

## RIBOSOMAL-DNA, MITOCHONDRIAL-DNA, CHROMOSOMAL, AND ALLOZYMIC STUDIES ON A CONTACT ZONE IN THE POCKET GOPHER, *GEOMYS*

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*Abstract.*—We studied 75 individuals of the Plains pocket gopher, *Geomys bursarius*, from eastern New Mexico, where the subspecies *major* and *knoxjonesi* hybridize. Each individual was examined for chromosome number, ribosomal DNA, mitochondrial DNA, and three protein systems for which reference parental populations were fixed for alternative alleles. Twenty individuals were indistinguishable from parental *major*, 14 individuals were indistinguishable from parental *knoxjonesi*, and 41 individuals had genotypes composed of combinations of character states that distinguish the two parental types. The parental types appear to represent discrete genetic entities that have restricted introgression across a narrow hybrid zone (width approximately 3 km, using the 20/80 criterion). Parental types overlap in geographic distribution near the center of the zone, and changes in mitochondrial DNA and the five nuclear markers are concordant across the zone. It is probable that there is premating isolation between *knoxjonesi* males and *major* females. The frequencies of individuals with certain genotypic combinations within our sample imply differential reproductive success of certain genotypes. We propose that  $F_1$ 's and highly heterozygous males are sterile and that hybrid females are less fertile than parental females. These postmating factors, along with premating isolation for one of the reciprocal crosses, probably account for the restriction of gene flow across the contact zone. The structure of the zone can be explained by the "dynamic equilibrium" model.

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Much recent work has focused on the genetic analysis of the speciation process. Interspecific differences have been repeatedly documented by examination of a variety of homologous genetic elements in pairs of closely related species (Smith et al., 1982; Avise, 1986). Such studies have revealed that speciation is not always correlated with changes in specific genetic markers (Templeton, 1981, 1982). The complexity of speciation virtually precludes a universal marker for that process.

Even if a precise speciation event could be shown to be correlated with a change in genetic markers, it would still be extremely difficult to sort out the differences that are actually critical to the speciation process from 1) those that evolved as a result of speciation but were not critical to the process itself, 2) differences that existed prior to the speciation events, and 3) differences

that accumulated since speciation. Studies of hybridization permit some subtle distinctions, but unfortunately, such studies have been of limited applicability, because natural hybridization is relatively uncommon and it is difficult to document the cause-and-effect roles of various factors in the maintenance of hybridization under natural conditions (Endler, 1977, 1982; Moore, 1977; Barton and Hewitt, 1985). Where zones of hybridization between taxa occur in nature, it is possible to document these genetic issues, albeit in an experimentally uncontrolled situation. Studies of zones of hybridization are among the only situations in which genetics of the speciation process can be examined in a setting where natural selection is still effective. We are not assuming that reinforcement can occur or that any hybrid zone will ultimately result in two totally isolated species. We are assuming that partial speciation between two populations can provide critical insight into the events leading to total isolation of previously conspecific lineages.

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The complex nature of the speciation process and the many alternative explanations of the factors determining the nature of hybrid zones clearly limit resolution based on any single marker. The more independent genetic systems that can be examined, the better the chance of finding changes that correlate with the speciation process. Additionally, when multiple data sets are generated from the same individuals, each data set can be used as a reference point to determine the extent to which a genetic component functions in an independent fashion (Nelson et al., 1987; Arnold et al., 1987). If the speciation process is ever to be dissected from the genetics of species differences, multiple data sets from the same individuals will be required.

In the present study, various genetic markers were studied in the analysis of a hybrid zone between two chromosomally characterized subspecies of the pocket gopher, *Geomys bursarius*. This contact zone was originally described by Pembleton and Baker (1978), who used diploid number and morphology to describe variation across the zone. They documented that *G. b. major* ( $2n = 72$ ) and *G. b. knoxjonesi* ( $2n = 70$ ) come into contact and hybridize in a narrow region where the darker-colored, endure soils inhabited by *major* interface with paler, wind-blown, sandy soils inhabited by *knoxjonesi*. Their results indicated a strong correlation between diploid number, pelage color, and soil color across the contact zone.

We resampled a portion of the transect studied by Pembleton and Baker (1978) and examined each individual for diploid number, mitochondrial haplotype, ribosomal DNA type, and allozymic variation at 37 presumptive loci. Individuals from the zone were compared with individuals of both taxa from reference populations collected at least 100 km from the locality of hybridization. Our goals were: 1) to document the extent to which the integrity of the gene pools associated with the chromosomal races were maintained; 2) to determine the extent to which each system under study documented the amount of hybridization and introgression characteristic of other markers; 3) to estimate the extent to which variation in the reciprocal crosses (*knoxjonesi* male  $\times$  *major* female vs. *major* male  $\times$  *knoxjonesi*

female) could account for characteristics of the contact zone; 4) to use the frequency and geographic distribution of the various genotypes found in the zone to evaluate the role of potential factors in maintaining the zone; and 5) to use the structure of the hybrid zone to make inferences about the genetics of the speciation process in the *Geomys bursarius* complex.

#### MATERIALS AND METHODS

Specimens were collected from natural populations using live-traps (Baker and Williams, 1972). In the contact zone reported by Pembleton and Baker (1978), animals were trapped, along a 6-km north-south transect along the De Baca-Roosevelt county line, 15.5 mi S and 3 mi E of Taiban, New Mexico. At this locality, the county line is marked by a barbed-wire fence, which permitted designation of an exact locality by distance either east or west of the fence. Animals were trapped on 18 days during May–July, 1986. Efforts were made to collect all individuals that constructed fresh mounds within a space of approximately 300 m east or west of the county line. Eleven individuals of *knoxjonesi* were collected along the right-of-way of U.S. highway 380, west of Brownfield, Terry Co., Texas, and nine individuals of *major* were collected along the right-of-way of U.S. highway 82, east of Idalou, Lubbock Co., Texas. These samples were used as references for the genetic characteristics of the two chromosomal races. Chromosomal and allozymic studies were conducted at Texas Tech University, and ribosomal DNA (rDNA) and mitochondrial DNA (mtDNA) studies were conducted in Alan Templeton's laboratory at Washington University. Initially, results from each system under study were scored for each animal without knowledge of the animal's geographic locality or its scores from other systems. Additionally, a subset of each system was reanalyzed in a blind test to ensure repeatability of methods.

