GENIC DIFFERENTIATION BETWEEN TWO CHROMOSOMAL RACES OF POCKET GOPHERS, GEOMYS BURSARIUS

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

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May, 1980

Bohlin, Raymond G., Genic Differentiation Between Two Chromosomal Races of Pocket Gophers, Geomys Bursarius. Master of Science (Biology), May, 1980, 60 pp., 5 tables, 11 illustrations, 52 titles.

Genic data from two chromosomal races of Geomys bursarius from a contact zone in central Texas indicated that the two races possessed distinct gene pools which would define them as separate species. Data from proteins encoded from 21 loci in this study substantiated this hypothesis. A pattern of alternately fixed alleles at the ADH-1, MDH-2, LDH-1, and IDH-1 loci with no apparent gene flow in zones of contact strongly suggested that these two races should be designated as separate species. Levels of heterozygosity and high F_{ST} values indicate that genomic structuring within Geomys is most heavily influenced by high levels of inbreeding and low migration rates. Fossorial rodents were suggested to undergo speciation primarily through parapatric means.

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CHAPTER I

INTRODUCTION

The amount of genic differentiation accompanying speciation has been the focus of many investigations into the genetic structure of natural populations (Ayala et al., 1975; Avise, 1976; Zimmerman et al., 1978). The primary conclusion at this point is that a wide range of patterns of genic differentiation exists, depending upon the taxonomic group being studied. This range indicates that not all speciation events are accompanied by large-scale genic reorganization (Avise, 1976). A major question is how this genic variation, as measured by gel electrophoresis, is being maintained in natural populations. Is it by adaptation and selection or by random and neutral processes (Lewontin, 1974; Selander, 1976)? This has led to a proliferation of studies attempting to relate patterns of genic variation to morphological, physiological, behavioral, and ecological parameters of the organism (Levins, 1968; Selander and Kaufman, 1973; Selander, 1976).

Fossorial rodents have been studied extensively to determine patterns of genomic structuring relative to their specialized habits of a total subterranean niche. A large amount of allozymic data for fossorial rodents has been obtained from three genera, Spalax (Nevo and Shaw, 1972),

Thomomys (Nevo et al., 1974; Patton et al., 1972; Patton and Yang, 1977; Patton and Feder, 1978) and Geomys (Selander et al., 1974; Penney and Zimmerman, 1976; Zimmerman and Gayden, 1979). The unique ecological and behavioral correlates which these rodents have in common include (1) the occupation of a uniform subterranean habitat, (2) the establishment of isolated populations based on soil types, (3) a high degree of territoriality, and (4) extremely low individual vagility. This results in an island model (Wright, 1943) and creates a mosaic distribution greatly affecting gene flow between local demes. As a result there is often extreme variation karyologically (Honeycutt, 1978) and genically (Zimmerman and Gayden, 1979).

The wide range of heterozygosities of fossorial rodents, from 2.3% (Zimmerman and Gayden, 1979) to 9.3% (Patton and Yang, 1977), has led to a number of hypotheses purporting to explain the genetic structure of fossorial rodent populations. Among these are selection for homozygosity due to a uniform environment (Nevo et al., 1974); random genetic drift (Penney and Zimmerman, 1976); founder effect (Selander et al., 1974); bottlenecking (Patton and Yang, 1977); and high levels of inbreeding with little migration (Zimmerman and Gayden, 1979).

Although any or all of these factors may play a role for an individual species, it is difficult to determine one strategy which explains genomic structuring in all fossorial

rodents. The high levels of genic heterogeneity found in a wide spectrum of fossorial rodents occupying a similar niche appears to contradict the hypothesis of selection for homozygosity in a uniform environment (Selander, 1976; Zimmerman and Gayden, 1979). Genetic drift, founder effect, and bottlenecking undoubtedly play a role in the genomic structuring of fossorial rodents as a result of low vagility and the formation of isolated populations. Zimmerman and Gayden (1979) summarized that perhaps restricted gene flow and high levels of inbreeding were most influential in determining patterns of genic variation in Geomys. This was based on high standardized variances of gene frequencies (F_{ST}) recorded for Thomomys bottae (Patton and Yang, 1971) and Geomys bursarius (Zimmerman and Gayden, 1979), whose F_{STS} were higher than any reported for an animal species. The standardized variance of gene frequencies (Wright, 1965) is an index of the probability that allelic frequencies at a given locus in a population are not drawn from one panmictic population. The higher the F_{ST}, the higher the probability that stochastic processes and inbreeding might have an effect on the genomic structure of the population.

Geomys bursarius

The karyological structure of G. bursarius has been studied extensively in recent years (Hart, 1971; Kim, 1972; Baker et al., 1973; Selander et al., 1974; Pembleton and

Baker, 1978; Honeycutt, 1978). Eight distinct chromosomal races of G. bursarius occur in Texas and adjacent states. These races are distinguishable by differences in diploid number (2N), fundamental number (FN), morphology of the X chromosome, and the degree of chromosomal polymorphism. Honeycutt (1978) has arranged the eight races into three groups based on similarities in the above given characteristics. The lutescens group (races A, B, C, D) consists of 2N ranging from 69 to 72, FN from 68 to 72, and an acrocentric X chromosome. The attwateri group (races F and G) has a 2N of 70, FN of 72 or 74, and the X chromosome is submetacentric. The breviceps group (races E, H) has a 2N of 74, FN of 72 or 74, and the X chromosome is submetacentric.

