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CHROMOSOMAL DISTRIBUTION OF SOME REPETITIVE DNA SEQUENCES IN POCKET GOPHERS (*GEOMYS*, *CRATOGEOMYS*, AND *THOMOMYS*) AS DETERMINED BY IN SITU HYBRIDIZATION

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Systematics and evolutionary biology are limited by the number of available genetic markers that can resolve questions concerning origin, recency of common ancestry, population genetic structure, and other problems. The recent advent of molecular biological techniques that provide resolution from DNA sequences holds considerable promise as a source for new genetic markers (Baker *et al.*, 1989). However, before such markers can be used with confidence, it is necessary to understand how they vary among taxa as well as the limits of the technique.

This study is an evaluation of localization of chromosomal position of specific DNA sequences in closely related taxa of the *Geomys bursarius* complex using *Cratogeomys castanops*, *Thomomys bottae*, and *Dipodomys ordii* as outgroups. These taxa were chosen based on results of a previous study of a hybrid zone between *G. bursarius* and *G. knoxjonesi* in which we have determined that chromosomal differences, allozyme differences (alcohol dehydrogenase, lactate dehydrogenase, peptidase), mitochondrial DNA restriction sites, and ribosomal cistron DNA restriction sites identify parental and hybrid populations (Baker *et al.*, 1989). From our analysis of this

hybrid zone, we hypothesized that both premating and postmating isolating mechanisms were operating. One means of testing these hypotheses would be to locate additional genetic markers that distinguish parental types and thus could further resolve the ancestry of potential hybrids. Because the critical species of *Geomys* differ in the amount and location of C- and G-band material (Qumsiyeh *et al.*, 1988), we chose to test highly repetitive sequences from geomyids as potential species-specific genetic markers. Using DNA probes isolated from *G. bursarius*, *G. pinetis*, and *Thomomys talpoides*, we have examined the chromosomal distribution of seven repetitive DNA sequences using biotinylated probes to determine if these provide resolution as genetic markers.

METHODS

Six of the seven probes were isolated from repetitive DNA sequences that formed bands in complete digests of DNA that were electrophoresed in an agarose gel. The bands were electroeluted from the agarose gels and were cloned in plasmid or cosmid vectors. Five of the probes (Geo 5, Geo 11, Geo 12, Geo 19, and Geo 48) were cloned from Hind III digests of *T. talpoides* DNA, and one probe (17-10) was cloned from Hind III digests of *G. pinetis* DNA. An additional probe (17-1) was screened from a *G. b. major* genomic library constructed in the cosmid vector pH79 using a *Mus musculus* 28S ribosomal gene clone (I-19 of Arnheim, 1979) as a probe. Clone 17-1 contains a 45 kb insert of the ribosomal DNA repeat (Davis, 1986).

These clones were sorted into families using Southern blot techniques (Southern, 1975). Each clone was used to construct a 32P labeled probe and was cross-hybridized (Table 1) to the remaining clones at a stringency condition of approximately 80 percent.

Individuals identified as either parental *Geomys bursarius* or *G. knoxjonesi* based on chromosomal, allozymic, mtDNA, and rDNA markers (Baker *et al.*, 1989) were selected for this study. Chromosome preparations were made following Baker *et al.* (1982) and were stored at 4°C. Slides were prepared from these cell suspensions by flame drying. Methods employed for *in situ* hybridization followed Hamilton *et al.* (1990). Outgroup taxa were selected following the method of Wichman *et al.* (1990): *Cratogeomys castanops*, which separated from *Geomys* approximately two to four million years ago (Russell, 1968a, 1968b), *Thomomys bottae*, which separated from *Geomys* and *Cratogeomys* approximately seven million years ago (Russell, 1968a, 1968b), and *Dipodomys ordii*, which separated from the Geomyidae approximately 20 to 40 million years ago.