*Chromosomal Studies and Preservation of Specimens.*—Most specimens were transported to Texas Tech University and karyotyped by the yeast-stress *in vivo* culture method of Lee and Elder (1980); however, some were karyotyped under field conditions as described by Baker et al. (1981).

Samples of liver, heart, kidney, and muscle were frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  pending analysis. Voucher specimens were prepared as skins and skeletons and deposited in The Museum, Texas Tech University.

Five diploid cells were counted and photographed for each individual. If these five cells were consistent in diploid number and chromosome morphology, no additional cells were counted. However, if there was variation in either diploid number or the number of banded chromosomes, additional cells were counted and photographed until we felt confident of the karyotype of the specimen.

Nucleolar organizer regions (NOR's) were visualized by the silver-staining method of Bloom and Goodpasture (1976), as modified by Bickham and Rogers (1985). This silver-staining method is thought to stain preferentially proteins associated with the sites of NOR's, where the rDNA is located.

*Mitochondrial and Ribosomal DNA.*—Both mtDNA and rDNA were visualized from Southern blots (Southern, 1975) of total cellular DNA. DNA isolation from skeletal muscle followed Hillis and Davis (1986).

Davis (1986) has shown that *major* and *knoxjonesi* differ at a *Bam*H I site in the internally transcribed spacer of the rDNA repeat between the 18S and the 5.8S genes. In *major*, *Bam*H I produces a diagnostic 3.4-kb fragment, while in *knoxjonesi* *Bam*H I produces 1.0-kb and 2.4-kb fragments, because an additional site is present. These fragments contain only coding-region and internally transcribed spacer sequences, and the restriction-site variation between *knoxjonesi* and *major* is unrelated to length variation in the nontranscribed spacer that has been reported in many other taxa (Arnheim et al., 1980; Arnheim et al., 1982; Williams et al., 1985; Learn and Schaal, 1987).

Davis (1986) also showed that *major* and *knoxjonesi* differ at mtDNA for *Bam*H I, *Bcl* I, and *Eco*R I sites. Total cellular DNA from each individual was digested to completion with each of these informative enzymes according to the supplier's directions (New England Biolabs). Electrophoresis, Southern transfer, and hybridization were described by Hillis and Davis (1986) and Davis (1986).

The mtDNA probe used for all hybridizations was pOrtho4, which contains a 13-kb *Pst* I fragment of the *Orthogeomys hispidus* mitochondrial genome in the vector pUC 12. This clone spans the most variable region of the *Geomys* mitochondrial genome and overlaps all three variable sites used in this study (Davis, 1986). The rDNA probe was pGb28s, which contains a 7-kb *Eco*R I fragment of the *major* rDNA repeat in the vector pUC 12. This clone overlaps all of the *Bam*H I fragments associated with the restriction-site difference separating the two taxa (Davis, 1986). Southern blots for *Eco*R I and *Bcl* I digests were probed only with pOrtho4, whereas Southern blots for *Bam*H I digests were probed sequentially with pGb28s and pOrtho4. All rDNA hybridizations used a twofold excess of radioactive probe to ensure complete saturation of all bound rDNA restriction fragments.

Autoradiography for mtDNA hybridizations used intensifying screens and generally followed the procedures of Hillis and Davis (1986). Autoradiography for rDNA hybridizations was carried out at room temperature without intensifying screens. The signal from each band on the resulting autoradiograph was thus directly proportional to the number of copies of that fragment on the Southern blot (Bonner, 1987). The intensity of each band was determined with an LKB soft laser scanning densitometer equipped with an integrator. The area under each peak was used as a measure to determine the relative copy number of a particular rDNA repeat type. To determine the exact relationship between the intensity of each band and the proportion of rDNA from each taxon, a standard curve was generated. DNA isolated from each individual of the *knoxjonesi* and *major* reference populations was combined to yield a single reference sample of *major* DNA and a single reference sample of *knoxjonesi* DNA. These two samples were mixed in a range of concentrations from 0 to 100% *major* and analyzed in exactly the same manner as the unknowns. A graph was generated of the proportion *major* in the mixture versus the ratio of the intensity of the 3.4-kb *major*-specific band to the intensity of the 1.0- and 2.4-kb *knoxjonesi*-specific bands. This standard curve was used to identify the proportion of *major* rDNA