Zimmerman and Gayden (1979) investigated levels of genic heterogeneity among 21 local populations (demes) of chromosomal races D and E in McClennan and Falls Counties Texas. The two races occur in parapatry along the Brazos River and are separated by 12 km. This was done in contrast to most other genetic studies which examine levels of genic heterogeneity in populations spread over a wide geographic area. The hypothesis under study was that the general biology of pocket gophers as previously discussed should lead to local differentiation as well as geographical differentiation. Mean genic identity, $\bar{I}$ (I values range from 0, where there are no common alleles, to 1.0 where all alleles are identical between two populations (Nei, 1972)),

between the two races was .696, while mean I values among populations of the chromosomal races were .891 for race D and .828 for race E. These are low values, indicating a higher degree of differentiation, compared to $\bar{I}$ values for local populations and subspecies of other taxonomic groups (Zimmerman et al., 1978). Also by showing more common alleles among races than between races, it is suggested that these two races can be separated genically as well as chromosomally in this region. Further evidence of clear genic differentiation was obtained by use of multivariate analysis. Differentiation among the 21 local populations of the two races was represented by a projection (three-dimensional plot) of these same populations with respect to the first three principal components extracted from a correlation matrix among allelic frequencies. Component I, influenced mainly by alternately fixed alleles at the LDH-1, ES-8, and IDH-1 loci, clearly separated the nine populations of race D from the twelve populations of race E.

The relatively high levels of interdemic and interracial heterogeneity found in G. bursarius, combined with high F_{ST} values, indicate that restricted gene flow and high levels of inbreeding might contribute to the genomic structuring of this species. There appears to be little selection operating for existence in a homogeneous environment among the proteins assayed. Although Zimmerman and Gayden (1979) found low levels of heterozygosity (2.3%), overall

levels of heterozygosity as summarized by Selander (1976) were not found to be significantly different than those for other rodents (6.0%). If selection in a homogeneous environment were operating, heterozygosities would be expected to be lower than for other rodents, and interpopulation heterogeneity would be low as well.

Objectives

This study was designed to (1) further clarify the factors affecting the genomic structuring of fossorial rodent populations; (2) determine whether the pattern of genic differentiation found by Zimmerman and Gayden (1979) is characteristic of the two chromosomal races over their entire range or whether it is strictly a local phenomenon; and if these genic patterns hold true, (3) determine if sufficient evidence exists to classify these two races as possessing separate gene pools, hence defining them as separate species. The hypothesis, then, is that genic variation within and between these two races is an indication of two separate gene pools. Attempts were made to determine patterns of genic expression and genic variation in developing embryos of Geomys bursarius. It was hoped that information on the genetic structure of the embryo population (next generation) would shed light on the role of genetic drift and inbreeding in this species by observing gene frequences shifting between generations.

CHAPTER II

MATERIALS AND METHODS

Two hundred forty-eight pocket gophers were collected from 25 populations in Texas, Oklahoma, and Louisiana (Figs. 1 and 2). Both chromosomal races are represented from over wide portions of their ranges. Chromosomal race E, G. b. sagitallus (Honeycutt, 1978), ranges from the Brazos River in central Texas, to western Louisiana, southwestern Arkansas, and eastern Oklahoma. Race D, G. b. major (Honeycutt, 1978) occurs from McClennan County in Central Texas, north to western Oklahoma, and west throughout northwest Texas. Hereafter, race E will be referred to as the eastern race and race D will be referred to as the western race.

Fourteen populations of the western race were obtained from the following localities, with sample sizes in parentheses: 1. Denton Co., TX, Jct. U.S. Hwy. 380 and FM 720 (14); 2. Denton Co., TX, 2.4 km W Spur 428 and FM 428 (17); 3. Denton Co., TX, 4.0 km N U.S. Hwy. 380 and U.S. Hwy. 377 (14); 4. Wise Co., TX, 9.7 km N Decatur (12); 5. Fischer Co., TX, 11.3 km N Sweetwater (16); 6. Hardeman Co., TX, 6.4 km N Quanah (7); 7. Wilbarger Co., TX, 1.6 km E Vernon (15) 8. Cotton Co., OK, 11.3 km SSW Randlett on St. Hwy. 36 (11); 9. Cleveland Co., OK, 1.4 km N Jct. St. Hwy. 9 and Jenkins

