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CLADISTICAL ANALYSIS OF CHROMOSOMAL EVOLUTION IN POCKET GOPHERS OF THE CRATOGEOMYS CASTANOPS COMPLEX (RODENTIA: GEOMYIDAE)

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The yellow-cheeked pocket gopher, *Cratogeomys castanops*, is a highly fossorial mammal. The genus *Cratogeomys* consists of seven species (Honeycutt and Williams, 1982), most of which occur in the transverse volcanic chain of central México. As currently recognized, one species, *C. castanops*, ranges northward as far as Colorado and Kansas (Fig. 1). Russell (1968) distinguished two subspecies groups, based on size, within *C. castanops*. Those with smaller crania were designated the *subnubilus* group and those with larger skulls were assigned to the *excelsus* group. Due to the distinctness of the two groups and lack of evidence of interbreeding between them, Russell (1968) suggested the possibility that they might represent distinct species.

An alternative view of the systematic relationships of subspecies of the *C. castanops* complex results from chromosomal data. Based on nondifferentially stained karyotypes, two chromosomal races are recognized within *C. castanops*. Berry and Baker (1972) suggested that these probably represent two distinct species, but the geographic boundaries of the two based on cytological evidence were different from those proposed by Russell (1968) based on skin and skull morphology. Berry and Baker (1972) reported that all *C. castanops* they examined from north of 25 degrees north latitude had a diploid number (2N) of 46 and a

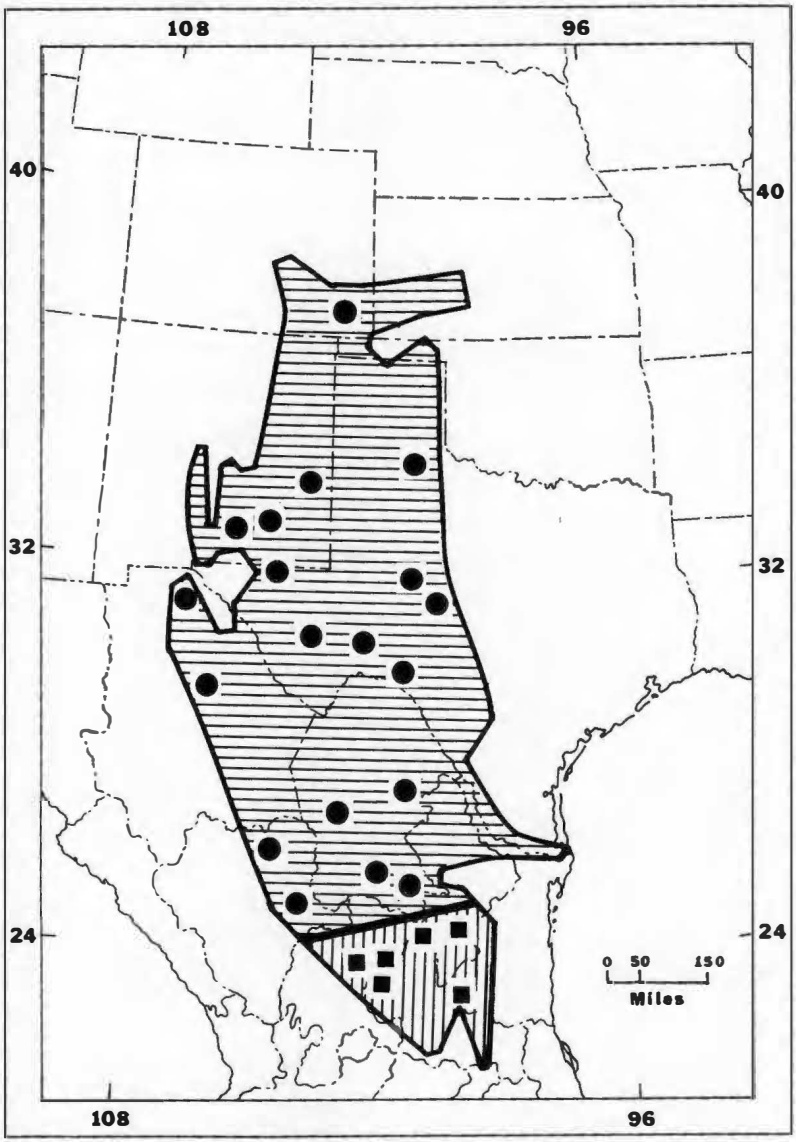

FIG. 1.—Diagramatic representation of the geographic distribution of the two cytotypes of *Cratogeomys castanops*. Circles represent the 2N = 46 cytotype and squares the 2N = 42 cytotype. Modified from Berry and Baker (1972:fig. 3).

fundamental number (FN = number of arms of the autosomal complement) of 86, whereas specimens of *C. castanops* taken south of 25 degrees north latitude possessed 2N = 42, FN = 78.