Specimens examined.—All specimens utilized in this study were collected from natural populations. Voucher specimens were prepared and have been deposited (identified by TK number) in The Museum, Texas Tech University. *Geomys bursarius major.*—TEXAS: Lubbock Co., 5.0 mi E Idalou (TK 30730, female); Lubbock, 0.8 mi SE jct. Loop 289 and Spur 331 (TK 30802, female); Lubbock, 2.8 mi SE jct. Loop 289 and Spur 331 (TK 30803, male); Lubbock, 2.6 mi SE jct. Loop 289 and Spur 331 (TK 30804, male); Garza Co., 14.0 mi S, 1.0 mi E Post (TK 30731, male). *Geomys knoxjonesi.*—TEXAS: Terry Co., 3.9 mi N Brownfield on Hwy 380 (TK 30772, female); 3.3 mi N Brownfield on Hwy 380 (TK 30776, female). NEW MEXICO: Roosevelt-De Baca Co. line, 16.0 mi S, 3.0 mi E Taiban (TK 30780, male). *Cratogeomys castanops.*—NEW MEXICO: De Baca Co., 11.0 mi S Taiban (TK 30768, female). *Thomomys bottae.*—NEW MEXICO: Los Alamos Co., White Rock (TK 24445, male). *Dipodomys ordii.*—TEXAS: Garza Co., 16.0 mi S, 5.0 mi E Post (TK 24436, female).

RESULTS

Cross-hybridization experiments of the seven clones indicated that three families of repetitive sequences are present (Table 1). The first family is comprised of clones Geo 5 and Geo 19, which show strong cross-hybridization indicating approximately 80 percent homology. The second family is comprised of clones Geo 12 and Geo 48. These two clones also cross-hybridize at a level indicating approximately 80 percent homology. The remaining three clones (Geo 11, 17-1, and 17-10) comprise the third family. These clones possess varying degrees of cross-hybridization depending on the pair-wise comparisons. Geo 11 hybridizes strongly to 17-1, but has moderate hybridization to 17-10. Clone 17-1 has moderate hybridization to both Geo 11 and 17-10. Clone 17-10 shows moderate hybridization to Geo 11, but does not appear to hybridize to 17-1.

Geo 5 probe.—In *G. b. major*, the Geo 5 probe (Fig. 1A) hybridized to all of the chromosomes in the complement; however, four pairs of smaller chromosomes appeared to hybridize over a smaller percentage of their length. Areas that hybridized less intensely included some centromeric, telomeric, and interstitial regions. There is variation in intensity within regions that hybridize and in some cases a slight banding pattern results. For *G. knoxjonesi* and *C. castanops* (Fig. 1B), the pattern observed appears to be the same as that described for *G. b. major*. In *T. bottae*, this probe hybridizes to all chromosomes, but with much greater difference in intensity. On 17 or more pairs of chromosomes, intense hybridization occurs in large

blocks at the telomeric regions, whereas hybridization occurs less intensely (or interspersed) in the remaining areas of these chromosomes, as well as the remainder of the karyotype. In *D. ordii*, hybridization occurs as faint blocks on about half of the chromosomes with less hybridization (interspersed) occurring on the remaining chromosomes.

Geo 48, 11, and 12 probes.—In both *G. b. major* (Fig. 2A) and *G. knoxjonesi*, these probes hybridized to all chromosomes producing a measles effect. The measles appearance results when probes hybridize in an interspersed manner (target sequence located in many sites in the genome, but in few copy numbers at each site) as opposed to being found in tandem arrays that produce large blocks of hybridization. The intensity of hybridization varied over all areas of the chromosomes, and all chromosomes appeared to have areas of hybridization. The smallest pair of acrocentric chromosomes hybridized more intensely than the remainder of the karyotype. A faint banding pattern was evident in some chromosomes. In *C. castanops*, the pattern is similar to that described in the two species of *Geomys*, except there appeared to be some small regions that did not hybridize, and the most intensely stained region is at the end of a small pair of acrocentric chromosomes and is not as intense as that found on the smallest pair of chromosomes in *Geomys*. In *T. bottae* (Fig. 2B), these probes hybridized to large blocks near the ends of at least 19 pairs of chromosomes, with no hybridization occurring on the remaining chromosomal regions. In *D. ordii*, hybridization occurred in faint blocks near the ends of about half the chromosomes, with little or no hybridization on the remaining chromosomes.

