

Robt J. Baker

Karyotypic analyses of the genus *Neotoma* (Cricetidae, Rodentia)¹

R. J. BAKER and J. T. MASCARELLO

Section of Cell Biology, Department of Biology, The University of Texas M. D. Anderson Hospital and Tumor Institute at Houston

Abstract. Examination of 154 specimens representing nine species of the genus *Neotoma* revealed diploid numbers of 38, 52, 54 and 56. Six species had the diploid number of 52. Extensive polymorphism was found in two species, *N. lepida* and *N. micropus*. Chromosomal variation in the subgenus *Neotoma* was primarily in Fundamental Number and not in diploid number.

Introduction

To understand trends in mammalian chromosomal evolution, including the sex-determining mechanisms and other cytogenetic phenomena, it is desirable to explore many diverse groups. A practical method is to first survey the somatic chromosomes of a given group and later apply other techniques to specific problems. The data presented here are the results of one such survey, the somatic chromosomes of the woodrats (genus *Neotoma*). In some cases our data already indicate that more information, such as meiotic behavior and DNA replication patterns, is needed. In the present report, however, only karyotypic analysis of somatic chromosomes are presented.

Trends in chromosomal evolution within several groups of mammals have recently been analyzed. The best-studied genus of cricetine rodents is *Peromyscus* (HSU and ARRIGHI, 1968). In *Peromyscus*, 19 species have the same diploid number (48), but the Fundamental

¹ Supported in part by a grant from the American Philosophical Society awarded to the senior author and National Science Foundation research grant GB-6657 awarded to Dr. T. C. Hsu.

Request reprints from: Dr. ROBERT J. BAKER, Department of Biological Sciences, Texas Technological College, Lubbock, TX 79409 (USA).

Number (FN) varies from 56 to 96. Not all members of this subfamily follow this pattern of evolution for a constant number of centromeres. For example, in *Reithrodontomys* the diploid number is found to vary from 38 to 50 (MATTHEY, 1961; SHELLHAMMER, 1967; HSU and BENIRSCHKE, 1968). In *Sigmodon*, the diploid number varies from 22 to 50 (ZIMMERMAN and LEE, 1968; HSU, in press).

Materials and methods

All specimens were collected from natural populations. Most preparations for karyotypic analysis were prepared from bone marrows using a modification of the blaze-dry method described by PATTON (1967). Some slides were made from cells of lung cultures, using the acetic-orcein, squash technique. In a few cases both materials from the same animal were used.

Results

We have examined 154 specimens representing nine species of the genus *Neotoma*. Diploid numbers found within the genus are 38, 52, 54 and 56. Six species have the diploid number of 52. Cytological data are summarized in the table, and a brief description of the chromosomes follows. Locality data and sample sizes are given in the addendum.

Chromosomal data for nine species of Neotoma

| | 2N | FN | X | Y | Specimens examined | |
|--------------------------|----|-------|---------|---------|--------------------|----|
| | | | | | ♂ | ♀ |
| Subgenus <i>Neotoma</i> | | | | | | |
| <i>N. floridana</i> | 52 | 56 | SM | ST | 3 | 5 |
| <i>N. micropus</i> | 52 | 50-56 | SM or A | ST or A | 18 | 18 |
| <i>N. albigula</i> | 52 | 58 | M or ST | ST | 28 | 32 |
| <i>N. lepida</i> | 52 | 60-66 | M? | ? | 10 | 9 |
| <i>N. stephensi</i> | 52 | 58 | ? | ? | 0 | 3 |
| <i>N. mexicana</i> | 52 | 50 | ST | ST | 6 | 10 |
| <i>N. fuscipes</i> | 56 | 82 | ? | ? | 2 | 3 |
| Subgenus <i>Teonoma</i> | | | | | | |
| <i>N. cinerea</i> | 54 | 60 | M | ST | 2 | 0 |
| Subgenus <i>Teanopus</i> | | | | | | |
| <i>N. phenax</i> | 38 | 50 | SM | SM | 2 | 3 |

A = acrocentric; M = metacentric; SM = submetacentric; ST = subtelocentric.