Results of a study on zoogeographic distribution of parasitic lice (genus *Geomydoecus*) on members of the *C. castanops* complex were completely compatible with patterns of the chromosomal races, but not with variation in cranial and body size (Hellenthal and Price, 1976).

Our study was designed to determine the nature and extent of chromosomal evolution that distinguishes the two cytotypes. By using another species of *Cratogeomys (C. gymnurus)* as an outgroup, we hoped to determine the direction of chromosomal evolution in this group. Cladistical analysis of the electrophoretic data for six species of *Cratogeomys* (Honeycutt and Williams, 1982) showed synapomorphies that indicate *C. zinseri, C. gymnurus, C. tylorhinus*, and *C. fumosus* form a clade, which is the sister group of *C. castanops*. The species *zinseri, gymnurus, tylorhinus*, and *fumosus* have indistinguishable nondifferentially-stained karyotypes (Berry and Baker, 1972), and any of these taxa would serve as an appropriate outgroup. Our ultimate goal was to determine if the chromosomal data are best interpreted as characteristic of that between two species and to determine which of the two cytotypes has undergone the most extensive chromosomal evolution.

Both G- and C-banding techniques were used in resolving the above questions. G-band patterns have been used to demonstrate types of chromosomal rearrangements and overall conservatism of gene sequences for mammals, especially as related to the euchromatic portion of the karyotype (Baker *et al.*, 1983*b*, 1987; Koop *et al.*, 1985; Stangl and Baker, 1984). Analysis of C-banding is used to determine variation in position and amount of constitutive heterochromatin. By using both banding techniques, it should be possible to determine which rearrangements have altered euchromatic chromosomal segments and which rearrangements involve only heterochromatic regions (constitutive heterochromatin regions are thought to be composed of highly repetitive DNA segments that probably do not act as reproductive isolating mechanisms).

We follow the taxonomy of *Cratogeomys* as proposed by Honeycutt and Williams (1982).

Materials and Methods

Specimens used in this study were collected from natural populations using live traps for pocket gophers (Baker and Williams, 1972). See the list of specimens examined for collecting localities.

Specimens were subjected to yeast stress for two to four days to increase mitotic activity and karyotyped by the method of Lee and Elder (1980). G-bands were obtained by trypsin digestion and Giemsa staining (Seabright, 1971, as modified by Baker *et al.*, 1982, and Baker and Qumsiyeh, 1987); C-band procedures were those described by Stefos and Arrighi (1971, as modified by Baker and Qumsiyeh, 1987).

Chromosomal pairs of the 2N = 42 cytotype were used as a standard numbering system to propose homology between cytotypes. A minimum of six G-banded spreads of chromosomes was examined for each animal to document the degree of consistency of banding patterns within an individual. Three to 10 mitotic spreads of each individual listed as examined were photographed and compared with those of other individuals studied. Spreads of chromosomes were photographed using 4 × 5 Kodak Plus-X film and enlarged to a standard size on Kodabromide F-5 paper. Chromosomes were arranged in order of decreasing size. Homologies were determined by side-to-side comparison of differential longitudinal staining patterns of each chromosome. Representative karyotypes (Figs. 2-7) for each cytotype were prepared from a cell with the correct diploid count for that cytotype. Figures that are a comparison between two cytotypes include a haploid complement from a single cell from each of the respective individuals.

Photographic negatives and voucher slides were deposited in the Department of Biological Sciences, Texas Tech University. Museum skins accompanied by partial body skeletons, as well as frozen samples of liver, heart, and kidney, are deposited as voucher specimens in The Museum, Texas Tech University.

Specimens examined (TK numbers are karyotype numbers used to reference tissues and voucher specimens).—*Cratogeomys castanops*, 2N = 46 cytotype.—Texas: TK 20395 (♀), TK 20397 (♂), Lubbock Co., 2 mi. N Loop 289 on University Ave.; TK 26238 (♀), TK 27143 (♀), TK 27144 (♂), Lubbock Co., 2 mi. W Loop 289 on 4th St.; TK 27145 (♂), TK 27146 (♀), TK 27147 (♂), TK 27149 (♂), Lubbock Co., 5 mi. N Loop 289 on University Ave.

Cratogeomys castanops, 2N = 42 cytotype.—Mexico: TK 27105 (♀), San Luis Potosí, 12 mi. W Cd. del Maiz; TK 27647 (♀), TK 27650 (♀), TK 27651 (♂), TK 27652 (♀), TK 27655 (♂), TK 27656 (♂), TK 27657 (♂), TK 27659 (♂), TK 27661 (♂), TK 27663 (♀), TK 27664 (♂), Zacatecas, 10.5 mi. S Concepción del Oro on Hwy. 54.

