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CLADISTICAL ANALYSIS OF CHROMOSOMAL EVOLUTION WITHIN THE GENUS NEOTOMA

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In this paper we present a cladistical analysis of 10 species of *Neotoma* in order to: 1) determine if chromosomal data can provide an hypothesis of phylogenetic relationships within the genus; 2) determine how chromosomal events are distributed among the species; and 3) compare chromosomal evolution as seen in *Neotoma* with that observed in other genera within the Cricetidae (Robbins and Baker, 1981; Baker and Barnett, 1981; Baker *et al.*, 1983). The extensive work of Mascarello and several coauthors (Baker and Mascarello, 1969; Baker *et al.*, 1970; Mascarello *et al.*, 1974 *a*, 1974*b*; Mascarello and Warner, 1974; Mascarello and Hsu, 1976; Mascarello, 1978) with *Neotoma* chromosomes provides basic karyotypic data that includes C and G-bands for *N. micropus*, *N. albigula*, *N. phenax*, *N. stephensi*, *N. lepida*, *N. devia*, *N. fuscipes*, and *N. cinereus*, as well as side-by-side comparisons between *N. micropus* and these seven other species of *Neotoma*. Additionally, we present G and C-band data for *N. mexicana* and *N. floridana*.

Based on shared sequences with *Peromyscus crinitus*, *N. micropus* was thought by Mascarello *et al.* (1974*a*) to have maintained most of the primitive G-band sequences for the genus *Neotoma*. Subsequent studies using outgroup methods substantiate that *Neotoma micropus* does have the probable primitive G-band karyotype for the genus *Neotoma* and that most of these G-band sequences may also be primitive for the family Cricetidae (Koop *et al.*, 1985; Haiduk *et al.*, personal communication). Therefore, starting with the proposed primitive G and C-band sequence for

the genus, we developed a parsimonious arrangement of chromosomal evolution within the genus.

Materials and Methods

Because of the extensive homology observed between *Neotoma* and *Peromyscus* and the availability of a standard chromosomal numbering system for *Peromyscus* (Committee for Standardization of Chromosomes of *Peromyscus*, 1977), we used this numbering system as a reference in comparing the G-band patterns of the various taxa. In some of the smaller chromosomes, numbers could not be assigned with certainty to a particular chromosome, and these are identified by a "?" in Fig. 3.

The G and C-bands of *N. mexicana* and *N. floridana* as well as additional *N. micropus* presented in this paper, were prepared from bone marrow suspensions (Lee and Elder, 1980). G-bands were developed by trypsin digestion and Giemsa staining (Seabright, 1971). C-bands were obtained using the procedure described by Stefos and Arrighi (1971).

Specimens examined—*Neotoma micropus*: 2♂, Texas, Garza Co., 1.5 mi. S Post; *Neotoma mexicana*: 1♂, 1♀, Texas, Jeff Davis Co., Madera Canyon, Davis Mtns.; *Neotoma floridana*: 1♂, 1♀, Texas, Wichita Co., 5 mi. E Burkburnett.

The G and C-bands of *Neotoma micropus* and *N. albigula* were presented by Mascarello *et al.* (1974*a*). The G and C-bands of *N. phenax* were presented by Mascarello *et al.* (1974*b*). The G and C-bands of *N. devia, N. fuscipes, N. lepida, N. stephensi*, and *N. cinereus* were presented by Mascarello and Hsu (1976).

Results

The G and C-banded karyotypes of *Neotoma micropus* and *N. floridana* were identical except in chromosome 3. In *N. micropus* there is a polymorphic heterochromatic short arm present, but in *N. floridana*, the heterochromatic short arm addition is fixed throughout most of its range (Birney, 1973). This result was predicted earlier on the basis of standard karyotypes (Baker and Mascarello, 1969; Baker *et al.*, 1970; and as discussed by Mascarello and Hsu, 1976; and Birney, 1973).

The G-banded karyotypes of *N. micropus* and *N. mexicana* differ in chromosomes 3, 17, 23, and 24 (Fig. 1). The difference in chromosome 3 involves the polymorphic heterochromatic short arm present only in *N. micropus*. Chromosomes 17 and 24 differ in