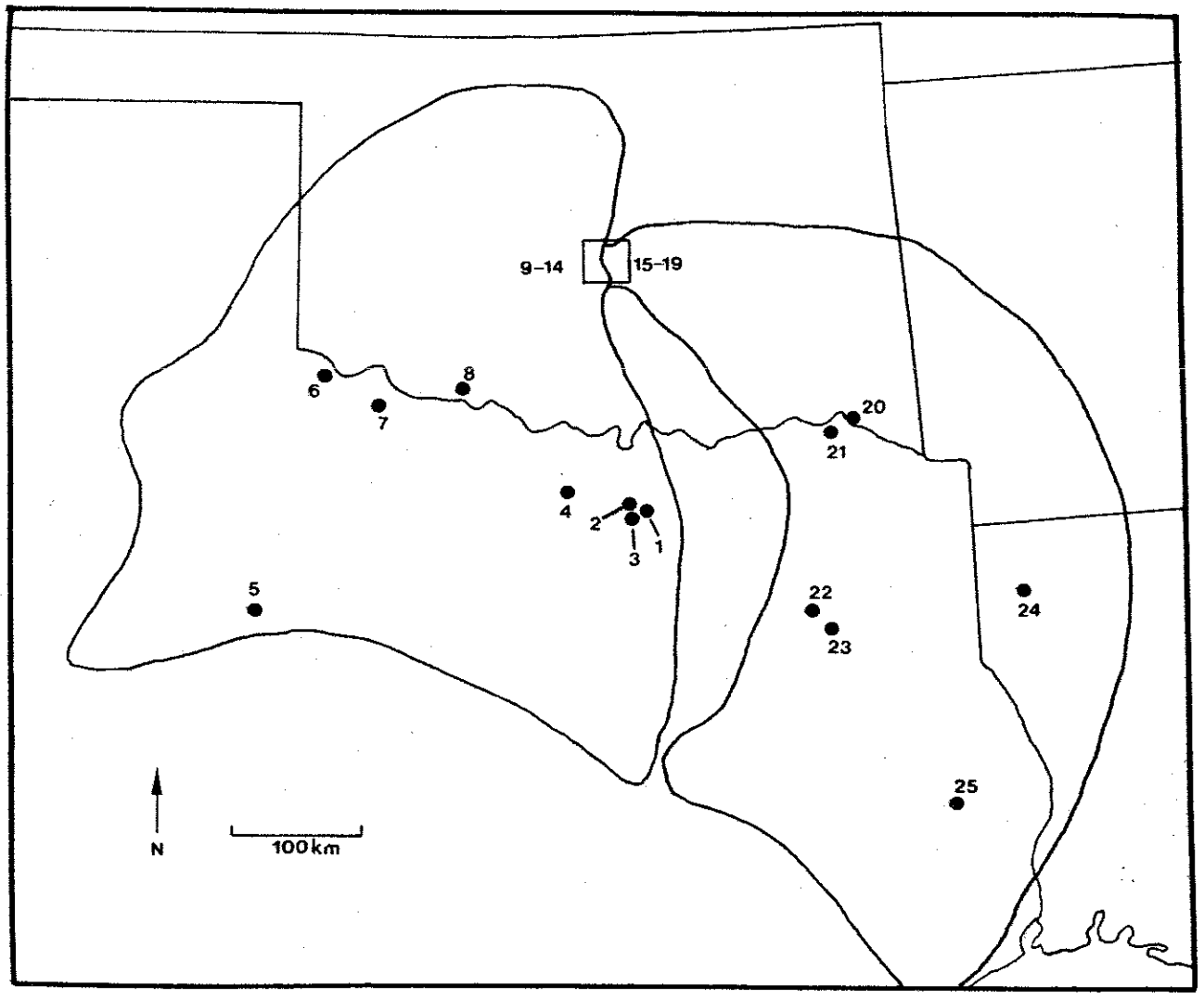

Fig. 1--Geographical locations for 25 populations of Geomys bursarius in Texas, Oklahoma, and Louisiana.

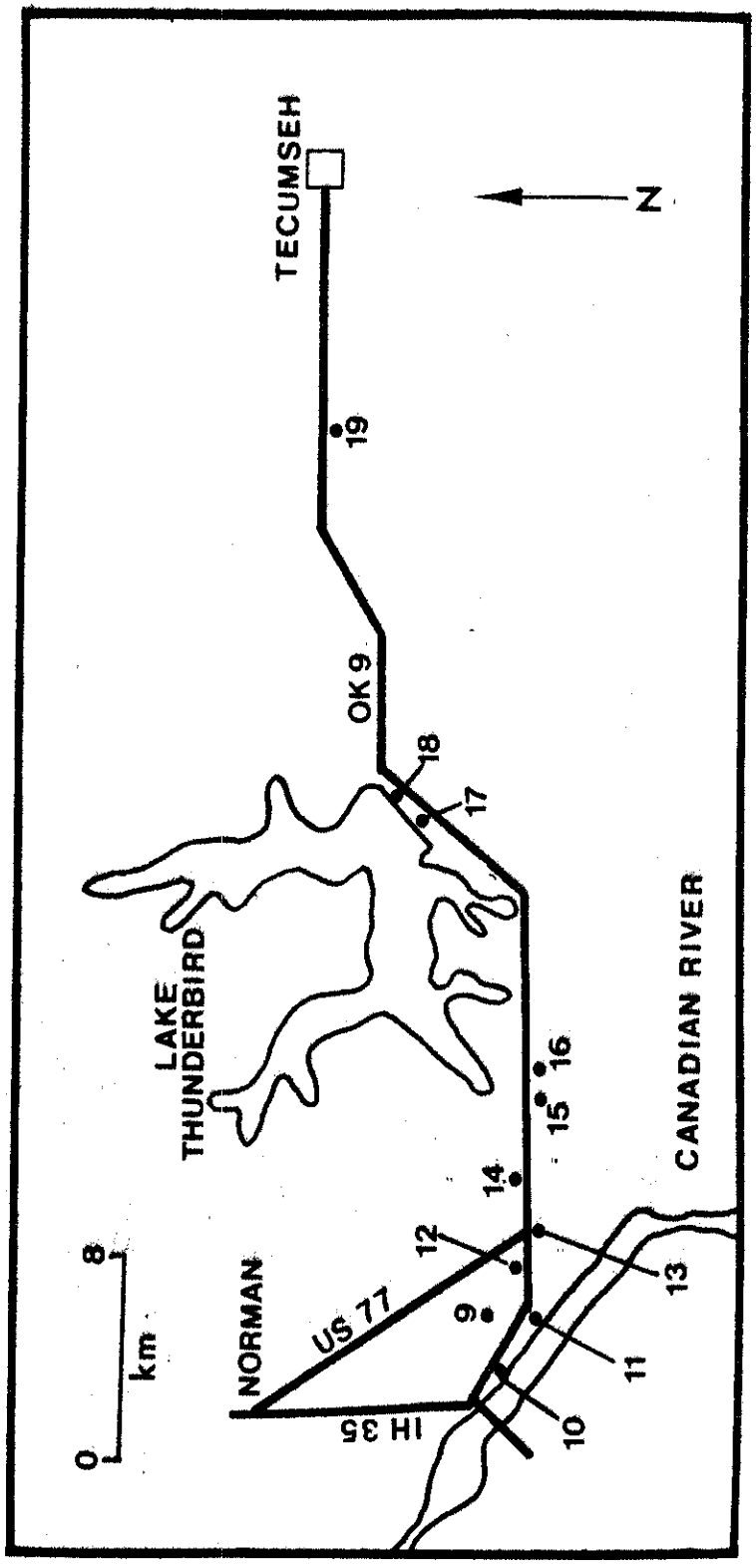

Fig. 2--Locations of populations 9 through 19 along a transect of the contact zone between the eastern and western races near Norman, Oklahoma.