Cratogeomys gymnurus, 2N = 38 cytotype.—Mexico: TK 28550 (♂), TK 28551 (♂), TK 28552 (♀), TK 28554 (♂), Michoacán, 9.4 mi. SE Patzcuaro.

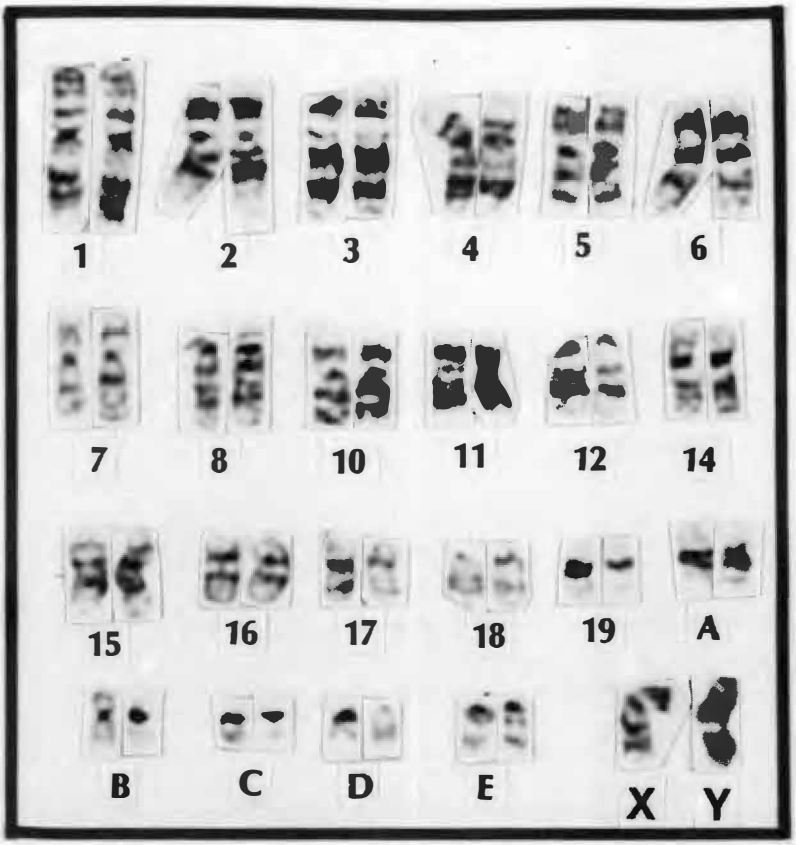

FIG. 2.—G-banded karyotype of a male *Cratogeomys castanops* (TK 27147) of 2N = 46 cytotype. X and Y chromosomes are illustrated at the lower right. Numbers represent proposed homology to chromosomes of the 2N = 42 cytotype.

RESULTS

The 2N = 46 cytotype of C. castanops.—All autosomes in this group are biarmed except two pairs. G-banding patterns for all chromosomes are shown in Figure 2 and C-bands are shown in Figure 3. One of the two pairs of acrocentrics is almost entirely heterochromatic, whereas heterochromatin is restricted to or near the centromeric regions on the remainder of the autosomal pairs. There is an interstitial C-positive band near the centromere on the long arm of the second largest chromosome. Also some interstitial C-bands were observed on several small chromosomes. As noted in Berry and Baker (1972), the sex chromosomes consist of a large metacentric or submetacentric X and a medium-sized

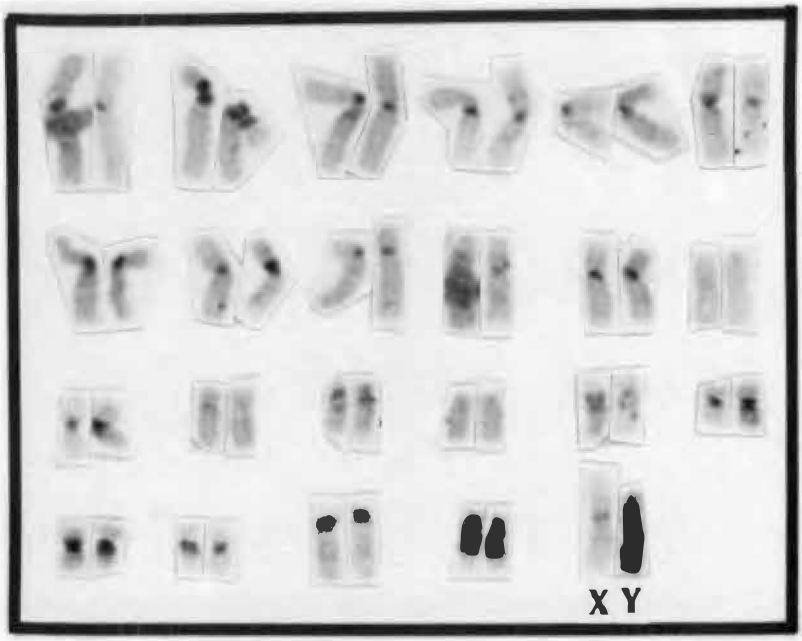

FIG. 3.—C-banded karyotype of a male *Cratogeomys castanops* (TK 20397), 2N = 46 cytotype, demonstrating distribution of heterochromatin.

acrocentric Y. The Y chromosome is almost entirely heterochromatic (Figs. 2 and 3).