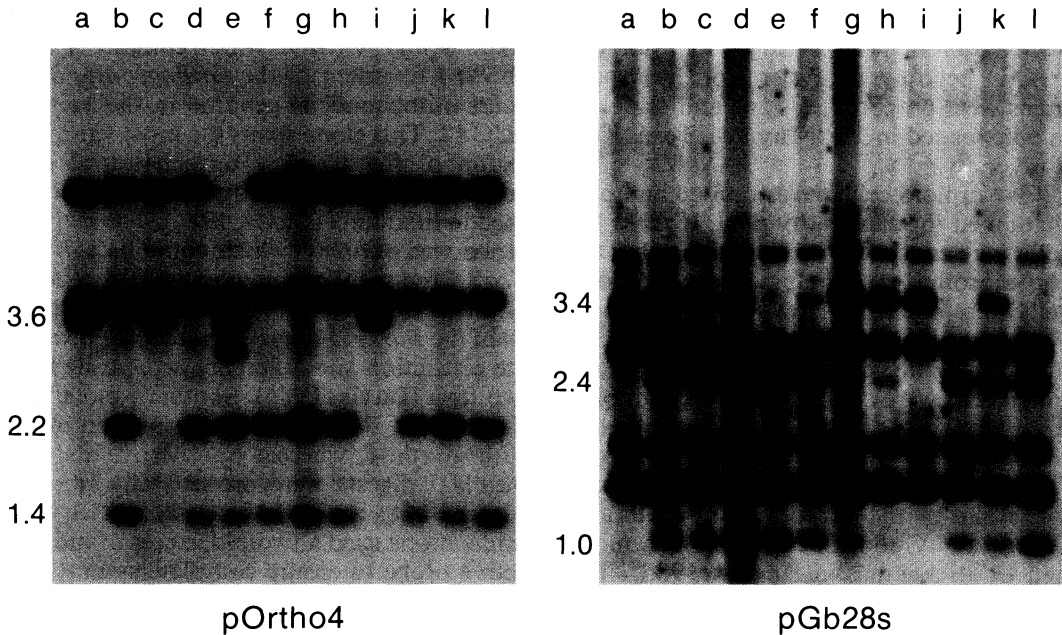


FIG. 1. Autoradiogram of a single Southern blot of *Bam*H I-digested total cellular DNA, sequentially hybridized to pOrtho4 (mtDNA) and pGb28s (rDNA). Numbers correspond to the size of each taxon-specific fragment in kilobases. Individuals a, c, and i have mtDNA characteristic of *major*, while all remaining individuals have the mtDNA characteristic of *knoxjonesi*. Individual e has a unique *Bam*H I restriction site that is unrelated to the variation distinguishing the two taxa. In the corresponding rDNA autoradiogram, individuals a and i have the rDNA typical of *major*, and individuals e, j, and l have the rDNA of *knoxjonesi*. All other individuals are of hybrid origin. A prolonged exposure was necessary to visualize the faint traces of hybridization in individuals resulting from repeated backcrossing (individuals f and h). Note that the mtDNA probe provides an excellent check on the completeness of the digestion, which is critical for the rDNA analysis.

in individuals from the hybrid zone. This method does not account for variation in genome size between the two taxa or for variation in copy number between individuals. A typical pair of rDNA and mtDNA autoradiograms, indicating both parental and hybrid rDNA types, is shown in Figure 1.

**Protein-Electrophoresis Systems.**—Preparation of heart, kidney, and liver tissue extracts and electrophoretic techniques followed Selander et al. (1971). Protein-staining techniques were as described in Selander et al. (1971) and Harris and Hopkinson (1977). The following loci were examined: *acid phosphatase*, *aconitase* (2 loci), *adenylate kinase* (2 loci), *albumin*, *alcohol dehydrogenase* (*Adh*), *creatine kinase* (3 loci), *esterase* (4 loci), *fumarase*, *glucose dehydrogenase*, *glucose phosphate isomerase*, *glucose-6-phosphate dehydrogenase*, *glutamate dehydrogenase*, *aspartate amino transferase* (2 loci),  $\alpha$ -*glycerophosphate dehydrogenase*,

*isocitrate dehydrogenase* (2 loci), *lactate dehydrogenase* (2 loci; parental populations were fixed for alternative alleles for *Ldh-1*), *malate dehydrogenase* (2 loci), *nucleoside phosphorylase*, *peptidase* (4 loci; parental populations were fixed for alternative alleles for *Pep-B-1*), *phosphoglucomutase*, *6-phosphogluconate dehydrogenase*, *sorbitol dehydrogenase*, and *superoxide dismutase*.

## RESULTS

**Chromosome Counts.**—All individuals from the reference sample of *knoxjonesi* had a diploid number ( $2n$ ) of 70, and all individuals from the reference sample of *major* had either a diploid number ( $2n$ ) of 72 or, if the diploid number was 71, the karyotype had an extra large biarmed chromosome not observed in animals from the contact zone (see Baker et al. [1983] for a description of this chromosomal polymorphism, which is not related to the variation that distinguishes the two taxa in the contact

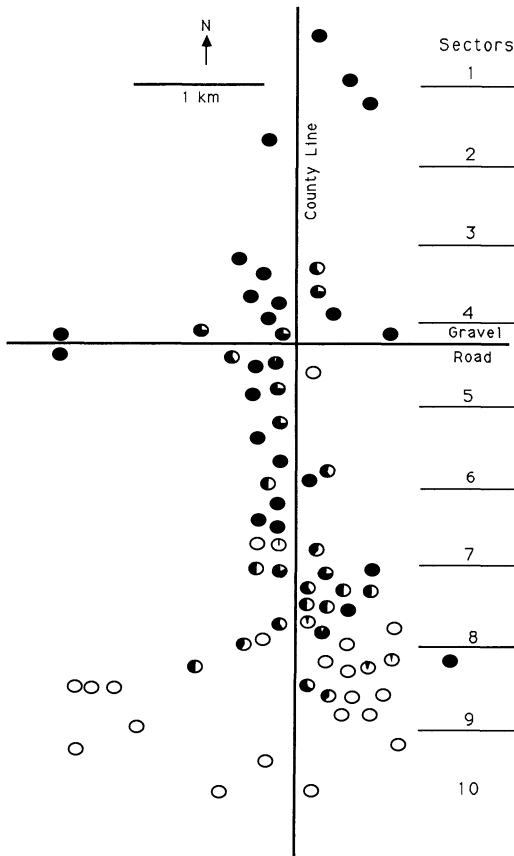


FIG. 2. Geographic distribution of 73 specimens of *Geomys* collected from the hybrid zone showing the composition of the ribosomal DNA of each individual. The closed portion of each circle represents the percentage of ribosomal DNA of *major* found in that individual. Sector divisions used to generate Figure 5 are numbered at right.

zone). In the contact zone,  $2n$  was 72 for 30 individuals, 71 for 23 individuals, and 70 for 22 individuals.