Geo 19 probe.—In *G. b. major* (Fig. 3A), this probe hybridized to all chromosomes in the complement with a major portion of the karyotype exhibiting intense hybridization. The remainder of the complement appeared to be relatively free of hybridization to this probe. The hybridization did not produce a distinct banding pattern. In *G. knoxjonesi* and *C. castanops*, the pattern of hybridization appeared to be similar to that seen in *G. b. major*. In *T. bottae*, Geo 19 produced the same pattern as probe Geo 5, with 17 or more pairs of chromosomes possessing large blocks of hybridization near the ends of chromosomes and the remaining chromosomes have a measles appearance. In *D. ordii* (Fig. 3B), a pattern similar to probe Geo 5 existed, with hybridization occurring in faint blocks near the ends of chromosomes and faint hybridization to the remaining chromosomes.

17-10 probe.—In *G. b. major* (Fig. 4A), this probe hybridized to all chromosomes and produced a banding effect for most chromosomes as

a result of differential intensity of hybridization. A few regions appeared to have little or no hybridization; the positions of absence of hybridization varied from chromosome to chromosome. For most chromosomes, there seemed to be a more intense area of hybridization juxtaposed to the centromere, but not including the centromere region proper. For *G. knoxjonesi*, the pattern was indistinguishable from that described for *G. b. major*. In *C. castanops*, hybridization of 17-10 produced banding of the chromosomes. Most chromosomal regions distinctly hybridized to the probe, and the regions juxtaposed to the centromere hybridized more intensely in most chromosomes. As in the two species of *Geomys*, a few chromosomal regions appeared to have little or no hybridization. In *T. bottae*, this probe was interspersed on all chromosomes and produced a measles affect. In *D. ordii*, faint blocklike regions near the ends of chromosomes appeared to hybridize to this probe.

17-1 probe.—In *G. b. major* this probe hybridized to 10 regions on small acrocentric chromosomes, and it also produced a measles affect over the remainder of the chromosomes in the karyotype. Based on other studies, these regions appeared to be similar to regions that hybridized to the 28S probe isolated from *Mus musculus* (Arnheim, 1979). In *G. knoxjonesi* (Fig. 4B), this probe produced a similar pattern to that observed in *G. b. major*. There are 10 regions on smaller chromosomes that hybridize intensely. In *C. castanops*, as with *G. b. major* and *G. knoxjonesi*, the 17-1 probe intensely hybridized to regions thought to be rDNA (two pairs of chromosomes), plus it produced a measles appearance on the remainder of the chromosomes (juxtaposed to the centromeric regions in two pairs of acrocentric chromosomes). This probe hybridized to all chromosomes in *T. bottae*, with the intensity varying within and among chromosomes. There are a few areas in the karyotype for which there is little or no hybridization. One telomeric region on a submetacentric pair hybridized more intensely than any other region in the karyotype. There are smaller blocks on two other pairs that also hybridized intensely. In *D. ordii*, this probe hybridized to about 50 percent of the chromosomal regions. Other chromosomes appeared to hybridize to the probe over their entire area and all chromosomes showed some hybridization. Some chromosomes appeared to have no hybridization over 90 percent of their chromosomal area. Up to three pairs of chromosomes had small bands that hybridized more intensely compared to other areas of hybridization.