Ave. (13); 10. Cleveland Co., OK, 4.5 km W Jct. U.S. Hwy 77 and St. Hwy. 9 (5); 11. Cleveland Co., OK, 1.9 km W Jct. U.S. Hwy. 77 and St. Hwy. 9 (5); 12. Cleveland Co., OK, 0.6 km W Jct. U.S. Hwy. 77 and St. Hwy. 9 (2); 13. Cleveland Co., OK, 0.8 km S Jct. U.S. Hwy. 77 and St. Hwy. 9(3); 14. Cleveland Co., OK, 2.9 km E Jct. U.S. Hwy 77 and St. Hwy. 9 (2).

Eleven populations of the eastern race were obtained from the following localities, with sample sizes in parentheses: 15. Cleveland Co., OK, 6.1 km E Jct. U.S. Hwy. 77 and St. Hwy. 9 (2); 16. Cleveland Co., OK, 7.2 km E Jct. U.S. Hwy. 77 and St. Hwy. 9 (3); 17. Cleveland Co., OK, 18.0 km E Jct. U.S. Hwy 77 and St. Hwy. 9 (3); 18. Cleveland Co., OK, 19.2 km E Jct. U.S. Hwy 77 and St. Hwy 9 (2); 19. Pottowatomie Co., OK, 32.4 km E Jct. U.S. Hwy. 77 and St. Hwy 9 (3); 20. McCurtain Co., OK, Jct. St. Hwy. 37 and Red River (8); 21. Red River Co., TX, 2.4 km NW Manchester (50); 22. Smith Co., TX, 6.4 km W Jct. U.S. Hwy. 69 and I-20 (3); 23. Smith Co., TX, 5.3 km S Jct. Loop 323 and U.S. Hwy. 69 (12); 24. Bossier Parish, LA, 1.1 km W Jct. U.S. Hwy. 71 and St. Hwy. 511 (16); 25. Tyler Co., TX, 8.05 km E Colmesneil (10).

Populations 9 through 19 were obtained in an attempt to reproduce the transect run by Baker and Glass (1951) in the northern contact zone between the races near Norman Oklahoma (Fig. 2).

Individual pocket gophers were secured with the use of Victor Gopher Traps (Woodstream Corporation). Gophers were sacrificed in the field and packed in dry ice for transportation to the laboratory. Individuals were sexed and measured for total length, tail length, and length of hindfoot. Kidney and a portion of liver were extracted, homogenized, and electrophoresed according to methods described by Selander et al. (1971) and Kilpatrick and Zimmerman (1976). In addition, esterases were run on the following system for better resolution. The electrode buffer was a 0.06M LiOH, 0.30M Boric Acid solution. The Gel buffer was a 0.29M Tris, 0.05M Citric Acid, 0.006M LiOH, and 0.03M Boric Acid solution diluted 1:20 with distilled water. Electrophoresis was carried out for 2.5 hours at 25 milliamps. Embryos were homogenized in toto in a 1:1 weight to volume dilution of tissue buffer. Skeletons of adults were saved for future comparisons of morphometric and genetic data. Individuals from populations 2, 10, 11 and 13 were captured alive and karyotyped according to the procedures of Lee (1969).

A 12% suspension of starch was used for all gels. Alleles were designated alphabetically in decreasing order of mobility. Proteins encoded by 20 loci were examined, including lactate dehydrogenase (LDH-1, LDH-2), 6-phosphogluconate dehydrogenase (6-PGD), isocitrate dehydrogenase (IDH-1, IDH-2), glutamate oxaloacetate transaminase (GOT-1,

GOT-2), leucine aminopeptidase (LAP-1), malic enzyme (ME-1), indophenol oxidase (IPO-1), malate dehydrogenase (MDH-1, MDH-2), α glycerophosphate dehydrogenase (α-GPD-1), alchohol dehydrogenase (ADH-1), sorbitol dehydrogenase (SDH-1), four esterases (ES-1, ES-2, ES-3, ES-4) and hemoglobin (Hb-α, Hb-β).

The four esterases were not scored according to conventional methods for mammals (Selander et al., 1971; Kilpatrick and Zimmerman, 1976). The higher resolution of the buffer system mentioned previously required a separate scoring system. Loci were designated with ES-1 having the greatest mobility and ES-4 being the least mobile. ES-1, ES-2, and ES-3 were monomers, with a single band representing homozygotes and a double band representing heterozygotes. ES-4 was a complex system of five bands which appeared to act as one system. Homozygosity for the a allele resulted in a five-band pattern, migrating faster than the five bands of the pattern resulting from homozygosity for the b allele. Heterozygotes were scored when five bands migrated the entire range of the aa and bb mobilities.

Frequencies of each allele detected by electrophoresis in a population were calculated. From these initial data, a number of indices were calculated for statistical analysis. Heterozygosity for a given locus (h) was calculated as the mean number of heterozygotes in a population.

Average heterozygosity (H) is the arithmetic mean of h

over all loci examined. H is an estimate of the total number of loci for which a randomly chosen individual will be heterozygous and is expressed as a proportion which may theoretically range from 0.0, no heterozygous loci, to 1.0, where all loci are heterozygous.