**rDNA.**—Representatives of *knoxjonesi* from the reference sample had three pairs of small acrocentric chromosomes with NOR's (two pairs with telomeric NOR's and one pair with an interstitial NOR), whereas representatives of *major* had four pairs of small acrocentric chromosomes with NOR's (three pairs with telomeric NOR's and one pair with interstitial NOR's). We therefore assumed that there are three unlinked locations for rDNA in *knoxjonesi* and four unlinked locations for rDNA in *major*. The rDNAs of the reference samples of *knoxjonesi* and *major* were identical to those de-

scribed by Davis (1986) as characteristic of these two taxa. Of the 75 specimens from the contact zone, 19 had only the 2.4- and 1.0-kb *Bam*H I fragments characteristic of *knoxjonesi*, 26 had only the 3.4-kb *Bam*H I fragment characteristic of *major*, and 30 had rDNA that was a mixture of parental types containing 1.0-, 2.4-, and 3.4-kb *Bam*H I fragments. The geographic pattern of rDNA variation is shown in Figure 2.

**Allozymes.**—Of the 37 protein systems examined, 15 were polymorphic (frequency of the common allele being less than 0.95). Three of these loci (*Adh*, *Ldh-1*, and *Pep-B-1*) exhibited fixed differences between the two reference populations. For the *Ldh-1* locus, 27 contact-zone individuals were homozygous for the allele characteristic of *major*, 22 were homozygous for the allele characteristic of *knoxjonesi*, and 26 were heterozygous. For the *Pep-B-1* locus, there were 25 individuals that were homozygous for the allele characteristic of the reference sample of *major*, 25 homozygous for the allele characteristic of the *knoxjonesi* reference sample, and 25 heterozygous for these alleles.

The situation with the *Adh* locus was more complex: a third allele, not found in either of the reference samples, was present in the contact zone. Apart from this allele, 19 individuals were homozygous for the allele characteristic of the *major* reference sample, 20 were homozygous for the allele characteristic of the *knoxjonesi* reference sample, and 25 were heterozygous for these alleles. Eleven individuals contained the third allele in combination with either the *major* allele (seven individuals) or the *knoxjonesi* allele (four individuals). Five of the individuals that had the *major* allele and the third allele were classified as *major* parental type, based on diploid number, rDNA, mtDNA, and the other two protein loci (*Pep-B-1* and *Ldh-1*); none of the individuals having the third allele was classified as *knoxjonesi* parental type, based on these five characters. All four individuals heterozygous for the third allele and the *knoxjonesi* allele were potential  $F_1$  individuals, based on diploid number, rDNA and the other two protein loci. These facts make it likely that the third allele had its origin in the population of *major*. Assuming that this is

true, 26 individuals in the contact zone were carrying only the *Adh* alleles characteristic of *major*, 20 individuals were homozygous for the allele characteristic of *knoxjonesi*, and 29 individuals were heterozygous for these alleles.

*mtDNA*.—The mtDNA of the reference samples of *knoxjonesi* and *major* were typical of those taxa as described by Davis (1986). The three diagnostic restriction-site differences between the two taxa were also 100% consistent in their classification of individuals from the hybrid zone. Of the 75 specimens from the contact zone, 47 had the mtDNA type of *knoxjonesi*, and 28 had the mtDNA of *major*. Although there were polymorphisms for *Bcl* I and *Bam*H I sites within both *major* and *knoxjonesi*, these polymorphisms did not affect our ability to identify those individuals as either *knoxjonesi* or *major*. The geographic distribution of individuals with the various mtDNA types is shown in Figure 3.

#### DISCUSSION

*Genetic Markers: Comparative Resolution*.—There were 75 individuals from the contact zone for which we had chromosomal, rDNA, *Ldh-1*, *Adh*, *Pep-B-1*, and mtDNA data. Of these, 14 had a diploid number ( $2n$ ) of 70 and rDNA, *Ldh-1*, *Adh*, *Pep-B-1*, and mtDNA characteristic of *knoxjonesi*. Twenty individuals had a diploid number ( $2n$ ) of 72 and rDNA, *Ldh-1*, *Adh*, *Pep-B-1*, and mtDNA characteristic of *major*. Because these two groups were identical to the reference samples of the pure parental types for all of the genetic markers (except *Adh* as described in Results), these two groups are considered to be parental *knoxjonesi* and *major*. Forty-one individuals had a combination of *knoxjonesi* and *major* characteristics, and we conclude that these individuals had a hybrid ancestry (Table 1).

With the complete data set, it is possible to evaluate the relative value of each marker in identifying hybrids. Of the 41 hybrid individuals, 23 were identifiable with nondifferentially stained karyotypes, 30 were identifiable with rDNA, 26 were identifiable with *Ldh-1*, 29 were identifiable with *Adh*, 25 were identifiable with *Pep-B-1*, and one was identifiable because it had the nuclear ge-

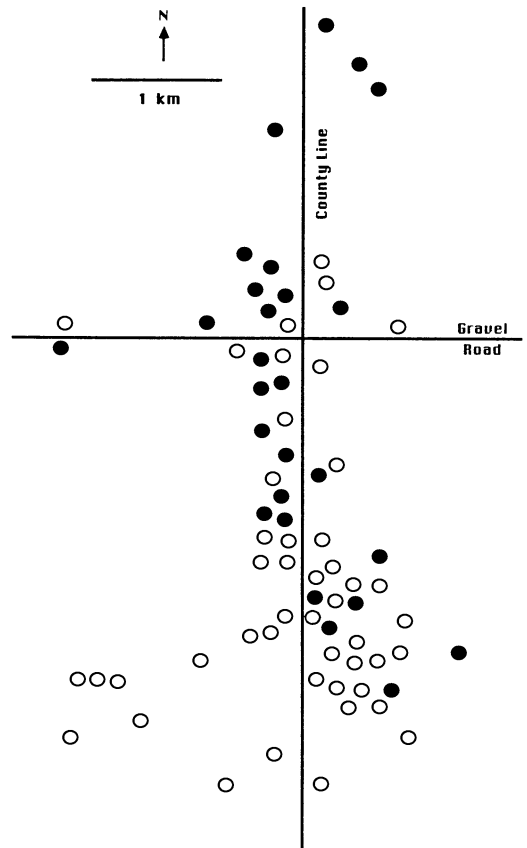


FIG. 3. Geographic distribution of specimens of *Geomys* collected from the hybrid zone showing the mtDNA type of each individual. Closed circles represent individuals with *major* mtDNA, and open circles represent individuals with *knoxjonesi* mtDNA.

nome of *major* and the mtDNA genome of *knoxjonesi*. The three protein data sets in combination identified 36 of the 41 hybrid individuals. The indication of hybridization by diploid number ( $2n = 71$ ) was always associated with other indicators documenting a hybrid origin, but only occurred in 23 out of 41 (56%) of hybrid animals. Thus, if the molecular data sets had been omitted, some hybrid individuals would have been misclassified as parental types. Each marker system (diploid numbers, rDNA, mtDNA, and three diagnostic protein loci) provided resolution on the extent and nature of hybridization. Because this is the foundation of our understanding of factors regulating the zone and how these factors relate to the speciation process, the value of multiple data sets on each individual is obvious.