The 2N = 42 cytotype of C. castanops.—All autosomes are biarmed except one small pair of acrocentrics. G-banding patterns for all chromosomes are shown in Figure 4 and C-bands are shown in Figure 5. The small pair of acrocentrics appears entirely heterochromatic. As in the 2N = 46 cytotype, interstitial C-band material is present on the second largest chromosome and an addition of a large block of heterochromatin is present on the third largest pair. The X chromosome is a large metacentric, and the Y chromosome is a medium-sized, heterochromatic acrocentric element (Figs. 4 and 5).

Outgroup.—The G- and C-banded karyotypes of *C. gymnurus* (2N = 38) are shown in Figures 6 and 7. All chromosomes are biarmed, and most heterochromatin is restricted to or near the centromeric regions. There are several interstitial C-positive bands on larger chromosomes (Fig. 7). The Y chromosome is almost entirely heterochromatic.

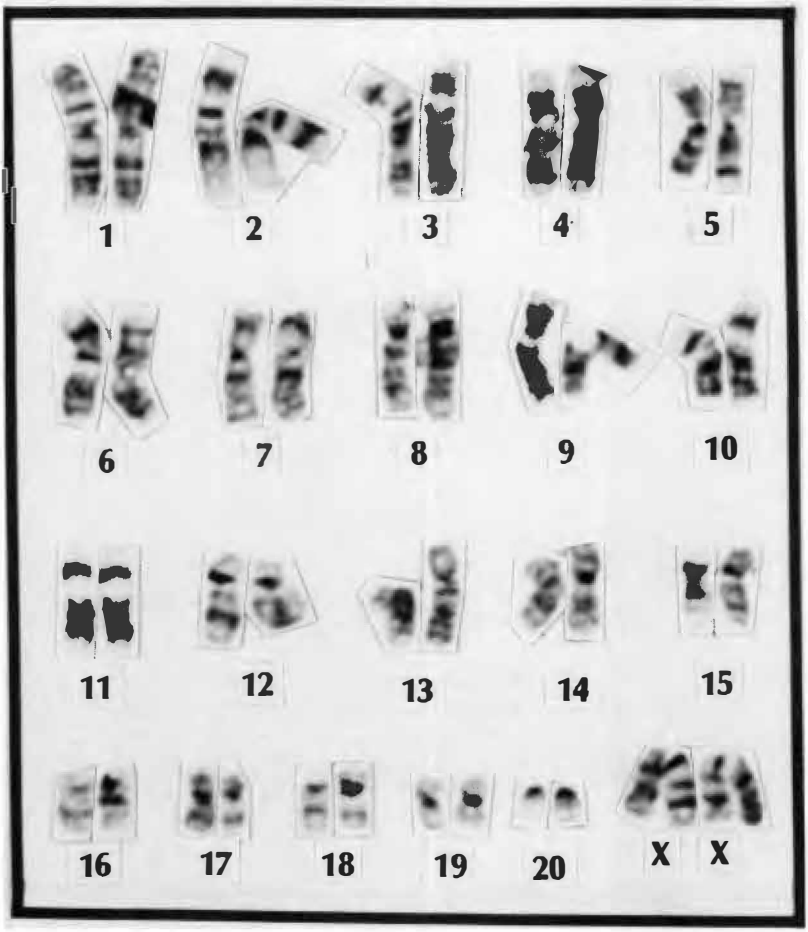

FIG. 4.—G-banded karyotype of a female *Cratogeomys castanops* (TK 27647), 2N = 42 cytotype. X chromosomes are illustrated at the lower right. The chromosomes of this cytotype are numbered in order to propose homology to other karyotypes examined.