DISCUSSION

Cross-hybridizations of the seven repetitive clones at 80 percent stringency using Southern hybridization techniques (Southern, 1975) indicated that some of the repetitive sequences shared homologous sequences with other clones (Table 1). For example, clones Geo 5 and Geo 19 shared at least 80 percent homology, and Geo 12 and Geo 48 shared at least 80 percent homology. The cross-hybridization data (Table 1) are compatible with the patterns of chromosomal location for these clones as revealed by *in situ* hybridization with one exception. Clone Geo 11 appeared to hybridize to the same chromosomal positions as did clones Geo 12 and Geo 48, but Geo 11 did not cross-hybridize to Geo 12 and Geo 48. However, it should be noted that these three clones produced a measles effect in *G. b. major*, *G. knoxjonesi*, and in *C. castanops*, indicating a dispersed pattern of distribution, and in *T. bottae* and *D. ordii* they produced hybridization in large blocks. It may be that Geo 11 hybridizes to regions adjacent to those that hybridize to Geo 12 and Geo 48, and produces patterns of hybridization that are difficult to discern from Geo 12 and Geo 48. A second possibility is that the repetitive sequences represented by Geo 11 and 17-10 are found in the nontranscribed spacer of the ribosomal clone 17-1. Alternatively, they may represent subunits of a larger repeat unit.

It also is possible that Southern blot techniques are more sensitive for detecting sequence similarity than *in situ* hybridization. Therefore, two sequences may cross-hybridize, but have a different pattern of chromosomal distribution as detected by *in situ* hybridization. Conversely, similar *in situ* patterns can occur for probes that possess no sequence similarity if those sequences are located in adjacent regions of the chromosomes.

The patterns of hybridization appeared to be identical for *G. b. major* and *G. knoxjonesi* across all seven probes examined. *C. castanops* and the two species of *Geomys* possessed similar patterns of hybridization for three of the seven probes (Geo 5, Geo 19, and 17-10). Probe 17-1, which contains the rDNA genes, hybridized to only two pairs of chromosomes in *C. castanops* as opposed to five pairs in the two species of *Geomys*. The superior resolution provided by *in situ* hybridization techniques is evident as only eight chromosomal regions possessing the ribosomal genes were observed by Baker *et al.* (1989), using silver staining methods in the nucleolar organizer regions. Probes Geo 11, Geo 12, and Geo 48, which produced similar hybridization patterns, did not hybridize to some