TABLE 1. Genotypic characteristics of the 41 hybrids examined in this study. M = genotype of *major*, K = genotype of *knoxjonesi*, H = heterozygous for *knoxjonesi* and *major* alleles, and U = unique allele, thought to have its origin in *major*. Voucher specimens are deposited in The Museum, Texas Tech University. Proportion of *major* genome was calculated from diploid number, *Ldh-1*, *Adh*, and *Pep-B-1* marker systems. These data are graphed as a frequency histogram in Figure 6. (See text for abbreviations.)

Individual	Voucher number	2n	Sex	Percentage <i>major</i> rDNA	mtDNA	<i>Ldh-1</i>	<i>Adh</i>	<i>Pep-B-1</i>	Proportion <i>major</i> genome
1	30682	72	M	100	M	M	M/U	H	7/8
2	30706	72	M	100	M	M	M	K	6/8
3	30701	72	M	100	M	M	H	M	7/8
4	30689	72	F	100	K	H	M/U	H	6/8
5	30696	72	M	100	K	M	M	M	8/8
6	30505	71	F	100	K	H	H	H	4/8
7	30653	72	M	97.5	K	M	M	H	7/8
8	30693	72	M	88.0	M	H	M	M	7/8
9	28193	72	F	85.3	K	M	H	M	7/8
10	30654	71	M	81.2	K	H	H	M	5/8
11	30510	72	F	80.0	K	H	H	H	5/8
12	30662	71	F	77.9	K	H	H	H	4/8
13	30695	71	F	77.5	K	H	H	K	3/8
14	30511	71	M	75.5	M	H	K/U	H	4/8
15	30700	71	F	74.0	M	H	H	H	4/8
16	30664	71	M	70.1	K	M	H	H	5/8
17	30507	71	F	66.4	K	H	H	H	4/8
18	30684	71	M	65.3	K	H	H	H	4/8
19	30659	71	M	65.0	K	H	K/U	H	4/8
20	30683	72	F	62.7	K	H	H	H	5/8
21	30673	71	M	61.9	K	H	H	H	4/8
22	30675	71	F	59.2	K	H	K/U	H	4/8
23	30508	71	F	55.3	K	H	H	H	4/8
24	30658	71	F	52.6	M	H	K	H	3/8
25	30679	71	F	52.3	K	H	H	H	4/8
26	30665	70	F	52.1	K	H	H	K	2/8
27	30687	71	M	51.7	K	H	H	H	4/8
28	30647	71	M	47.1	K	H	H	H	4/8
29	30681	71	M	45.4	K	H	H	H	4/8
30	30650	71	F	38.7	K	H	H	K	3/8
31	30661	71	M	35.9	K	H	K/U	H	4/8
32	30690	71	M	34.6	K	H	H	H	4/8
33	30655	70	F	13.2	K	K	K	K	0/8
34	30657	70	M	11.7	K	K	K	K	0/8
35	30678	71	F	8.3	K	K	K	K	1/8
36	30648	70	M	5.6	K	K	K	K	0/8
37	28196	70	F	0.0	M	K	H	K	1/8
38	30692	71	M	0.0	K	H	H	H	4/8
39	30686	70	F	0.0	K	K	K	H	1/8
40	30702	70	M	0.0	K	K	H	K	1/8
41	30704	70	M	0.0	K	K	H	K	1/8

Variation found at each of four markers (diploid number, *Ldh-1*, *Adh*, and *Pep-B-1*), should segregate as for a single locus, and if these markers are not linked, then they would be expected to assort independently. The chromosomal marker appeared in the heterozygous condition at a lower frequency than was observed for heterozygous allozyme markers (23 vs. 26, 25, and 29 for the proteins; Table 1). The G- and C-band chromosomal characteristics of these two

taxa are described by Qumsiyeh et al. (1988), and although the nondifferentially stained karyotypes vary in number only by a single pair of chromosomes, the data from G- and C-band chromosomes clearly document that the two karyotypes differ by several other rearrangements (including, but not restricted to, additions of heterochromatic segments). The extent to which negative heterosis may result in postmating isolation is probably due to the effects of several chro-

mosomal rearrangements and not just the one implied by nondifferentially stained karyotypes (Baker et al., 1987).

*Ribosomal DNA as a Marker for Hybridization Studies.*—Ribosomal genes (rDNA) appear to hold considerable promise as markers for studies of hybridization (Arnold et al., 1987). Several points are critical to understanding their value and limitations. First, ribosomal genes in mammals (and most eukaryotes) are mid-to-highly repetitive sequence elements occurring in tandem arrays (Britten and Kohne, 1968). The large number of copies (200–750 copies per diploid genome) (Henderson et al., 1976; Winking et al., 1980; Quirk and Henderson, 1985; Arnold et al., 1987) allows easy analysis through genomic Southern experiments. Second, the copies on homologous and nonhomologous chromosomes evolve together in what has been termed “concerted gene evolution” (Zimmer et al., 1980; Dover and Coen, 1981). From the limited data available to date, interspecific variation for rDNA restriction-site markers appears to greatly outweigh intraspecific variation.