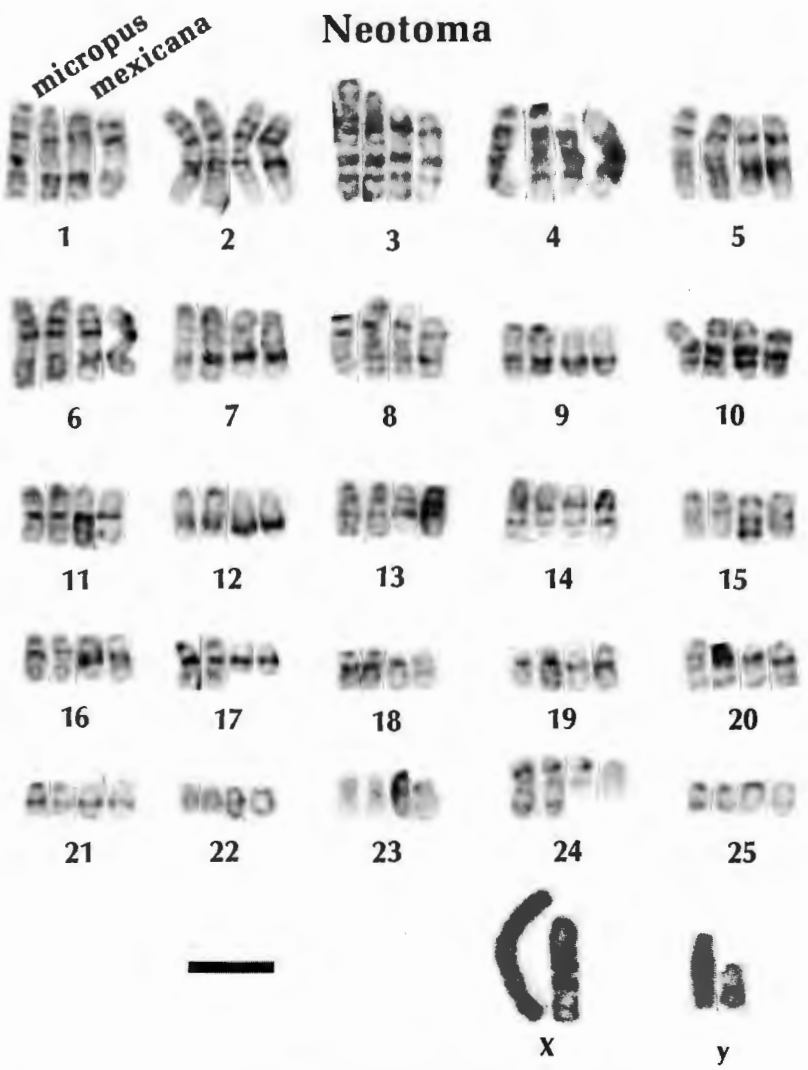

FIG. 1.—A comparison of diploid G-banded chromosomes of *Neotoma micropus* and *N. mexicana*. Chromosomes are numbered according to the standard chromosomal numbering system for *Peromyscus* (Committee for standardization of chromosomes of *Peromyscus*, 1977).

that euchromatin distal to the centromere in the long arm appears to be missing in *N. mexicana*. In chromosome 23 the biarm condition in *N. micropus* seems to be rearranged to the acrocentric condition in *N. mexicana*; however, because of the presence of so few bands in 23, we are unsure of proposed homology. Because the standard

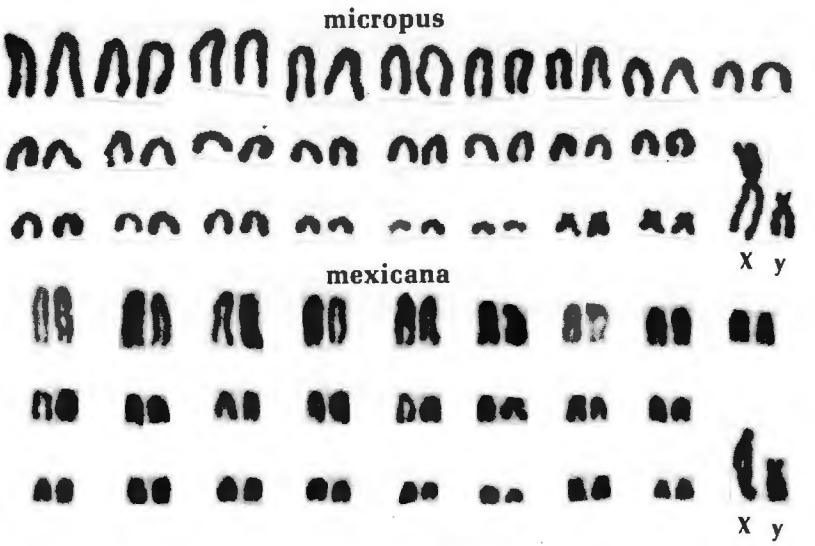

FIG. 2.—A comparison of standard chromosomes of *Neotoma micropus* (2N=52, FN=54) and *Neotoma mexicana* (2N=52, FN=54).

karyotypes of *N. mexicana* and *N. micropus* (except for the polymorphic heterochromatic short arms and the X chromosome) appeared identical, the differences in G-band sequences were not predictable from standard karyotypes (Fig. 2).