Normalized genic identity (Nei, 1972) between two populations at the j locus is defined as

$$Ij = \frac{\Sigma x_i y_i}{(\Sigma x_i^2 \Sigma y_i^2)^{\frac{1}{2}}}$$

where x_i and y_i represent the frequencies of the ith allele in populations X and Y, respectively. For all loci in a sample, the overall genetic identity of X and Y is defined as

$$I = \frac{J_{xy}}{(J_x J_y)^{\frac{1}{2}}}$$

where J_x, J_y and J_{xy} are the arithmetic means over all loci of x_i^2, y_i^2, $\Sigma x_i \Sigma y_i$, respectively. I is an estimate of the number of alleles which two populations share in common, is expressed as a proportion, and may theoretically range from 0.0, where there are no alleles in common, to 1.0, where all alleles are identical.

Genetic distance can be calculated using I, where

$$D = -\ln I$$

D is an estimate of the number of codon substitutions per locus since the two populations diverged. D is expressed in terms of a proportion, which when multiplied by 100, yields

the number of codon substitutions per 100 loci.

The standardized variance of frequencies at a locus across all populations is calculated as

$$F_{ST} = S_p^2 / \bar{p}\ (1-\bar{p})$$

where $\bar{p}$ is the arithmetic mean of the allelic frequencies of a given locus for all populations, and S_p^2 is the unstandardized variance of p. F_{ST} for monomorphic loci is always equal to zero (only one allele). Where there are two alleles, F_{ST} will be the same when calculated with either frequency. However, if there are more than two alleles present, an F_{ST} must be calculated for each set of allelic frequencies. A mean $\bar{F}_{ST}$ is an index of the probability that genic frequencies at a given locus in populations are not drawn from one panmictic population. The higher the F_{ST}, the greater the effect inbreeding may have on maintaining interdemic heterogeneity.

Multivariate analyses were performed, using gene frequencies on NT-SYS programs (Rohlf et al., 1969). Populations were clustered, using an unweighted pair-group method on the correlation matrix. Genic differentiation is represented by a projection of all populations with respect to the first three principal components extracted from the correlation matrix of gene frequencies. A shortest minimally connected network was computed in the original character space and was utilized to connect most similar localities in the three-dimensional plot.

CHAPTER III

RESULTS

Genic Variation

Of the 21 loci examined, nine were found to be monomorphic in all 25 populations: LAP-1, IDH-2, MDH-1, ME-1, GOT-2, LDH-2, IPO and the two hemoglobin loci. Within the western race, MDH-2 and ADH-1 were monomorphic and within the eastern race IDH-1, LDH-1, and α-GPD were monomorphic. The allelic frequencies for the polymorphic loci within the western race are listed in Table 1. GOT-1, α-GPD, 6-PGD, LDH-1, ES-1, and ES-4 showed only slight variation, exhibiting fixation for a common allele in most populations. IDH-1, ES-2, ES-3, and SDH-1 were polymorphic, at least to some degree, in almost all populations. The allelic frequencies for the polymorphic loci within the eastern race are provided in Table 2. MDH-2, GOT-1, 6-PGD, ES-1, ES-4, and ADH-1 were only mildly polymorphic, exhibiting fixation for a common allele in most populations. ES-2, ES-3, and SDH-1 showed high degrees of polymorphism in almost all populations. Due to inconsistent results, genic data for ADH-1 from populations 16, 22 and 25 were unattainable. Similar difficulty was encountered with SDH-1 in populations 15, 22 and 25.

TABLE 1

ALLELIC FREQUENCIES AND F_{ST} VALUES FOR POLYMORPHIC LOCI IN G. B. MAJOR (WESTERN RACE)

POPULATION	n	IDH-1		GOT-1		∝-GPD			6-PGD	
		a	b	a	b	a	b	c	a	b
* 1	14	1.00	0.00	0.36	0.64	0.00	1.00	0.00	0.93	0.07
* 2	17	0.94	.06	.41	.59	.00	1.00	.00	.88	.12
* 3	14	1.00	.00	.39	.61	.00	1.00	.00	.89	.11
* 4	12	0.88	.12	.00	1.00	.00	1.00	.00	1.00	.00
* 5	16	.34	.66	.00	1.00	.12	0.88	.00	1.00	.00
6	7	.64	.36	.00	1.00	.00	1.00	.00	1.00	.00
* 7	15	.50	.50	.00	1.00	.00	1.00	.00	1.00	.00
* 8	11	.27	.73	.00	1.00	.00	1.00	.00	1.00	.00
* 9	13	.54	.46	.00	1.00	.08	0.88	.04	1.00	.00
10	5	.80	.20	.00	1.00	.00	1.00	.00	1.00	.00
11	5	.80	.20	.00	1.00	.00	1.00	.00	1.00	.00
12	2	.25	.75	.00	1.00	.00	1.00	.00	1.00	.00
13	3	.83	.17	.00	1.00	.00	1.00	.00	1.00	.00
14	2	.50	.50	.00	1.00	.00	1.00	.00	1.00	.00
F_{ST}		0.428		0.324		0.093	0.106	0.040	0.080	