If the rDNA is located only on one pair of chromosomes, it should be inherited as a single-copy gene, unless there is crossing over within repeated copies. If the rDNA is distributed on two or more pairs of nonhomologous chromosomes and if the segregation of nonhomologous chromosomes containing rDNA genes is random, the net effect would be an increase in the number of possible Mendelian combinations that could be produced through subsequent production of offspring by hybrids. Additionally, as the number of nonhomologous chromosomes having ribosomal genes increases and as crossing over within the repeated segments increases, the probability of producing a reconstituted parental type would decrease. These unique characteristics of rDNA allow it to provide a relatively continuous estimate of the degree of reconstitution of parental types within a hybrid zone (Table 1), unlike proteins, mtDNA, or diploid number, which are inherited as discrete characters. In the two taxa under study here, there are three pairs of NOR's (sites of rDNA) in *knoxjonesi* and four pairs in *major*. The range of rDNA values found in the hybrids in this study (Table 1, Fig. 2) in-

dicates that Mendelian segregation and possibly crossing-over occurred in these individuals. Nonetheless, normal Mendelian segregation and crossing-over are probably not the only phenomena acting on rDNA loci in the hybrid zone. Of the 41 hybrids, 36 could be identified using all three protein markers in concert. Theoretically, the rDNA markers (four or more loci segregating in hybrid individuals) should have identified an equal or even larger proportion of the hybrids. In fact, rDNA identified 30 of the 41 hybrids, less than the 36 identified by allozymes. There are two possible explanations for this observation. First, the rDNA loci may not segregate as independent blocks. NOR regions are known to associate nonrandomly in some species (Arnheim and Southern, 1977), and it may be that this causes the parental rDNA loci to preferentially segregate in meiosis. Second, it is possible that some of the forces postulated to explain concerted evolution (such as gene conversion) may be acting to homogenize the variants more rapidly than expected.

*Distribution of Parental Types Within the Zone.*—The data show extensive hybridization in the zone of contact (of the 75 individuals studied, we concluded that 41 were hybrids). Nonetheless, there were individuals that, based on the character states of diploid number, rDNA, mtDNA, *Adh*, *Ldh-1*, and *Pep-B-1*, appeared to be parental *knoxjonesi* or parental *major*, and these parental types were sometimes trapped within a few meters of each other (Fig. 4). Individuals identified as pure parental types do not, however, overlap more than a few hundred meters within the zone.

*Change of Markers Across the Zone.*—To demonstrate the change in character states across the zone, we divided the zone into ten equal sectors and calculated the relative frequency of each character state within each sector (Fig. 5). This graph can be interpreted as showing that the change in biparentally inherited genetic markers is concordant across the zone. A maximal 20/80 estimate of the width of the zone (Hafner, 1982) would be from the northern end of sector 4 to the southern end of sector 9, or a distance of approximately three kilometers.

*mtDNA Variation and Evidence for a Pre-mating Isolating Mechanism.*—Starting at the north end of the zone and moving south,

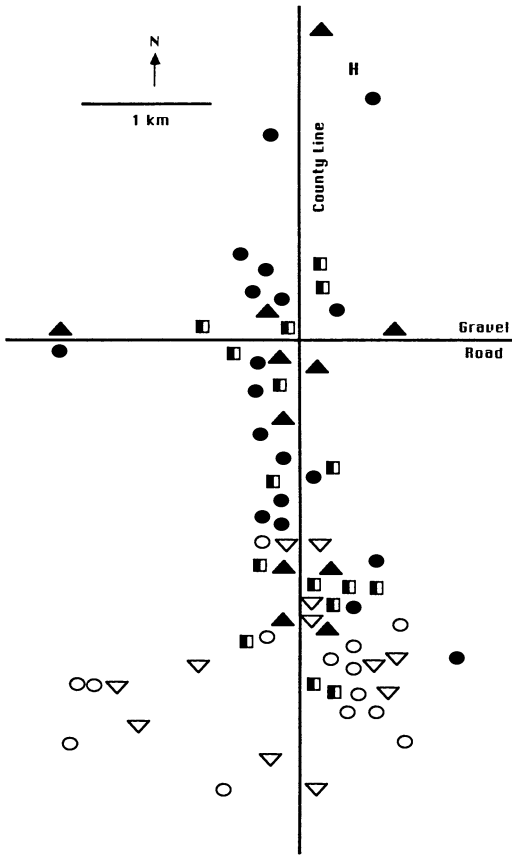


FIG. 4. Geographic distribution of the various genotypic classes found in the hybrid zone. Legend: ● = parental *major*; ○ = parental *knoxjonesi*; ■ = individuals heterozygous for diploid number, *Adh*, *Ldh-1*, and *Pep-B-1*; ▲ = individuals homozygous for *major* alleles at some, but not all, loci; ▽ = individuals homozygous for *knoxjonesi* alleles at some, but not all, loci; H = the single individual that was fixed for *major* alleles at three marker systems and for *knoxjonesi* alleles at the remaining marker system.

the shift from *major* to *knoxjonesi* character states occurs for mtDNA before it occurs for any of the nuclear markers (Fig. 5). Of the 41 hybrid individuals, 33 have *knoxjonesi* mtDNA; this frequency is significantly higher than that expected, based on a null hypothesis of a 50:50 split ( $\chi^2 = 15.24$ ,  $d.f. = 1$ ,  $P < 0.001$ ). The simplest explanation for the high percentage of hybrids with *knoxjonesi* mtDNA is that crosses of *knoxjonesi* females with *major* males are far more common than the reciprocal cross. One test of this hypothesis would be to examine a series of  $F_1$  individuals. An  $F_1$  would have the following characteristics:  $2n = 71$ , het-