DISCUSSION AND CONCLUSIONS

Haploid complements of G-banded karyotypes of *C. castanops* of 2N = 42 type and *C. gymnurus* (2N = 38) are compared in Figure 8. The proposed homologies between the two species are shown in box A. The remainder of the haploid complements for which homology is less probable are shown in box B. The G-banded karyotype of *C. gymnurus*, the outgroup taxon of this study, shows homology of chromosome numbers 1-13 when

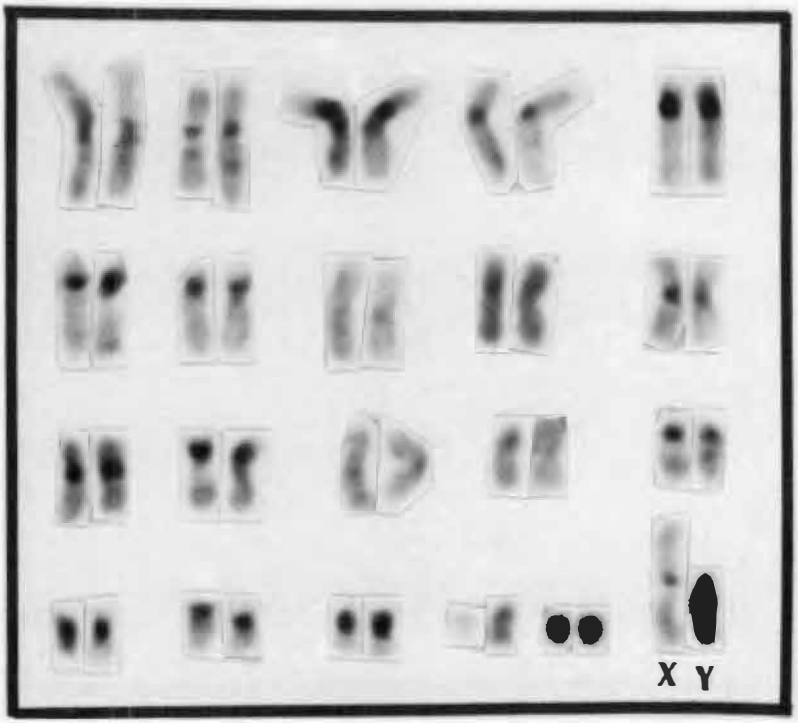

FIG. 5.—C-banded karyotype of a male *Cratogeomys castanops* (TK 27657) of 2N = 42 cytotype. X and Y chromosomes are illustrated in the lower right.

compared with the 2N = 42 cytotype of *C. castanops*. A pericentric inversion distinguishes the short arm of chromosome number 3 of *C. gymnurus* and *C. castanops*. Based on cladistical analysis, it is assumed that the homologies between *C. castanops* (2N = 42) and *C. gymnurus* (2N = 38) are primitive elements for the *castanops* group.

Haploid complements of G-banded karyotypes of the two chromosomal races of *C. castanops* are compared in Figure 9. Box A of Figure 9 presents the chromosomes that appeared to be completely homologous between the two cytotypes. Chromosomes 10-12 in box B appear partially homologous but some rearrangements occurred in at least one arm of each proposed homologous chromosome. It appears probable that inversions occurred that distinguish these three pairs of chromosomes. The remainder of the haploid complements for which homology is uncertain are shown in box C. The G-banded karyotypes of the two cytotypes of *C. castanops* indicate complete homology of chromosome

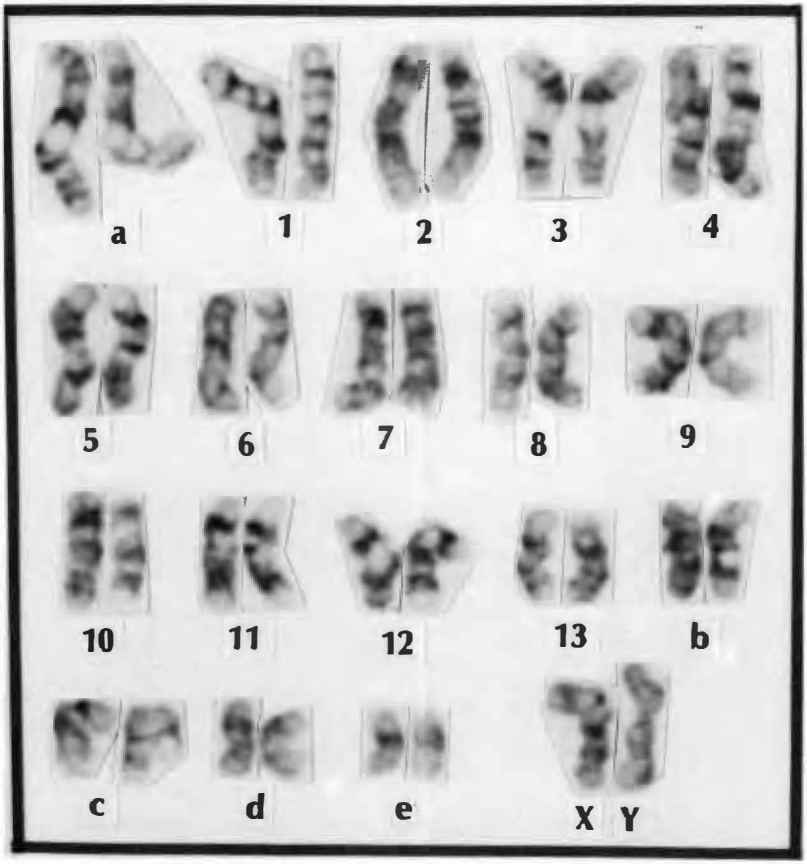

FIG. 6.—The G-banded karyotype of a male *Cratogeomys gymnurus* (TK 28550), 2N = 38. X and Y chromosomes are illustrated at the lower right. Numbers under chromosomes indicate proposed homology to the 2N = 42 cytotype of Fig. 4.