* POPULATIONS USED IN F_{ST} CALCULATION

TABLE 1 Continued

POPULATION	n	LDH-1		ES-1			ES-2		
		a	b	a	b	c	a	b	c
* 1	14	0.00	1.00	0.14	0.86	0.00	0.82	0.11	0.07
* 2	17	.09	0.91	.12	.88	.00	.38	.38	.24
* 3	14	.00	1.00	.00	.89	.11	.50	.43	.07
* 4	12	.08	0.92	.25	.75	.00	.29	.29	.42
* 5	16	.00	1.00	.00	1.00	.00	1.00	.00	.00
6	7	.00	1.00	.00	1.00	.00	0.07	.93	.00
* 7	15	.00	1.00	.00	1.00	.00	.27	.53	.20
* 8	11	.00	1.00	.00	1.00	.00	.73	.27	.00
* 9	13	.00	1.00	.00	1.00	.00	.31	.23	.46
10	5	.00	1.00	.00	1.00	.00	.40	.60	.00
11	5	.00	1.00	.00	1.00	.00	.60	.40	.00
12	2	.00	1.00	.00	1.00	.00	.50	.50	.00
13	3	.00	1.00	.00	1.00	.00	.67	.33	.00
14	2	.00	1.00	.00	1.00	.00	.75	.25	.00
F_{ST}		0.075		0.153	0.121	0.112	0.329	0.145	0.219

*POPULATIONS USED IN F_{ST} CALCULATION

TABLE 1 Continued

POPULATION	n	ES-3 a	ES-3 b	ES-3 c	ES-4 a	ES-4 b	SDH a	SDH b
* 1	14	0.21	0.46	0.32	0.79	0.21	0.40	0.60
* 2	17	.44	.38	.18	.88	.12	.92	.08
* 3	14	.29	.64	.07	1.00	.00	.73	.27
* 4	12	.17	.75	.08	0.92	.08	.00	1.00
* 5	16	.41	.16	.44	1.00	.00	.41	0.59
6	7	.29	.43	.28	0.57	.43	.29	.71
* 7	15	.03	.97	0.00	1.00	.00	.27	.73
* 8	11	.14	.73	.14	0.82	.18	.86	.14
* 9	13	.38	.58	.04	1.00	.00	.79	.21
10	5	.00	.80	.20	1.00	.00	1.00	.00
11	5	.00	.90	.10	1.00	.00	1.00	.00
12	2	.25	.75	.00	1.00	.00	1.00	.00
13	3	.00	.00	1.00	1.00	.00	0.00	1.00
14	2	.00	1.00	0.00	1.00	.00	.00	1.00
F_{ST}		0.110	0.257	0.170	0.112		0.430	
$\bar{F}_{ST}$ =		0.184						

*POPULATIONS USED IN F_{ST} CALCULATION

TABLE 2

ALLELIC FREQUENCIES AND F_{ST} VALUES FOR POLYMORPHIC LOCI IN G.B. SAGITALLUS (EASTERN RACE)

POPULATION	n	MDH-2		GOT-1			6-PGD	
		a	b	a	b	c	a	b
15	2	0.00	1.00	0.00	1.00	0.00	0.00	1.00
16	3	.00	1.00	.00	1.00	.00	.00	1.00
17	3	.00	1.00	.00	1.00	.00	.00	1.00
18	2	.00	1.00	.00	1.00	.00	.00	1.00
19	3	.00	1.00	.00	1.00	.00	.00	1.00
*20	8	.00	1.00	.00	1.00	.00	.94	.06
*21	50	.00	1.00	.00	1.00	.00	1.00	.00
22	3	.00	1.00	.00	1.00	.00	1.00	.00
*23	12	.42	0.58	.00	0.92	.08	1.00	.00
*24	16	.06	.94	.00	1.00	.00	1.00	.00
*25	10	.00	1.00	.00	0.80	.20	1.00	.00
F_{ST}		0.386		0.145			0.061	

*POPULATIONS USED IN F_{ST} CALCULATION

TABLE 2 Continued

POPULATION	n	ES-1			ES-2			ES-3		
		a	b	c	a	b	c	a	b	c
15	2	0.00	1.00	0.00	0.25	0.75	0.00	0.00	1.00	0.00
16	3	.00	1.00	.00	.50	.50	.00	.00	0.67	.33
17	3	.00	1.00	.00	.67	.33	.00	.17	.50	.33
18	2	.00	1.00	.00	.00	.00	1.00	1.00	.00	.00
19	3	.00	1.00	.00	.00	1.00	.00	.33	.67	.00
*20	8	.12	.88	.00	.00	1.00	.00	.00	1.00	.00
*21	50	.03	.97	.00	.00	1.00	.00	.00	1.00	.00
22	3	.00	.67	.33	.33	.00	.67	.83	.17	.00
*23	12	.00	1.00	.00	.21	.42	.37	.46	.37	.17
*24	16	.0	1.00	.00	.09	.75	.16	.16	.59	.25
*25	10	.20	.80	.00	.25	.20	.55	.20	.70	.10
F_{ST}			0.118		0.138	0.577	0.342	0.164	0.377	0.127

*POPULATIONS USED IN F_{ST} CALCULATION

TABLE 2 Continued

POPULATION	n	ES-4		ADH		SDH			
		a	b	b	c	a	b	c	d
15	2	1.00	0.00	1.00	0.00	..	..	..	..
16	3	1.00	.00	..	..	1.00	0.00	0.00	0.00
17	3	1.00	.00	1.00	.00	1.00	.33	.33	.33
18	2	1.00	.00	0.50	.50	0.50	.50	.00	.00
19	3	1.00	.00	.00	.00	1.00	.00	.00	.00
*20	8	1.00	.00	.94	.06	0.50	.50	.00	.00
*21	50	1.00	.00	1.00	.00	.64	.34	.02	.00
22	3	1.00	.00	..	..	..	..	..	..
*23	12	1.00	.00	1.00	.00	.75	.25	.00	.00
*24	16	0.59	.41	0.78	.22	.54	.46	.00	.00
*25	10	.50	.50	..	..	..	..	..	..
F_{ST}		0.424		0.166		0.052	0.055	0.020	
$\bar{F}_{ST}$ =		0.225							

*POPULATIONS USED IN F_{ST} CALCULATION

The proportion of loci heterozygous per individual (H) ranged from 0.00%, in population 19 of the eastern race, to 8.89%, in population 6 of the western race (Table 3). $\bar{H}$ within the western race was 6.1%, while within the eastern race $\bar{H}$ was 3.9%. Although heterozygosity appears to be greater in the western race, the difference is not significant ($P[t]>0.20$), indicating that the patterns of heterozygosity within the two groups are not dissimilar. $\bar{H}$ for all 25 populations was 5.1%, which is well within the range of those found for other rodents and not appreciably different from the mean of 5.2% for fossorial rodents (Selander, 1976).