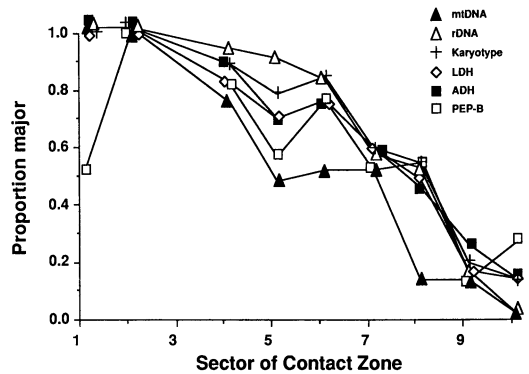


FIG. 5. Changes in character states for six marker systems across the hybrid zone. A sector represents one-tenth of the 6-km distance examined in our study. (Sector 1 is the northernmost sector). Sample sizes (sector) are as follows: 2 (1), 2 (2), 0 (3), 8 (4), 11 (5), 6 (6), 6 (7), 17 (8), 17 (9), and 4 (10).

erozygous at *Ldh-1*, *Adh*, and *Pep-B-1*, and approximately 50% *major* rDNA. Some variation of rDNA frequency type beyond 50% is necessary to correct for any copy-number variation in rDNA; we chose 40–60% *major* rDNA as limits for  $F_1$ 's. Individuals with the characteristics of an  $F_1$  could be produced in an  $F_2$  cross. However, because the other genotypes that should also be produced in an  $F_2$  cross occur so infrequently in the zone (see maintenance of the zone below), we conclude that the production of the  $F_1$  phenotype through  $F_2$  crosses is not occurring at a high frequency in the zone. Individuals having a genotype fitting our definition of an  $F_1$  could also be produced by an  $F_1 \times$  parental-type backcross, but with the chromosome, protein, and rDNA markers, the regeneration of an  $F_1$  type would occur only once in 32 offspring even if the rDNA segregated as a single locus. It therefore appears that the vast majority of the individuals classified as  $F_1$ 's by our definition should in fact be true  $F_1$  hybrids.

Of the 41 hybrids, only six fit our criteria for an  $F_1$ , and all had the mtDNA characteristic of *knoxjonesi*. If the rDNA variation acceptable for an  $F_1$  is expanded to 35–65% *major*, then nine individuals fit the criteria for an  $F_1$  hybrid, and all have the mtDNA of *knoxjonesi*. Given the missing cell (none with *major* mtDNA) a chi-square test is inappropriate. Therefore, we tested the null hypothesis that the  $F_1$ 's are equally likely to

have *knoxjonesi* or *major* mtDNA using the binomial probability,  $(0.5)^N$ , where  $N$  is the number of animals with *knoxjonesi* mtDNA. All tests were significant at the 5% level, regardless of the  $F_1$  criteria for rDNA variation. We interpret these data to indicate that successful crosses of *knoxjonesi* males with *major* females do not occur with appreciable frequency.

It has been suggested (Asmussen et al., 1987) that asymmetry in reciprocal crosses may be due to an incompatibility between the cytoplasmic genome of one parental type and the nuclear genome of the other. Such an incompatibility would be a postmating rather than a premating isolating mechanism. The existence of one individual (number 37 in Table 1) with a *major* mtDNA genome and an almost fully reconstituted *knoxjonesi* nuclear genome as well as several individuals with *major* mtDNA and complex mixtures of nuclear genomes (some similar to those of an  $F_1$  hybrid; Table 1) indicates that such a cytoplasmic-nuclear genome incompatibility is not the most probable explanation for the asymmetry observed in *Geomys*. Although these data do not absolutely rule out alternative explanations, they do lend weight to the hypothesis that a significant mechanism affecting introgression in these pocket gophers is premating isolation between *knoxjonesi* males and *major* females. The observed bias in  $F_1$  production could easily explain the slightly faster north-south shift from *major* to *knoxjonesi* for mtDNA (Fig. 5).

Prior to the availability of a maternally inherited marker such as mtDNA, this intermediate state of speciation where one cross rarely occurs while the other is relatively common could not be easily documented. We hypothesize that this state of partial isolation through sexual selection will prove common in higher vertebrate taxa that have incomplete isolating mechanisms.

*Maintenance of the Zone and Evidence for a Postmating Isolating Mechanism.*—Moore (1977) outlined two explanations of hybrid zones that provide mechanisms for the maintenance and stability of a narrow zone. The first, termed the “dynamic-equilibrium model” (Meise, 1928; Mayr, 1963; Bigelow, 1965; Barton, 1979; Barton and Hewitt, 1981, 1985) uses a dynamic balance

between gene flow into the hybrid zone from parental types and selection against hybrids to explain the maintenance of a narrow hybrid zone. This model would predict that if hybridization extended beyond the  $F_1$  stage, successful reproduction would be biased toward reconstitution of the parental genomes through repeated breeding to parental types.

The second, a “hybrid-superiority model” (Moore, 1977) is regulated by the width of the ecotone where the hybrids are more fit. In a zone maintained by hybrid superiority, a stable hybrid population would exist, and the more fit hybrid individuals would breed with each other producing a broader array of genotypes than would be possible from repeated crossing with parental types. Both of these models are compatible with Endler’s descriptive formula (number 3.11 in Endler [1977 p. 62]), which explains such clines as a balance between dispersal and selection gradients.

To test these models, we determined the genotype of each individual based on the four single-locus markers (diploid number, *Ldh-1*, *Adh*, and *Pep-B-1*) used in this study (Table 2). This classification scheme gives rise to nine categories ranging from pure *major* to pure *knoxjonesi*. Figure 6 shows the high number of potential  $F_1$ -type individuals ( $N = 17$ ) relative to the total number ( $N = 20$ ) of individuals comprising the other six genotype classes that could be produced by subsequent hybridization. The low number of individuals in the  $1/4$  and  $3/4$  classes suggests that individuals in the middle five categories must be contributing relatively few offspring to the hybrid population. For example, contrast the observed frequency of the different classes in Figure 6 with the predicted genotypic ratio (1:4:6:4:1) expected from a cross of an  $F_1$  to either parental type. Additional support for the idea that most individuals in the middle classes have a low level of fertility comes from the fact that only one of the 41 hybrid individuals (number 2 in Table 1) is homozygous for a *major* allele at one locus and for a *knoxjonesi* allele at another locus (an  $F_1 \times F_1$  cross should produce 43% homozygous for alternative markers). All hybrids except individual number 2 in Table 1 (homozygous for *major* alleles at three markers and homozygous for *knoxjonesi* alleles at the

TABLE 2. Frequency of the hybrid genotypes found in the sample of 41 individuals. M = *major*, K = *knoxjonesi*, H = heterozygous for *major* and *knoxjonesi*, and U = unique allele.