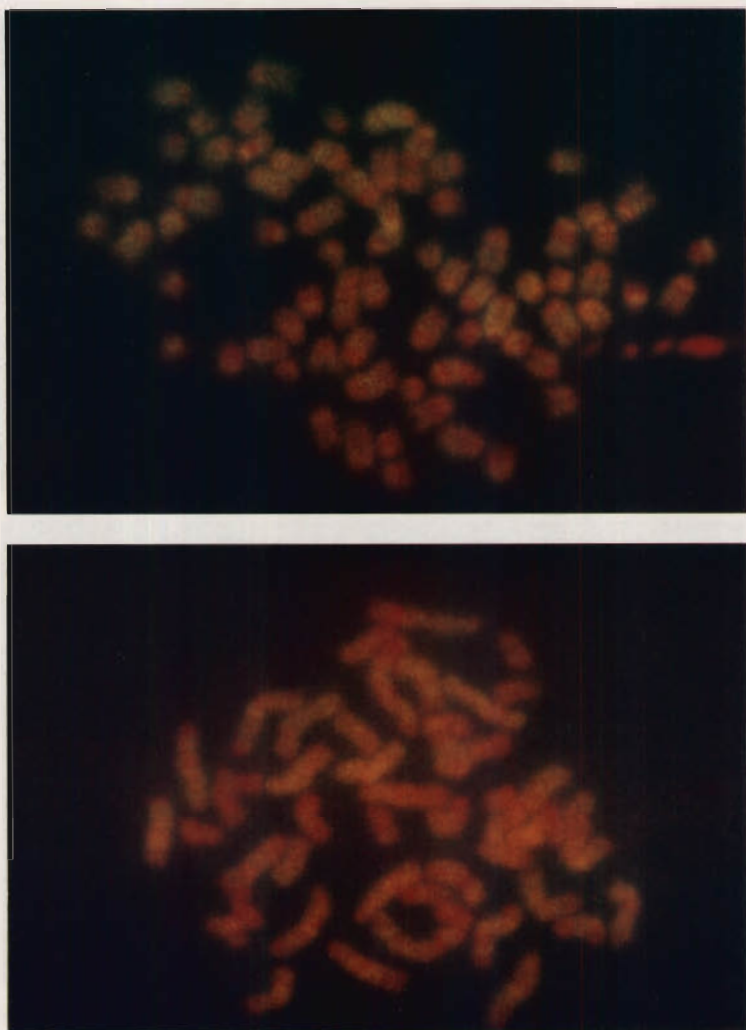


FIG. 1.—*In situ* hybridization of a biotin labeled sequence (Geo 5) isolated from *T. talpoides* to metaphase chromosomes of *G. b. major*, TK 30802 (A, above) and *C. castanops*, TK 30768 (B, below). Areas of hybridization appear yellow or yellowish green. Both spreads show a slight banding pattern and an absence of hybridization on some portions of the smaller chromosomes.

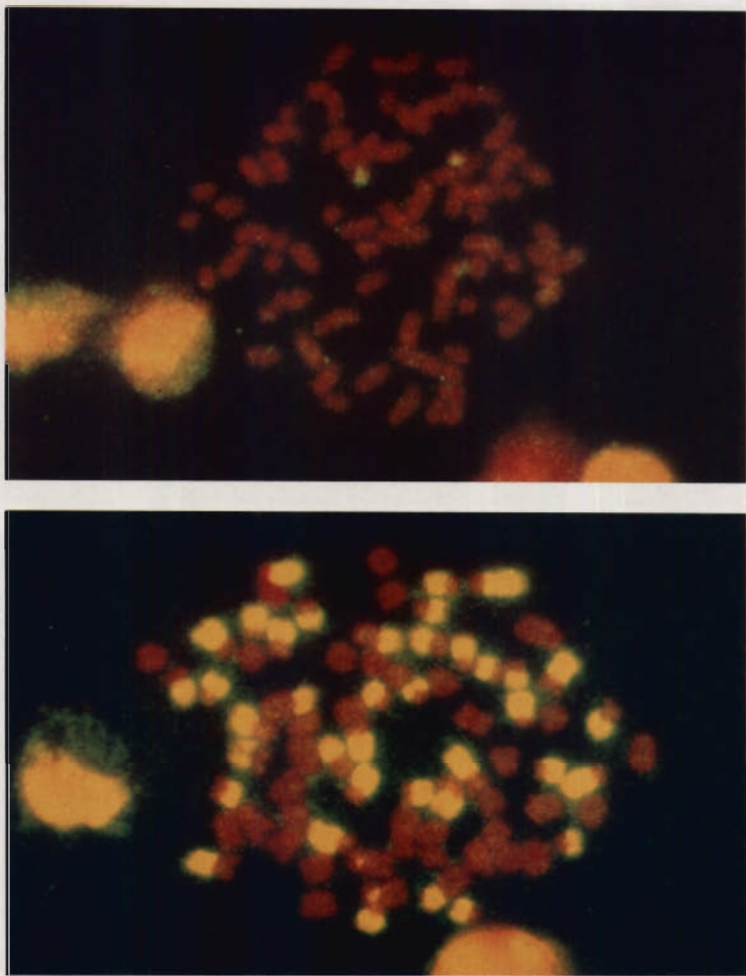


FIG. 2.—*In situ* hybridization of a biotin labeled sequence (Geo 48) isolated from *T. talpoides* to metaphase chromosomes of *G. b. major*, TK 30772 (A, above) and *T. bottae*, TK 24445 (B, below). In *G. b. major*, the smallest pair of acrocentric chromosomes has an intense band of hybridization, whereas, the remainder of the genome hybridizes in an interspersed manner (measled). In *T. bottae*, Geo 48 produces large blocks of hybridization on 19 pairs of chromosomes. The measled appearance in this photograph is primarily a result of background hybridization.

