Robbins and Baker (1981) to suggest that these forms represent a radical departure from the conservation of euchromatic segments that characterizes peromyscine rodents. Arnold et al. (1983) proposed that these species represent an example of karyotypic megaevolution (Baker and Bickham, 1980). The present study documents that some (7–10 autosomal pairs) of the primitive acrocentric arm sequences are present and unaltered in the larger chromosomes of the taxa with low diploid numbers. Improved identification of banding sequences allowed an estimate of amount and type of chromosomal rearrangement and provided resolution of phylogenetic relationships among the low diploid number forms. In this example, data on differentially-stained chromosomes have provided a magnitude of information that could not have been afforded by standard karyotypic preparations. Banding studies of pteropodid and phyllostomid bats (Patton and Baker, 1978; Baker and Bickham, 1980; Haiduk et al., 1981; Haiduk and Baker, 1982) and oryzomyine rodents (Baker et al., 1983) are additional examples that suggest caution in interpreting chromosomal evolution based on standard chromosomal morphology.

In a recent paper, Warner (1983) questioned the systematic value of cladistically analyzed G-band data from taxa with extensive repatterning of the karyotype and suggested that in such cases morphological and biochemical data are more informative in assessing phylogenetic relationships at specific and generic taxonomic levels. In the case of *Reithrodontomys*, it is clear that classical morphology and standard karyology could not resolve the systematic problem of the relationship of *R. raviventris*. The evolutionary origin and relationships of *R. raviventris* would not have been revealed if the earlier studies had been accepted uncritically and biochemical data were unavailable. We conclude that there are cases where G- and C-band studies of taxa with extensively repatterned karyotypes will provide the critical data set for assessing phylogenetic relationships and solving systematic problems.

ACKNOWLEDGMENTS

We thank M. D. Carleton, K. Nelson, F. B. Stangl, Jr., and an anonymous reviewer for critically reading the manuscript and K. Nelson, M. Newcomer, J. L. Patton, D. S. Rogers, F. B. Stangl, Jr., and D. Tolliver for aid in collecting specimens. Cornelio Sanchez-H. assisted in obtaining permits to collect in Mexico. Collection of *R. raviventris* was conducted under Federal Permit PRT 2-9368 issued to H. S. Shellhammer. We thank the Federal Wildlife Permit Office, Washington, D.C., for the prompt issue of that permit. This research was supported in part by the Institute of Museum Research, and NSF Grant DEB 80-04293 to R. J. Baker.