N	2n	Genotype			Percentage major genome
		<i>Ldh-1</i>	<i>Adh</i>	<i>Pep-B-1</i>	
3	70	K	K	K	0/8
3	70	K	H	K	1/8
1	70	K	K	H	1/8
1	70	H	H	K	2/8
1	71	K	K	K	1/8
1	71	H	K	H	3/8
2	71	H	H	K	3/8
13	71	H	H	H	4/8
4	71	H	K/U	H	4/8
1	71	H	H	M	5/8
1	71	M	H	H	5/8
2	72	H	H	H	5/8
1	72	H	M/U	H	6/8
1	72	M	M/U	H	7/8
1	72	M	M	K	6/8
2	72	M	H	M	7/8
1	72	M	M	H	7/8
1	72	H	M	M	7/8
1	72	M	M	M	8/8

other) can be explained as a result of repeated crosses involving at least one parental type.

What are the potential explanations for the low number of hybrids (1 of 41) that are fixed for alternative parental alleles? It has been noted that in hybrids there is often differential fertility between the sexes and that the heterogametic sex is most often infertile (Haldane, 1922). If  $F_1$  males and other highly heterozygous males were sterile in these gophers, then  $F_1$  females could only mate with a parental type or the progeny of a backcross. Pembleton and Baker (1978) examined the reproductive condition of five males with a hybrid karyotype ( $2n = 71$ ) from this same zone and found no sperm in either the epididymis or the testis. The elimination of the majority of males in the central region of Figure 6 as breeders would help explain the lack of  $F_2$  as well as the lack of some  $F_1 \times$  parental crosses. It also seems likely that at least a portion of female hybrids must have reduced fertility, because if  $F_1$ -like females bred primarily with one parental type or the other, then the expected genotypic ratio would be 1:4:6:4:1. However, the empirical data do not support the hypothesis that such a backcross has oc-

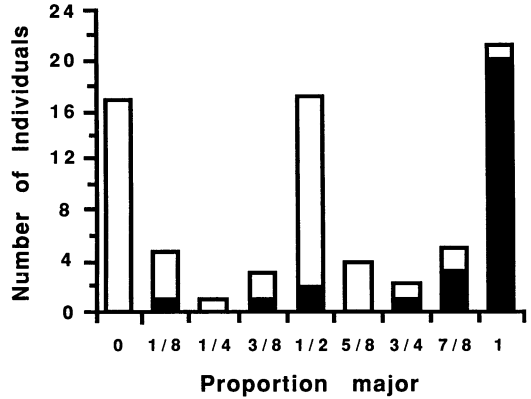


FIG. 6. Histogram of the frequency of individuals with proportions of the *major* genome totaled from diploid number, *Adh*, *Ldh-1*, and *Pep-B-1* marker systems (see Tables 1 and 2). The solid portion of each bar represents the number of individuals in the class that had the mtDNA of *major*.

curred frequently in our sample. There is only one individual in the  $1/4$  class and two in the  $3/4$  class, one of which (number 2 in Table 1) must be the product of a hybrid  $\times$  hybrid cross, not a backcross. The genotypes that are expected to occur with the greatest frequency in a backcross make up the least frequent class in our sample.

If individuals in the  $3/8$ – $5/8$  *major* range (Fig. 6) have reduced fertility, it would mean that many of these hybrid types (24 of 41 hybrids in our sample) must be regenerated each generation. This is even more striking considering that only nine of the 41 hybrid individuals could be classified as  $F_1$ 's on the basis of all data sets. This means that 8 of the 17 individuals in the  $1/2$  *major* class are not the progeny of a pure parental cross. The most probable parentage of these individuals involves highly backcrossed animals (the  $1/8$  and  $7/8$  *major* categories). If these highly backcrossed individuals are fertile, then they are able to reproduce effectively only with the two parental types or each other. Crosses of  $1/8$  *major* to pure *major*,  $7/8$  *major* to pure *knoxjonesi*, and  $1/8$  *major* to  $7/8$  *major* (Fig. 6) would produce individuals in the  $3/8$ – $5/8$  *major* range.

If the origin of the different classes in the hybrid zone follows this scenario, then there should be close geographic association of individuals that are heterozygous for all marker systems (and thus potentially ster-

ile) and the parental types required to produce these individuals. The geographic distribution of individuals having the various genotypes (see Fig. 4) reveals a geographic distribution of genotypes that is consistent with the explanation of continuous production of highly heterozygous genotypes. We conclude that these data are most compatible with the predictions of the dynamic-equilibrium model (Moore, 1977) and the dispersal-selection model (Endler, 1977) for maintaining the zone.

### Conclusions

These two taxa of pocket gophers represent discrete genetic entities that interact in a narrow hybrid zone. The levels of introgression in mtDNA and nuclear markers are concordant, and a 20/80 definition of the zone corresponds to a width of three kilometers. Parental types overlap in our sample over a few hundred meters. It is probable that premating isolating mechanisms block the mating of male *knoxjonesi* with female *major*. Postmating isolation, involving a low level of fertility in  $F_1$  individuals and other highly heterozygous hybrids, probably is also operating. It is postulated that all highly heterozygous hybrid males are sterile and that highly heterozygous hybrid females experience a reduced level of fertility. The relatively high numbers of these individuals are believed to be generated through crosses between parental types and through crosses between highly backcrossed individuals and parental types. The structure of the zone is best explained by the "dynamic equilibrium" model. These results indicate that *major* and *knoxjonesi* are acting like biological species with gene pools that are effectively isolated.

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