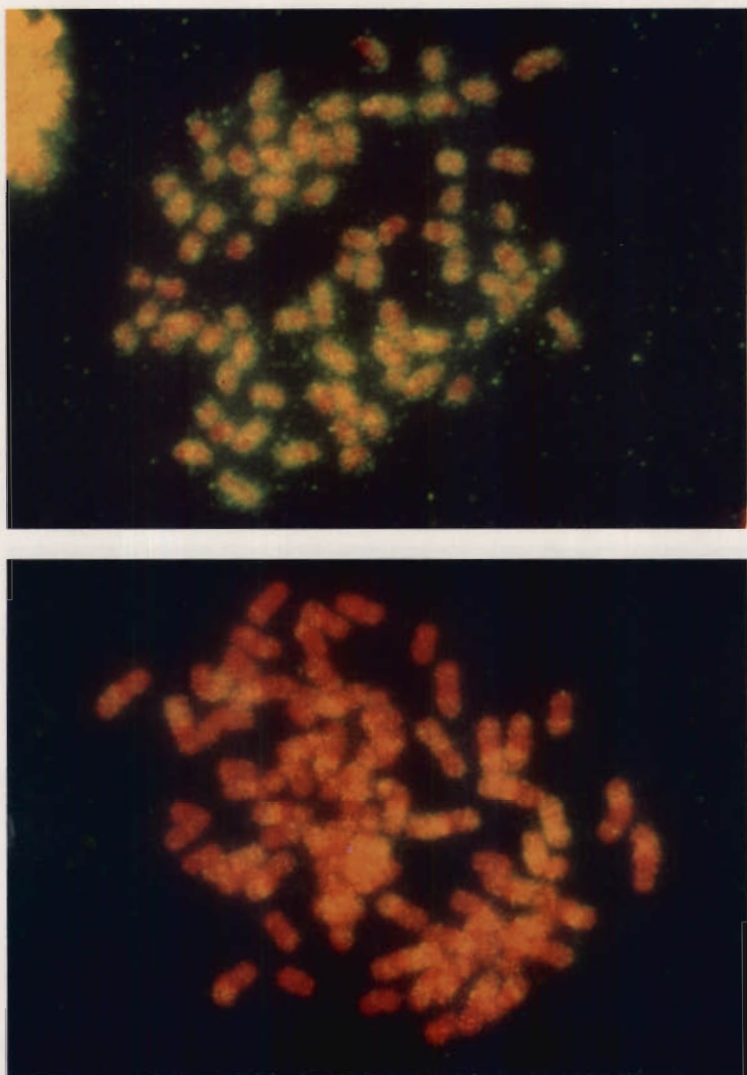


FIG. 3.—*In situ* hybridization of a biotin labeled sequence (Geo 19) isolated from *T. talpoides* to *G. b. major*, TK 30731 (A, above) and *D. ordii*, TK 24436 (B, below) metaphase chromosomes. In *G. b. major*, notice the absence of hybridization on portions of many of the chromosomal elements. In *D. ordii*, the hybridization of this probe is less intense than in geomyids and the intensity (and amount) of hybridization varies with the diploid complement.