Neotoma floridana (ORD). Figure 1; $2N = 52$, $FN = 56$. The autosomes consist of one large and two small pairs of distinctly banded elements plus a series of graded acrocentrics ranging in size from large to small. The X is a large submetacentric and the Y, a medium-sized subtelocentric. The chromosomes of the specimen from Florida were indistinguishable from those of the Oklahoma specimens. The chromosomes of this species were first described by CROSS (1931) and later by MATTHEY (1953).

Neotoma micropus BAIRD. Figure 2; $2N = 52$, $FN = 50$ to 56 . The most common and widely distributed karyotype for this species is very similar to that described for *N. floridana* (compare Figs. 1



Fig. 1. Representative karyotype of a male *Neotoma floridana* from the vicinity of Stillwater, Oklahoma.

Fig. 2. Representative karyotype of a male *Neotoma micropus* from Kermit, Winkler Co., Texas.

and 2). In some populations the number of large biarmed elements varies from one to four, but the diploid number remains constant at 52. This variation will be discussed in detail elsewhere.

All males examined by us have a medium-sized submetacentric Y. Specimens of this species studied by HSU and BENIRSCHKE (1968) were reported to have a small acrocentric Y. However, there is in the karyotype of the male a medium-sized submetacentric which may be the Y (T. C. HSU, personal communication).

Neotoma albigula HARTLEY. Figure 3; $2N = 52$, $FN = 58$. The autosomes of this species contain two large pairs and two small pairs



Fig. 3. Representative karyotype of a male *Neotoma albigula* from the town of Vicam, Sonora, Mexico.

Fig. 4. Representative karyotype of a male *Neotoma lepida* from Endira, Baja California, Mexico.

of biarmed chromosomes. The X is a large submetacentric. The Y is usually large and subtelocentric, but in two specimens from Texas, it is acrocentric. In a specimen from central Sonora the Y is as large as the X.

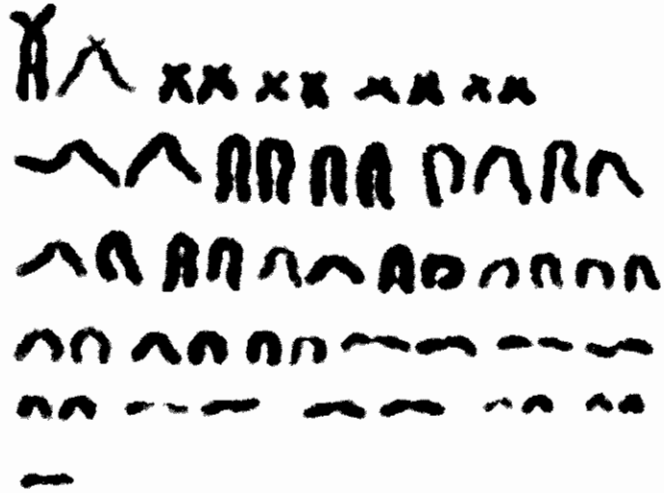
Five subspecies were sampled, including the melanistic *N. a. melas* from New Mexico and *N. a. seri* from Isla Tiburon, and all specimens have four large biarmed autosomes. In some specimens these elements do not seem to match. For example, if the X of the specimen from Vicam, Sonora, is submetacentric (Fig. 3), as in other specimens, then two of the autosomes do not match in centromere placement.

Neotoma lepida THOMAS. Figure 4; $2N = 52$, $FN = 60$ to 66 . Our data reveal no karyotype which can be termed 'typical' for the species. All specimens have a diploid number of 52, but where larger sample sizes are available from a general locality, several different combinations of biarmed as opposed to unarmed elements appear. Patterns of population variations also vary with geographic localities. No pair of chromosomes was consistently heteromorphic in the males; therefore, the sex chromosomes cannot be positively determined by morphology alone. In many male specimens the largest biarmed element appears to be one of the heteromorphic elements, and it may be the X (Fig. 4). The greatest number of large- to medium-sized biarmed elements is 12. This system will be discussed in detail elsewhere.