Geographic Variation

Although overall patterns of genic variation and heterogeneity were similar within all twenty-five populations, there are definitive patterns of variation between the western and eastern races. Three loci exhibit patterns of alternately fixed alleles between eastern and western races: ADH-1, MDH-2, and LDH-1. A fourth locus, IDH-1, shows a similar pattern, with fixation or predominance of alternate alleles in the two races. ADH-1 and MDH-2 patterns are represented in Figures 3 and 4. Within western populations, 1 through 14, fixation has occurred for the ADH_a and $MDH\text{-}2_a$ alleles. In eastern populations, 15 through 25, the ADH_b and $MDH\text{-}2_b$ alleles predominate but are not

TABLE 3

HETEROZYGOSITIES FOR 25 POPULATIONS OF *GEOMYS BURSARIUS* IN TEXAS, OKLAHOMA AND LOUISIANA

WESTERN RACE		EASTERN RACE	
POPULATION	H	POPULATION	H
1	0.054	15	0.025
2	.047	16	.050
3	.060	17	.079
4	.048	18	.048
5	.074	19	.000
6	.088	20	.019
7	.074	21	.007
8	.082	22	.017
9	.077	23	.074
10	.067	24	.064
11	.043	25	.047
12	.048		
13	.016		
14	.071		
$\bar{H}$ = .06 ± .005 S.E.		$\bar{H}$ = .039 ± .008 S.E.	
OVERALL MEAN = 0.051 ± .005 S.E.			

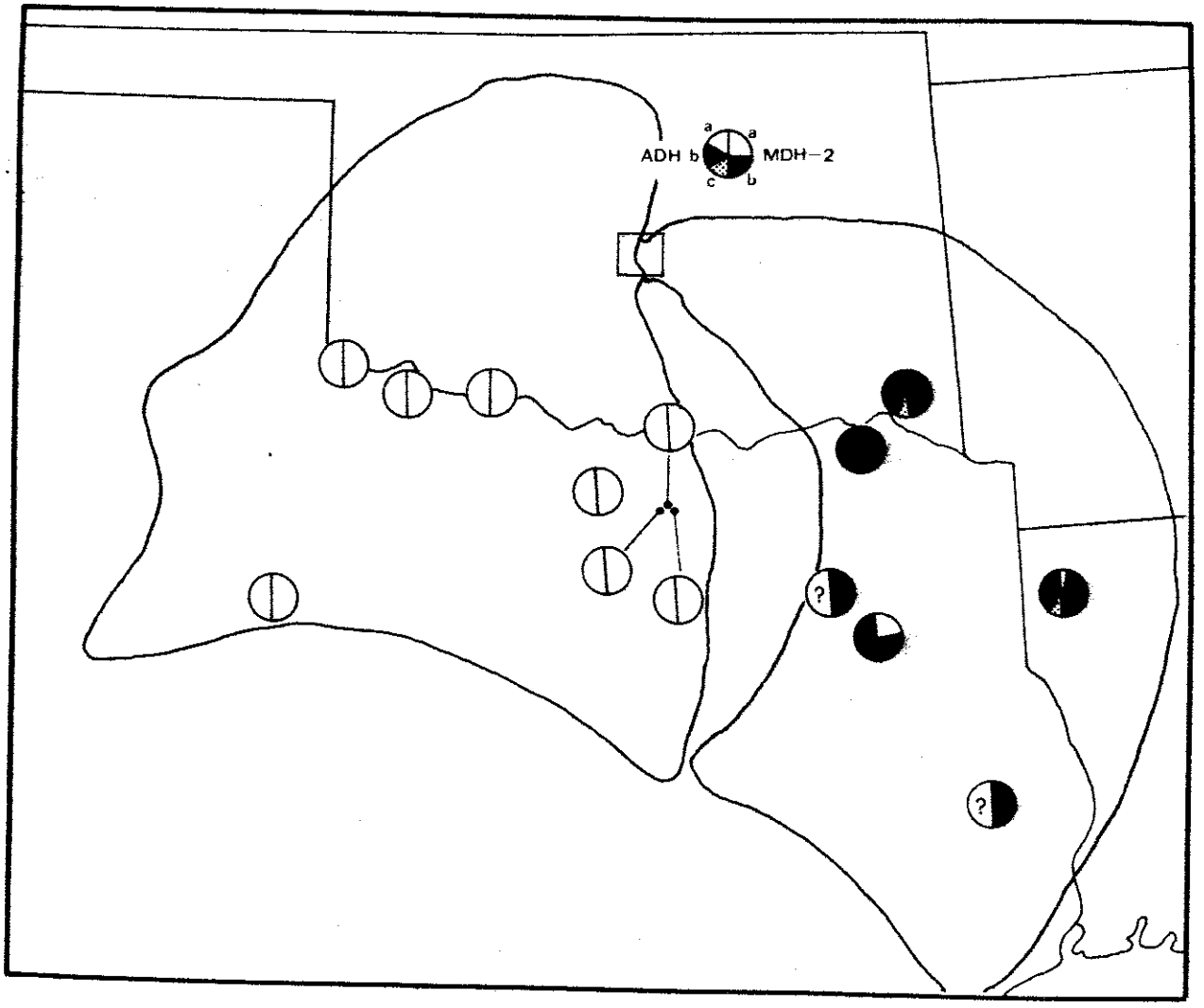

Fig. 3--Gene frequencies for ADH-1 and MDH-2 of populations 1-8 of the western race and 20-25 of the eastern race.