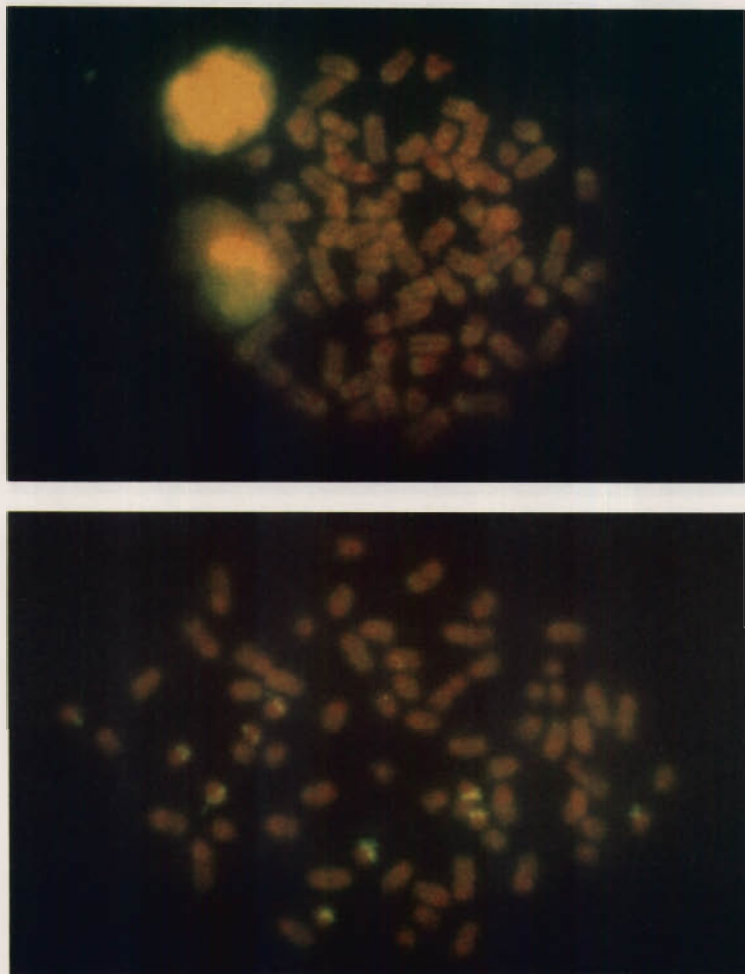


FIG. 4.—*In situ* hybridization of biotin labeled sequences to metaphase chromosomes of *G. b. major*, TK 30802 (A, above) and *G. knoxjonesi*, TK 30780 (B, below). *G. b. major* chromosomes are hybridized with the 17-10 probe isolated from *G. pinetis*. *G. knoxjonesi* chromosomes are hybridized with the 17-1 probe isolated from *G. b. major*. There are 10 areas that hybridized more intensely than in the remainder of the genome.

TABLE 1.—Cross-hybridization of repetitive clones (rows by columns) using the techniques of Southern (1975). Hybridization values of strong indicates approximately 80 percent homology.

| | Geo 5 | Geo 11 | Geo 12 | Geo 19 | Geo 48 | 17-1 | 17-10 |
|--------|--------|----------|--------|--------|--------|----------|----------|
| Geo 5 | strong | none | none | strong | none | none | none |
| Geo 11 | weak | strong | none | none | weak | moderate | moderate |
| Geo 12 | weak | none | strong | weak | strong | none | none |
| Geo 19 | strong | none | none | strong | none | none | none |
| Geo 48 | weak | none | strong | none | strong | none | none |
| 17-1 | none | strong | none | none | none | strong | none |
| 17-10 | none | moderate | none | none | weak | moderate | strong |

chromosomal regions in *C. castanops*, and did not hybridize as intensely to chromosomes as was found in *Geomys*.