LITERATURE CITED

- ARNOLD, M. L., L. W. ROBBINS, R. K. CHESSER, AND J. C. PATTON. 1983. Phylogenetic relationships among six species of *Reithrodontomys*. *J. Mamm.*, 64:128-132.
- BAKER, R. J., AND J. W. BICKHAM. 1980. Karyotypic evolution in bats: evidence of extensive and conservative chromosomal evolution in closely related taxa. *Syst. Zool.*, 29:239-253.
- BAKER, R. J., B. F. KOOP, AND M. W. HAIDUK. 1983. Resolving systematic relationships with G-bands: a study of five genera of South American cricetine rodents. *Syst. Zool.*, 32:403-416.
- BLANKS, G. A., AND H. S. SHELLHAMMER. 1968. Chromosome polymorphism in California populations of harvest mice. *J. Mamm.*, 49:726-731.
- CARLETON, M. D., AND P. MYERS. 1979. Karyotypes of some harvest mice, genus *Reithrodontomys*. *J. Mamm.*, 60:307-313.
- COMMITTEE FOR STANDARDIZATION OF CHROMOSOMES OF *PEROMYSCUS*. 1977. Standardized karyotype of deer mice, *Peromyscus* (Rodentia). *Cytogenet. Cell Genet.*, 19:38-43.
- DIXON, J. 1908. A new harvest mouse from the salt marshes of San Francisco Bay, California. *Proc. Biol. Soc. Washington*, 21:197-198.
- . 1909. A new harvest mouse from Petaluma, California. *Univ. California Publ. Zool.*, 5:271-273.
- ENGSTROM, M. D., R. C. DOWLER, D. S. ROGERS, D. J. SCHMIDLY, AND J. W. BICKHAM. 1981. Chromosomal variation within four species of harvest mice (*Reithrodontomys*). *J. Mamm.*, 62:159-164.
- FISLER, G. F. 1965. Adaptations and speciation in harvest mice of the marshes of San Francisco Bay. *Univ. California Publ. Zool.*, 77:1-108.
- HAIDUK, M. W., R. J. BAKER, L. W. ROBBINS, AND D. A. SCHLITTER. 1981. Chromosomal evolution in African megachiropterans: G- and C-band assessment of the magnitude of change in similar standard karyotypes. *Cytogenet. Cell Genet.*, 29:221-232.
- HAIDUK, M. W., AND R. J. BAKER. 1982. Cladistical analysis of G-banded chromosomes of nectar feeding bats (Glossophaginae: Phyllostomidae). *Syst. Zool.*, 31:252-265.
- HALL, E. R. 1981. The mammals of North America. Second edition. John Wiley and Sons, New York, 2:601-1181 + 90.
- HOOPER, E. T. 1944. San Francisco Bay as a factor influencing speciation in rodents. *Misc. Publ. Mus. Zool., Univ. Michigan*, 59:1-89.
- . 1952. A systematic revision of the harvest mice (genus *Reithrodontomys*) of Latin America. *Misc. Publ. Mus. Zool., Univ. Michigan*, 77:1-255.
- HSU, T. C., AND K. BENIRSCHKE. 1968. An atlas of mammalian chromosomes. Springer-Verlag, New York, 2: Folio 67.
- LEE, M. R., AND F. F. B. ELDER. 1980. Yeast stimulation of bone marrow mitosis for cytogenetic investigations. *Cytogenet. Cell Genet.*, 26:36-40.
- NELSON, K., R. J. BAKER, H. S. SHELLHAMMER, AND R. K. CHESSER. 1984. Test of alternative hypotheses concerning the origin of *Reithrodontomys raviventris*: genetic analysis. *J. Mamm.*, 65:668-673.
- PATTON, J. C., AND R. J. BAKER. 1978. Chromosomal homology and evolution of phyllostomatoid bats. *Syst. Zool.*, 27:449-462.
- ROBBINS, L. W. 1981. Sex chromosome polymorphisms in *Reithrodontomys montanus* (Rodentia: Cricetidae). *Southwestern Nat.*, 26:201-202.
- ROBBINS, L. W., AND R. J. BAKER. 1980. G- and C-band studies on the primitive karyotype for *Reithrodontomys*. *J. Mamm.*, 61:708-714.
- . 1981. An assessment of the nature of chromosomal rearrangements in 18 species of *Peromyscus* (Rodentia: Cricetidae). *Cytogenet. Cell Genet.*, 31:194-202.
- ROGERS, D. S. 1983. Phylogenetic affinities of *Peromyscus* (*Megadontomys*) *thomasi*: evidence from differentially stained chromosomes. *J. Mamm.*, 64: 617-623.
- ROGERS, D. S., E. J. HESKE, AND D. A. GOOD. 1983. Karyotypes and a range extension of *Reithrodontomys* (Cricetidae: subgenus *Aporodon*) from Mexico. *Southwestern Nat.*, 28:372-374.
- SEABRIGHT, M. 1971. A rapid banding technique for human chromosomes. *Lancet*, 2:971-972.
- SHELLHAMMER, H. S. 1967. Cytotaxonomic studies of the harvest mice of the San Francisco Bay region. *J. Mamm.*, 48:549-556.
- . 1969. Supernumerary chromosomes of the harvest mouse, *Reithrodontomys megalotis*. *Chromosoma* (Berlin), 27:102-108.
- STEFOS, K., AND F. E. ARRIGHI. 1971. Heterochromatic nature of the W chromosome in birds. *Experimental Cell Res.*, 68:228-231.
- WARNER, R. M. 1983. Karyotypic megaevolution and phylogenetic analysis: New World nectar-feeding bats revisited. *Syst. Zool.*, 32:279-282.

Submitted 29 November 1983. Accepted 25 April 1984.