numbers 1-8 and 14-19 and incomplete homology of chromosomes 9-13 and 20 in the 2N = 42 cytotype and 10-12 A, B, C, D, and E in the 2N = 46 cytotype.

The two chromosomal races currently recognized as a single species share 14 pairs of autosomes (1-8, and 14-19), that appear to be unchanged between the two. Additionally, the X chromosome has an homologous portion that appears unchanged. Pairs 10-12 appear to be altered by a pericentric inversion, and because one condition for these three chromosomal pairs is shared by the outgroup (*C. gymnurus*) and the 2N = 42 cytotype, it is concluded that the primitive condition is found in the 2N = 42 cytotype, and that all three chromosomes are derived in the 2N =

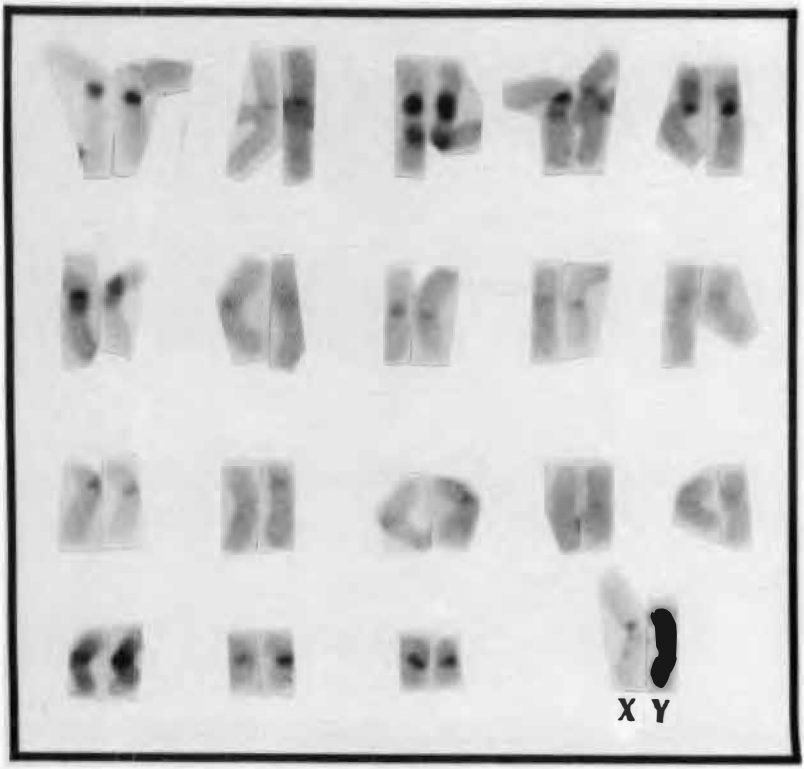

FIG. 7.—C-banded karyotype of a male *Cratogeomys gymnurus* (TK 28550). X and Y chromosomes are illustrated at the lower right.

46 cytotype (Figs. 2 and 10). Moreover, the two cytotypes are distinguished by rearrangements that change pairs 9, 13, and 20 of the 2N = 42 cytotype into pairs A, B, C, D, and E of the 2N = 46 cytotype (or vice versa). However, because 9 and 13 are shared by the outgroup and the 2N = 42 cytotype, the condition for most if not all of the A, B, C, D, and E chromosomes of the 2N = 46 cytotype probably is derived. Because there are few bands on some of these smaller elements, it is a matter of conjecture as to how 9, 13, and 20 were rearranged into A, B, C, D, and E. Probably three or more rearrangements were required to produce the observed differences. Therefore, the 2N = 42 cytotype and the 2N = 46 cytotype probably are distinguished by six or more rearrangements, and most if not all were established in the 2N = 46 cytotype.