In *Thomomys*, Geo 11, Geo 12, and Geo 48 hybridized to at least 19 pairs of chromosomes in large blocks at the ends of chromosomes producing similar patterns of hybridization. Geo 5 and Geo 19 produced blocks of intense hybridization in *Thomomys* typical of DNA sequences that are arranged in the genome as tandem repeats. The general appearance for these probes is like that described above for Geo 11, Geo 12, and Geo 48, except there is additional hybridization that is interspersed on the remaining chromosomes. Probe 17-10 produces an unique pattern as it is entirely dispersed over all chromosomes and does not hybridize in blocks. Probe 17-1 appears to consist of two different elements—one is interspersed over most or all of the genome, whereas the other hybridizes to the ribosomal cistron in discrete units. Rodent ribosomal DNA units consist of two major segments—a 45S transcription unit coding for the 18S, 5.8S, and 28S ribosomal RNAs and a nontranscribed spacer. Studies in *Rattus* have shown that the nontranscribed spacer regions contain highly repeated sequences (Braga *et al.*, 1985). These regions may have sequence similarity to certain interspersed sequences found throughout rodent genomes that results in the measles hybridization patterns depicted by probe 17-1. The discrete blocks of hybridization are expected given the repetitive sequence organization of mammalian rDNA (Gerbi, 1985). Variation among taxa may reflect variation in ribosomal DNA organization.

In *D. ordii*, all seven probes produced hybridization to the ends of approximately half of the chromosomes. Hybridization occurred in faint blocks as was seen in *T. bottae*. This suggests that these clones

are tandemly repeated in *D. ordii* as opposed to the interspersed patterns present in *Geomys* and *Cratogeomys*.

Several of the *in situ* hybridized probes produced banding patterns on chromosomes in *G. b. major* and *G. knoxjonesi*. It appears likely that these banding patterns reflect the G-band regions as was documented by Baker and Wichman (1991) for the *Mys* retrotransposon in *Peromyscus leucopus*, and the L1 family of long interspersed repeated sequences (LINES) in human chromosomes (Korenberg and Rykowski, 1988). Both retrotransposons and LINES have open reading frames for reverse transcriptase, and are thought to accumulate in the genome through parasitic DNA behavior using reverse transcriptase. Therefore, it would be of interest to determine if the elements (Geo 5, Geo 11, Geo 12, Geo 48, and 17-10), that produce banding in *Geomys* have a sequence that could code for reverse transcriptase.

Our observations document differences between the genome organization of *Geomys* and *Cratogeomys* as compared to *Thomomys*. The probes Geo 5, Geo 11, Geo 12, Geo 48, and Geo 19 appear interspersed in *Geomys* and *Cratogeomys*, whereas these probes hybridize like that expected for tandem repeats in *Thomomys*. This may reflect in part the observations of Patton and Sherwood (1982) and Sherwood and Patton (1982) concerning the differences in DNA content among various species of *Thomomys*, whereas Bradley *et al.* (1991) did not observe such differences in the DNA content between *G. b. major* and *G. knoxjonesi*. It is significant that the methods we used did not produce probes that visualized the major blocks of heterochromatin found in the *Geomys* karyotype (Qumsiyeh *et al.*, 1988). This indicates either blocks of heterochromatin evolved independently in *Geomys* and *Thomomys* or that the DNA sequences have diverged to the point that they no longer cross-hybridize. Qumsiyeh *et al.* (1988) concluded that the pattern of blocks of heterochromatin distinguished the two species of *Geomys*, and it is possible that such probes would provide further resolution of hybrid individuals. Utilization of such probes in concert with restriction digests, Southern blot (Southern, 1975), and fingerprinting techniques as employed by Longmire *et al.* (1988) on falcons also could generate additional resolution of hybrid individuals.

Phylogenetic screening (Wichman *et al.*, 1985) permits documentation of the relative rates of evolution of DNA probes. The most distantly related taxon, *D. ordii*, separated from the common ancestor of the pocket gophers (remainder of the taxa examined) some 20 to 40 million years ago. As all seven of the probes examined in this study

produced distinct hybridization to *D. ordii*, none of these probes appears to be as rapidly evolving as those found in other rodent taxa such as *Peromyscus* (Wichman *et al.*, 1985, 1990).

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