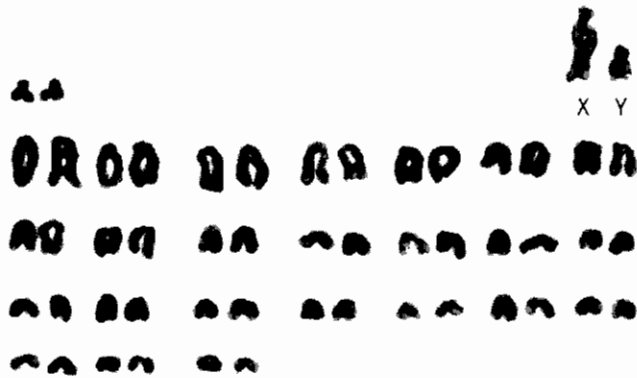
Neotoma stephensi GOLDMAN. Figure 5; $2N = 52$ or 53 , $FN = (58)$. This is the only species in which a specimen was found having an odd number of chromosomes in its somatic cells (Fig. 5). The three females examined have four pairs of medium to small biarmed elements and one pair of large subtelocentric elements. With only females examined, the sex chromosomes could not be determined.

Neotoma mexicana BAIRD. Figure 6; $2N = 52$, $FN = 54$. Except for a pair of small biarmed chromosomes, the autosomes are acrocentric. The X is a large subtelocentric and the Y, a medium-sized subtelocentric. Several of the populations sampled (representing five different subspecies) are isolated on mountain tops. This would seem to be an ideal situation for a variety of chromosomal types to be found within a species, but no chromosomal variation was found.

Neotoma fuscipes BAIRD. Figure 7; $2N = 56$, $FN = 82$. Compared to the chromosomes of the other species examined, all chromosomes are medium- to small-sized, with at least 15 pairs of biarmed elements. No distinctly heteromorphic elements were found in males. It was difficult to obtain spreads of this species that were not overcontracted.



5

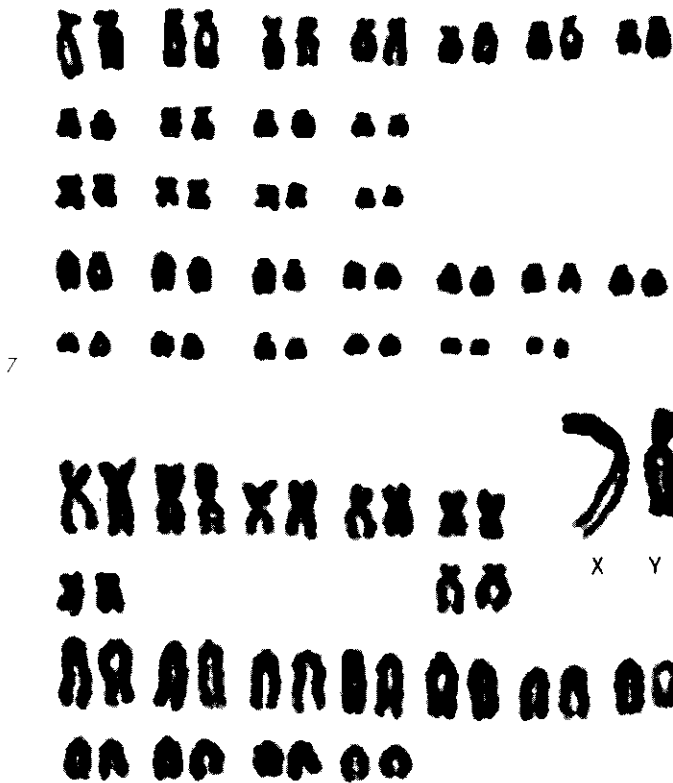


6

Fig. 5. Representative karyotype of a female *Neotoma stephensi* from the Hualapai Mtns, Mohave Co., Arizona (note diploid number of 53).

Fig. 6. Representative karyotype of a male *Neotoma mexicana* from the Graham Mtns, Graham Co., Arizona.

In one 200-g specimen, injected with 0.20 ml of a 0.05% solution of Velban and sacrificed after one hour, approximately 90% of the spreads obtained were overcontracted. In diploid number, Fundamental Number and general morphology of the chromosomes, this species is outstanding when compared to other species of *Neotoma* examined.



7

8

Fig. 7. Representative karyotype of a female *Neotoma fuscipes* from Irvine, California.

Fig. 8. Representative karyotype of a male *Neotoma phenax* from the northern border of Sinaloa, Mexico, on Highway 15.