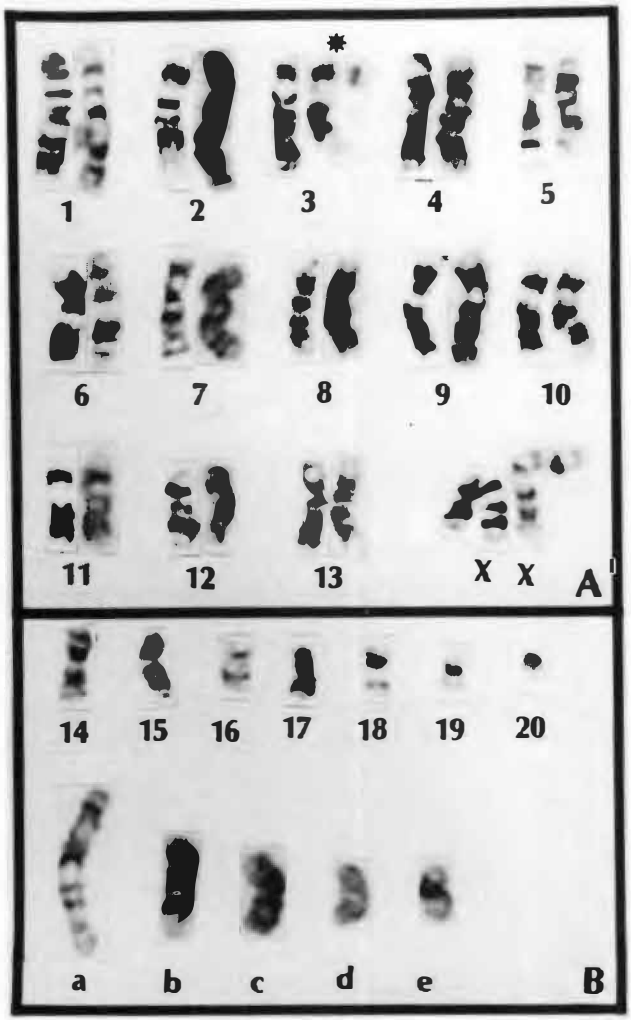

FIG. 8.—Composite comparison of haploid karyotypes of *Cratogeomys castanops* (2N = 42 cytotype, TK 27647) and *Cratogeomys gymnurus* (2N = 38, TK 28550) showing proposed G-band homologies. The chromosome on the left side of each comparison is from a representative of 2N = 42 *Cratogeomys castanops*, whereas the right chromosome is from a representative of *Cratogeomys gymnurus*. Box A contains 13 pairs for which we propose G-band homology. In box A, inversion (*) distinguishes the upper arm of chromosome number 3 of these two species and on pair 13 there is also an extra band in the chromosome of *castanops*. The remainders of the haploid complements for which homology is unknown are shown in box B. (Numbered chromosomes are from *castanops* and those lettered are from *gymnurus*.)