The cladogram of chromosomal change in ten species of *Neotoma* is shown in Fig. 3. In examining the C and G-banded chromosomes of *N. micropus, N. mexicana, N. devia, N. floridana, N. lepida, N. albigula, N. phenax, N. stephensi, N. fuscipes,* and *N. cinereus,* several derived G and/or C-band sequences appeared to be shared. In chromosome 3, a heterochromatic short arm addition was found to be polymorphic in *N. micropus* (Baker *et al.*, 1970), *N. floridana* (Birney, 1973), and *N. lepida* and *N. devia* (Mascarello and Hsu, 1976; Mascarello, 1978), but fixed in *N. albigula.* Although the presence of a heterochromatic arm addition is a derived state, the fact that it is highly polymorphic precludes us from rigidly defining a group on the basis of this character alone. For example, we cannot exclude *N. mexicana* from this group because it may have been fixed for the primitive condition after having been polymorphic for the heterochromatic short arm addition.

Three other shared derived G-band sequences were apparent. In chromosome 2 of *N. cinereus* and *N. fuscipes,* the proximal one-third of the acrocentric primitive G-band sequence was missing,

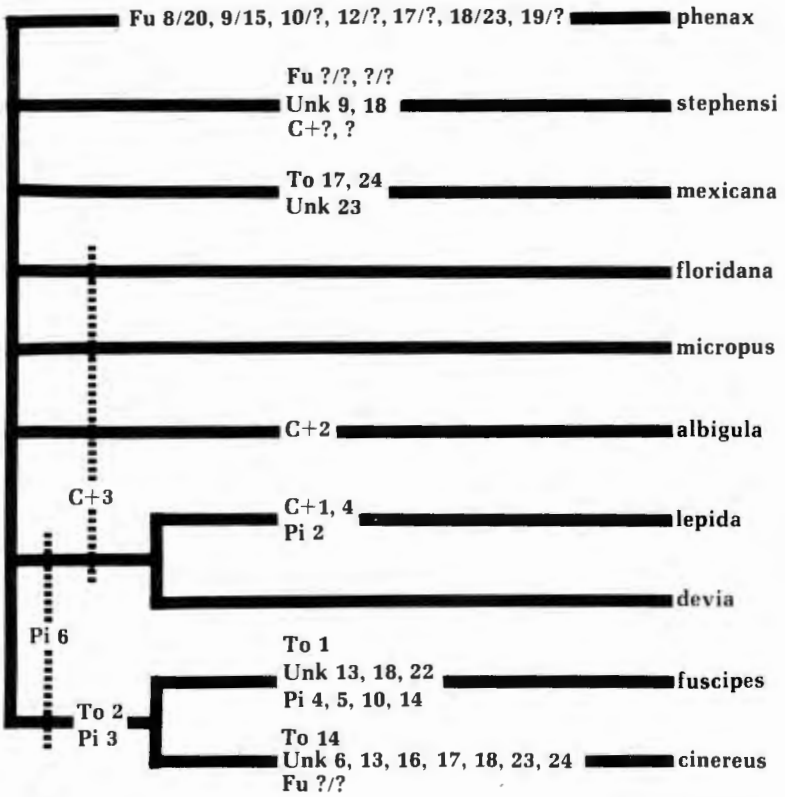

FIG. 3.—Cladistical analysis of chromosomal evolution in ten species of woodrats. Numbers identify proposed homology to Fig. 1 and to the standard G-band karyotype for *Peromyscus*.

presumably by a translocation. In chromosome 3 of *N. cinereus* and *N. fuscipes*, a change in centromere position presumably by a pericentric inversion was identified. In chromosome 6 of *N. lepida*, *N. devia*, and *N. fuscipes*, a presumed pericentric inversion was also identified.

We found no other shared derived G-band sequences in the chromosomes in which we were sure of homology.

DISCUSSION

In ten species of *Neotoma* a minimum of 42 independent chromosomal rearrangements are needed to construct the most parsimonious phylogenetic tree (Fig. 3). This assumes heterochromatic short arm additions to chromosome 3 can be explained as evolving only once. Within *Neotoma* the only group