Neotoma cinerea (ORD). $2N = 54$, $FN = 60$. The autosomes consist of one pair of medium submetelocentric and three pairs of small biarmed elements plus a graded series of 44 acrocentrics. The X is a large submetelocentric and the Y, a medium-sized submetelocentric.

Neotoma phenax (MERRIAM). Figure 8; $2N = 38$, $FN = 50$. The autosomes consist of six pairs of large to medium submetelocentric, one pair of medium-sized submetelocentric, and 11 pairs of large- to medium-sized acrocentric chromosomes. The X is a very large submetelocentric and the Y, a large submetelocentric. The X and Y are larger than any of the autosomes.

Discussion

In six of the seven species studied in the subgenus *Neotoma*, the diploid number is stabilized at 52. Even in species which have polymorphic populations, the diploid number remains unchanged. Only one of the 141 specimens examined from these six species had a diploid number other than 52. This aberrant case was an individual of *N. stephensi*, which had a chromosomal constitution of $2N + 1$. This non-Robertsonian variation is similar to that reported for a closely related genus, *Peromyscus* (HSU and ARRIGHI, 1968), except in that genus the common diploid number is 48.

One species, *N. fuscipes* of the subgenus *Neotoma*, has a diploid number of 56. Its FN is 82. Neither the diploid number nor the FN of this species, when compared with those of other species in subgenus *Neotoma*, can be explained by the Robertsonian process.

The two other species studied, *N. cinerea* (subgenus *Teonoma*) and *N. phenax* (subgenus *Teanopus*) are each placed in a separate subgenus. Available karyological data reflect the divergence of these two respective lines of evolution from that of the subgenus *Neotoma* by different diploid numbers of 54 and 38, respectively.

The *N. fuscipes* of the subgenus *Neotoma*, whose diploid number deviates from the common number of 52, was once accorded subgeneric rank (subgenus *Homodontomys*) on the basis of dental characters (HALL and KELSON, 1959). It was later placed as a species of subgenus *Neotoma* in a study of comparative bacula structure (BURT and BARKALOW, 1942). If chromosomal data only were used to indicate degree of divergence, the recognition of subgenus *Homodontomys* would be valid. However, the trends discussed above may be strongly altered by karyotypic data from the species of *Neotoma* which have not been studied. Even if these trends are supported by additional karyotypic data, the subgeneric rank should be constructed on a combination of characters rather than divergence in a single character. These data do suggest that a re-evaluation of the status of *N. fuscipes* in relationship to other members of the subgenus *Neotoma* is in order.

Over half of the autosomes of all *Neotoma* studied (except *N. fuscipes*) are acrocentric. In *N. mexicana*, all but one pair are acrocentric. Several genera of cricetid rodents contain one or more species with a diploid number of 48, 50 or 52, their karyotypes containing a large number of acrocentrics. For example, *Baiomys taylori* ($2N = 48$) had all autosomes acrocentric (HSU and BENIRSCHKE, 1967);

Peromyscus boylei ($2N = 48$), 20 pairs of acrocentrics (HSU and ARRIGHI, 1968); *Onchomys torridus* ($2N = 48$), 9 pairs of acrocentrics (HSU and BENIRSCHKE, 1968); *Reithrodontomys fulvescens* ($2N = 50$), all autosomes acrocentric (HSU and BENIRSCHKE, 1967); *Sigmodon hispidus* ($2N = 52$), 24 pairs of acrocentrics (HSU and BENIRSCHKE, 1968); and *Microtus agrestis* ($2N = 50$), all autosomes acrocentric (MATTHEY, 1950). These data suggest that the primitive karyotype of cricetid rodents had a diploid number in the range of 48 to 50 and the autosomes were composed largely of acrocentrics.

Our preliminary data indicate that the chromosomes of genus *Neotoma* are of great interest to cytologists and evolutionists besides their value in taxonomic studies. Probably the most startling phenomenon is the variability in Fundamental Number within a species. In *N. lepida* and *N. micropus*, polymorphism not only exists among populations but also within a population. Such cases of polymorphism were so drastic and obvious that there was no room for doubt. For example, a female *N. micropus* showed three large biarmed chromosomes. These karyotypes even obscure the determination of the sex elements by morphology alone.