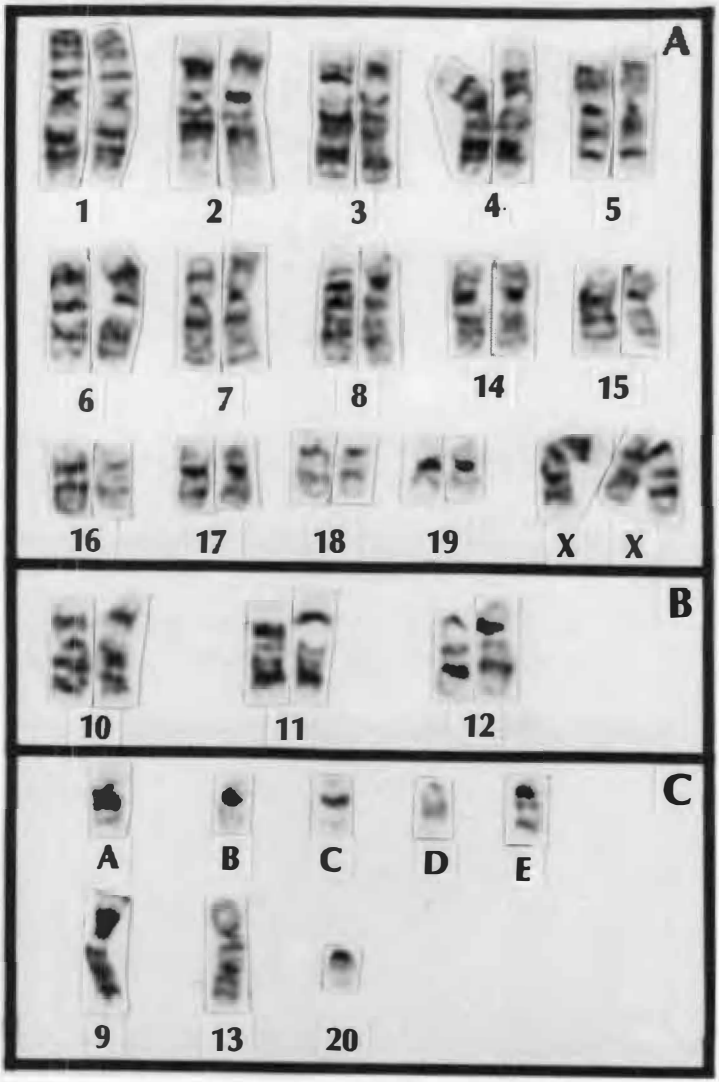

FIG. 9.—Composite comparison of haploid karyotypes of the two cytotypes of *Cratogeomys castanops* depicting proposed homologies. The chromosome on the left side of each comparison is from a representative of the 2N = 46 (TK 27147), FN = 86 cytotype from Lubbock County, Texas, whereas the right chromosome is from the 2N = 42 (TK 27647), FN = 78 cytotype from Zacatecas, México. Box A presents chromosome pairs that appear completely homologous. Pairs 10-12 in Box B appear partially homologous. Box C presents the remainder of the haploid complements, for which homologies could not be determined (numbered chromosomes from the 2N = 42 cytotype, lettered chromosomes from the 2N = 46 cytotype).

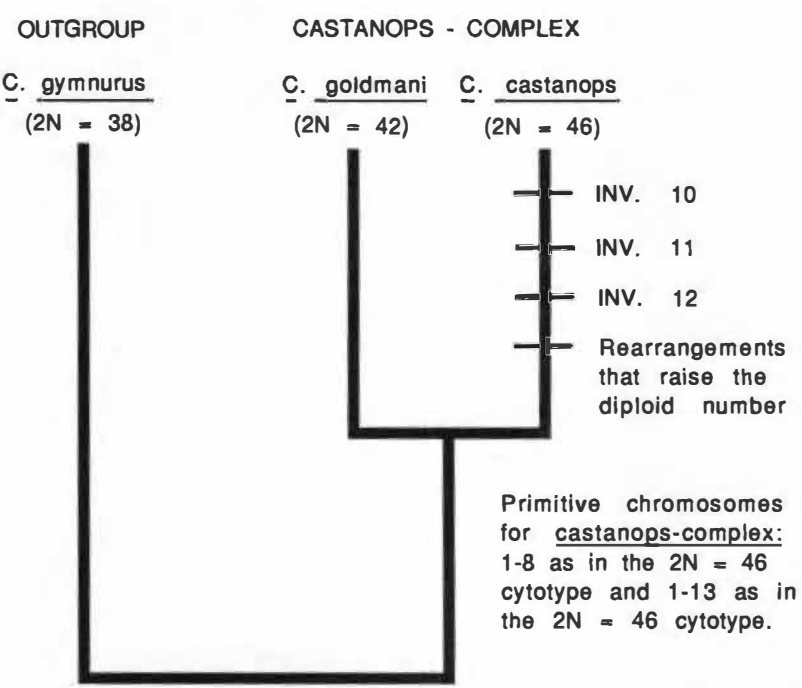

FIG. 10.—Cladistical analysis of the cytotypes of *Cratogeomys castanops* complex, using *Cratogeomys gymnurus* as an outgroup.

Are these chromosomal differences sufficient to produce genetic isolation? In the absence of breeding data, the matter is conjectural, but this is certainly more chromosomal rearrangement than usually distinguishes two closely related species of mammals (see Fig. 10). At this time, it probably is best to recognize two species in order to emphasize that the two cytotypes represent discrete genetic entities. If two species are recognized, the northern taxon would be *C. castanops* (Baird, 1852) (2N = 46 cytotypes) and the southern species (2N = 42 cytotype) probably would be *C. goldmani* Merriam (1895). There is a problem in assigning a name to the 2N = 42 cytotype (Berry and Baker, 1972). The published type locality of *C. goldmani* is well within the range of the 2N = 42 cytotype, but, although individuals collected in the 1970s from near the type locality (Canitas, Zacatecas) of *goldmani* had a 2N = 42, they differ morphologically from the type specimen and paratypes of *goldmani* that were available to Merriam (1895) at the time he described *goldmani*. Therefore, it is not totally clear what the karyotype of

the type specimens of *C. goldmani* might have been. If it should prove that the holotype of *goldmani* is actually from the 2N = 46 cytotype, then *C. subnubilus* (Nelson and Goldman, 1934) would be the oldest available name for the 2N = 42 species.

Although it is attractive to propose an adaptive role for the karyotype, data documenting such a role are few and have been reviewed by Baker *et al.* (1983*a*). Robbins *et al.* (1983) suggested that in *Peromyscus* and *Onychomys* there was a positive correlation between number of derived rearrangements in a species and the total geographic range occupied by that species. A similar correlation also may be found in *Cratogeomys*, where *C. castanops* is the most widely distributed of the species. *C. castanops* is the only species of the genus to occur outside the Mexican central core region. It is tempting to suggest that the rearrangements found in the 2N = 46 *castanops* may have been involved in a cause-and-effect relationship with genetic changes that facilitated invasion of a new geographic region for this complex of gophers, but such is only an hypothesis.

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