clearly distinguished is *N. fuscipes* and *N. cinereus*, identified by translocation in chromosome 2 and a pericentric inversion in chromosome 3. Another group may be indicated by either a fixed or polymorphic heterochromatic arm addition to chromosome 3. However, although this rearrangement groups *N. floridana*, *N. micropus*, *N. albigula*, *N. devia*, and *N. lepida*, it does not exclude any of the other taxa. A shared derived G-band sequence in chromosome 6 is found in *N. lepida* and *N. fuscipes*. This may represent a convergent event, because *N. lepida*, *N. devia*, and *N. albigula* are grouped together on the basis of one synapomorphy (C+3) and *N. fuscipes* is grouped with *N. cinereus* on the basis of two other synapomorphies (To2, Pi3). Additional support for the contention of convergence in chromosome 6 comes from examining the tree presented by Robbins and Baker (1981) for 18 species of *Peromyscus*. In their *Peromyscus* cladogram, chromosome 6 was rearranged six times, two of which were very similar to that seen in *N. lepida* and *N. fuscipes*, and three of which were convergent reversals back to the acrocentric primitive condition. This would seem to indicate that chromosome 6 has a "hotspot" for chromosome breakage and within the neotomine-peromyscine group, changes in chromosome 6 must be used with care in systematic studies.

Traditional systematic studies (Hall, 1981; Burt and Barkalow, 1942) have placed *N. cinereus* in a monotypic group in the subgenus *Teonoma*, *N. phenax*, in a monotypic subgenus, *Teanopus*, and the other eight species of *Neotoma* examined in this study in the subgenus *Neotoma*. From our chromosomal analysis, we cannot comment on the subgeneric status of *N. phenax*; however, our data suggest alternative systematic hypotheses: 1) *N. cinereus* be placed in the subgenus *Neotoma* or 2) that both *N. fuscipes* and *N. cinereus* be placed in the subgenus *Teonoma*.

Within *Neotoma* there were two cases where identical standard karyotypes of two taxa were found to differ in their G-band karyotypes. The first was noted by Mascarello and Hsu (1976) in *N. albigula* and *N. lepida*. The other is *N. micropus* and *N. mexicana* (Figs. 1 and 2). These two cases demonstrate a need for caution in assuming homology based on standard karyotypes.

Chromosomal evolution within *Neotoma* is conservative (*N. micropus* and *N. floridana*) and extensive (*N. cinereus* and *N. fuscipes*). The types of rearrangements that we could document in the ten species of *Neotoma* include 8 pericentric inversions, 6 translocations, 9 fusions, 6 heterochromatic arm additions, and 13 unidentified events. Seven of the 9 centric fusions found within

TABLE 1.—*Comparison of types and numbers of chromosomal rearrangements in* Peromyscus, Onychomys, Neotoma, *and the largest 12 chromosomes of* Oryzomys. *N equals species examined, Fu/Fi equals centric fusion or fissions, To equals translocation or other (duplications or deletions), Pi equals pericentric inversions, Pa equals paracentric inversions, C+ equals heterochromatic short arm additions, Unk equals unidentified events, Tot equals total rearrangements, and Avg Eu/sp equals average euchromatic rearrangements per species.*

Genus	*N*	Fu/Fi	To	Pi	Pa	C+	Unk	Tot	Avg/sp	Avg Eu/sp
Peromyscus	18	0	0	26	0	34	0	60	3.3	1.5
Onychomys	3	0	0	0	0	25	0	25	8.3	0.0
Neotoma	10	9	6	8	0	6	13	42	4.2	3.6
*Oryzomys***	11	13	21	8	1	0	12	55	5.0	5.0

**Only the largest 12 pairs of chromosomes were analyzed.

the genus are found in the *N. phenax* lineage. As for the general unresolved nature of the cladogram in Fig. 3, there are at least two possible explanations. First, radiation of the 10 species of *Neotoma*, for which G-band data are available, occurred at approximately the same time from a basal "*Neotoma*" stock. This explanation would predict that with the exception of *fuscipes* and *cinereus*, each of the various species would be equally related to each other. Alternatively, some of the species may have shared a common ancestry after separating from the other species examined; however, during the period of common ancestry, chromosomal evolution occurred only in the *fuscipes* and *cinereus* lineage.

In *Peromyscus* and *Onychomys*, Robbins *et al.*, (1983) noted a strong correlation between the range of a species and the number of rearrangements that had occurred within a species. Using their methods (Robbins *et al.*, 1983) and the available data for the genus *Neotoma*, we found no correlation between the geographic range and the number of rearrangements found in a species.

In Table 1, the magnitude and types of chromosomal evolution for four genera of the family Cricetidae are compared. From this table, it appears that karyotypic orthoselection (White, 1975) is not as obvious in the genus *Neotoma* as it is in *Peromyscus* and *Onychomys*. In *Neotoma*, many types of rearrangements occur, including centric fusions and translocations which are absent in *Peromyscus* and *Onychomys*. The average number of euchromatic rearrangements per species within *Neotoma* (3.6) is higher than in *Peromyscus* (1.5) and *Onychomys* (0.0) but not as high as found in *Oryzomys* (5.0) (Baker *et al.*, 1983).

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