As mentioned, this investigation was initiated as a karyotype survey of the genus *Neotoma*, so that for simplicity and for rapidity, no provision was taken for other phases of investigation. Our results now demand the following approach:

1. Analysis of meiotic behavior of individuals with obvious polymorphism.
2. Autoradiography with tritiated thymidine to study DNA replication patterns for possible identification of sex chromosomes.
3. Analysis of selected populations to determine the extent of variability.
4. Analysis of offspring populations from mating of karyologically different individuals.
5. Introduction of individuals to wild populations different in chromosomal constitutions.

Some of these experiments are now in progress, using ear fragments for culture to save the live specimens.

Acknowledgements

We wish to acknowledge the assistance and advice of Dr. E. L. COCKRUM of the University of Arizona, under whose direction this project was started. Dr. T. C.

Hsu, M. D. Anderson Hospital and Tumor Institute at Houston, has been of continual academic assistance and has permitted us to use in our study specimens examined by him. Mr. GOTTHARD SCHUSSLER, of the University of California at Irvine, collected the California specimens. Other investigators who collected specimens for this study were Mr. JAMES L. PATTON, Dr. GARY BATEMAN, Dr. LEE CHRISTIANSON and Mr. DUKE DOSTER, then of the University of Arizona, Dr. ROSS DINGMAN, of the University of San Diego for Men, Dr. LARRY BROWN, then of the University of Wyoming, Mr. GEORGE ROGERS, of Oklahoma State University, Mr. GENE LOPEZ, Mr. ROBERT MARTIN, Mr. WALTER CONLEY, Mr. BRENT DAVIS, Mr. TONY MOLLHAGEN, Mr. DALE BERRY, Mr. PAUL RAMSEY, Mr. FRANK JUDD and Mr. DAVID SCHIMIDLY, of Texas Technological College, and Dr. MAURICE BURKHOLDER, of Boise, Idaho. The technical assistance of Mr. JOHN D. CARNES, of M. D. Anderson Hospital and Tumor Institute at Houston, is also acknowledged.

Addendum

Specimens examined

Voucher specimens are deposited in either the Collection of Mammals, Department of Biology, Texas Technological College, Lubbock, or Collection of Mammals, Department of Biological Sciences, University of Arizona, Tucson.

Neotoma floridana. Oklahoma: Payne Co., 10 mi W of Stillwater (two males, five females). Florida: vicinity of Tampa (one male).

Neotoma micropus. Texas: Garza Co., Post (11 males, 11 females); Lamb Co., 3 mi N of Fieldton (one female); Winkler Co., 2 mi N of Wink (one male, two females); 8 mi SSE of Kermit (one male); Lynn Co., 2.5 mi S, 1 mi W of Tahoka (two males); 9 mi N of Odessa (one male, one female); Cameron Co., 11 mi E of Brownsville (two males, three females).

Neotoma albigula. Texas: Garza Co., 6 mi S of Southland (two males). New Mexico: Lincoln Co., 3.8 mi W of Carrizozo (one female); Grant Co., Silver City golf course (one male). Arizona: Pima Co., Catalina Foothills, northern end of Tucson's Campbell Ave. (three males, one female); Tucson Jct. of 22nd St. and Old Spanish Trail (one male); Sahuarita Butte (three females); Santa Cruz Co., 1 mi S of Pena Blanca Rd. on Ruby Rd. (one female); Gila Co., Pinal Mtns, Upper Pinal Campground (one female); Pinal Co., Desert Biology Station (two males, three females); Yuma Co., 2.3 mi E, 1.3 mi S of Stone Cabin (one male, three females); Cochise Co., 2 mi E of Douglas (one male); Chiricahua Mtns, Cave Creek Canyon, Sunny Flat picnic area (one female); Chiricahua Mtns, 1 mi S of Portal Ranger Station (one male, three females); 2 mi NW of Dos Cabezas (five males, two females); Texas Canyon on Interstate Route 10 (two males, one female). Sonora: Isla Tiburón, Ensenada del Perro (two males, one female); 16 mi N of Navajoa on Hwy 15 (one male, two females); 7 mi E, 1 mi S of Vicam (one male, two females); 43 mi N of El Oasis (two females); 1 mi W of Aribabi (one male); Hwy marker 1672 on Hwy 15 (one male, two females). Sinaloa: Hwy marker 1672 on Hwy 15 (two males); northern bank of Rio Fuerte on Hwy 15 (three females).

- PATTON, J. L.: Chromosome studies of certain pocket mice, genus *Perognathus* (Rodentia, Heteromyidae). *J. Mammal.* 48: 27-37 (1967).
- SHELLHAMMER, H. S.: Cytotaxonomic studies of the harvest mice of the San Francisco Bay region. *J. Mammal.* 48: 549-556 (1967).
- ZIMMERMAN, E. G. and LEE, M. R.: Variation in chromosomes of the cotton rat, *Sigmodon hispidus*. *Chromosoma, Berl.* 24: 243-250 (1968).

Manuscript received 8 October 1968; accepted for publication 12 November 1968.

Neotoma lepida. Arizona: Mohave Co., Black Mtns, 7.5 mi NE of Oatman (two males); Black Mtns, 9.2 mi NE of Oatman (one male, one female); Yuma Co., Horse tank, 2.3 mi E, 3 mi S of Stone Cabin (one female); 5 mi S of Yuma Proving Grounds turnoff on Hwy 95 (one male, one female); 11.2 mi N of Yuma Proving Grounds turnoff (one female). California: Orange Co., University of California at Irvine Ranch (five males, two females). Baja California: 4 mi S of Endira (one male, two females); 3 mi S of Old Mill Bahia San Quintin (one female).

Neotoma stephensi. Arizona: Mohave Co., 7.3 mi SSE of Kingman (two females); 5.2 mi N of Kingman, by Stockton Hill Rd. (one female).

Neotoma mexicana. Arizona: Pima Co., Santa Catalina Mtns, 1 mi E of Bear Wallow (one male); Santa Catalina Mtns, 1/4 mi S of road to Big Lookout (one female); Graham Co., Graham Mtns, 4 mi N of Ladybug Saddle (three males, three females); Coconino Co., NW side of San Francisco Peaks (one female); Apache Co., Phelps' Botanical Area (three females); Sheep's Crossing (one female). New Mexico: Lincoln Co., 3.8 mi W of Carrizozo (one female), Colorado: Jefferson Co., 4 mi S, 1 mi E of Pine (one male). Durango: 42 mi W of El Salto (one male).

Neotoma fuscipes. California: Orange Co., University of California at Irvine Ranch (two males, three females).

Neotoma cinerea. Wyoming: (one male). Montana: (one male).

Neotoma phenax. Sinaloa: km marker 1672 on Hwy 15 near Sonoran border (two males, three females).

References

- BURT, W. H. and BARKALOW, F. S.: A comparative study of the bacula of wood rats (subfamily Neotominae). *J. Mammal.* 23: 287-297 (1942).
- CROSS, J. C.: A comparative study of the chromosomes of rodents. *J. Morph.* 52: 373-401 (1931).
- HALL, E. R. and KELSON, K. R.: The mammals of North America, Vol. 2 (The Ronald Press Co., New York 1959).
- Hsu, T. C.: Robertsonian fusion between homologous chromosomes in a natural population of the least cotton rat, *Sigmodon minimus* (Rodentia, Cricetidae). *Experientia* (in press).
- Hsu, T. C. and ARRIGHI, F. E.: Chromosomes of *Peromyscus* (Rodentia, Cricetidae). I. Evolutionary trends in 20 species. *Cytogenetics* 7: 417-446 (1968).
- Hsu, T. C. and BENIRSCHKE, K.: An atlas of mammalian chromosomes, Vol. I (Springer-Verlag, New York 1967).
- Hsu, T. C. and BENIRSCHKE, K.: An atlas of mammalian chromosomes, Vol. II (Springer-Verlag, New York 1968).
- MATTHEY, R.: Les chromosomes sexuels géants de *Microtus agrestis* L. *Cellule* 53: 163-184 (1950).
- MATTHEY, R.: Les chromosomes des Muridae. *Rev. suisse Zool.* 60: 225-283 (1953).
- MATTHEY, R.: Etudes de cytogenétique et de taxonomie chez les Muridae (Rodentia) *Reithrodontomys megalotis dychei* Allen, *Hypogeomys antimena* Grand., *Neofiber alleni* True. *Mammal.* 25: 145-161 (1961).