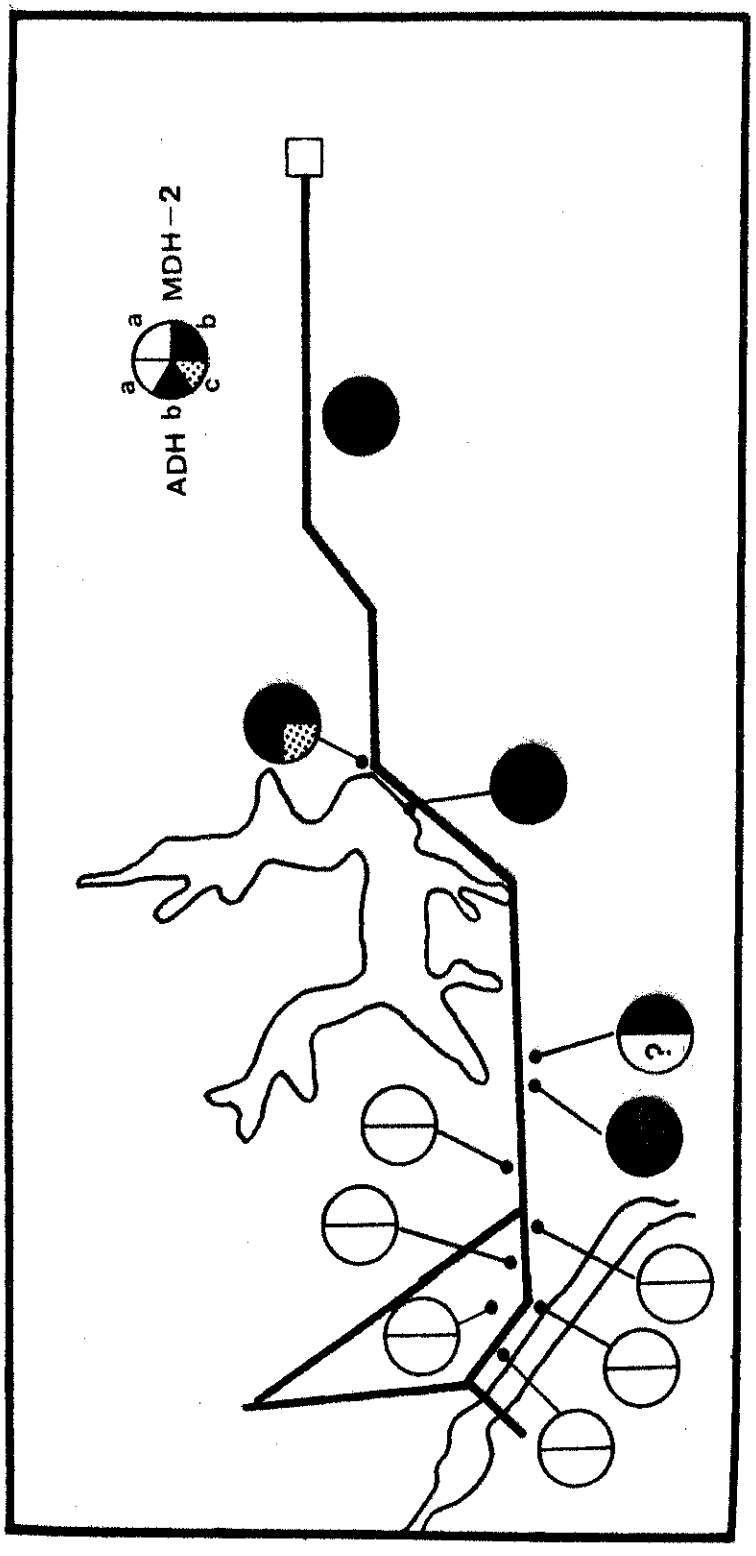

Fig. 4--Gene frequencies for ADH-1 and MDH-2 of populations 9 through 19 near Norman, Oklahoma.

fixed in all populations. The ADH_a allele does not appear in any of the eastern populations. The source of polymorphism is the presence of a third allele, ADH_c, in a few eastern localities. The MDH-2_a allele appears in only two of the eleven eastern populations, at low frequencies, while in all other eastern populations the MDH-2_b allele is fixed. LDH-1 and IDH-1 patterns are represented in Figures 5 and 6. The LDH-1_b allele is fixed in most western populations except 2 and 4. The LDH-1_a allele occurs in only two populations, 2 and 4, at frequencies of less than 10%. Within the eastern populations the LDH-1 allele is found exclusively. The IDH-1 pattern is slightly different in that there is a high degree of polymorphism within the western populations. Within eastern populations, only the IDH-1_b allele occurs. For each pair of alleles at the four loci, there is a similar pattern of variation. ADH-1 and MDH-2 show complete fixation for the a and b alleles, respectively, in all western populations, while there is a small degree of polymorphism in eastern populations. LDH-1 and IDH-1 are fixed for the a and b alleles, respectively, in the eastern populations, while in the western populations there is some degree of polymorphism at both loci. At the contact zone near Norman, Oklahoma, the different patterns of allelic frequencies are separated by only 3.2 km.

Additional analysis of this differentiation is represented by projecting the twenty-five populations with respect

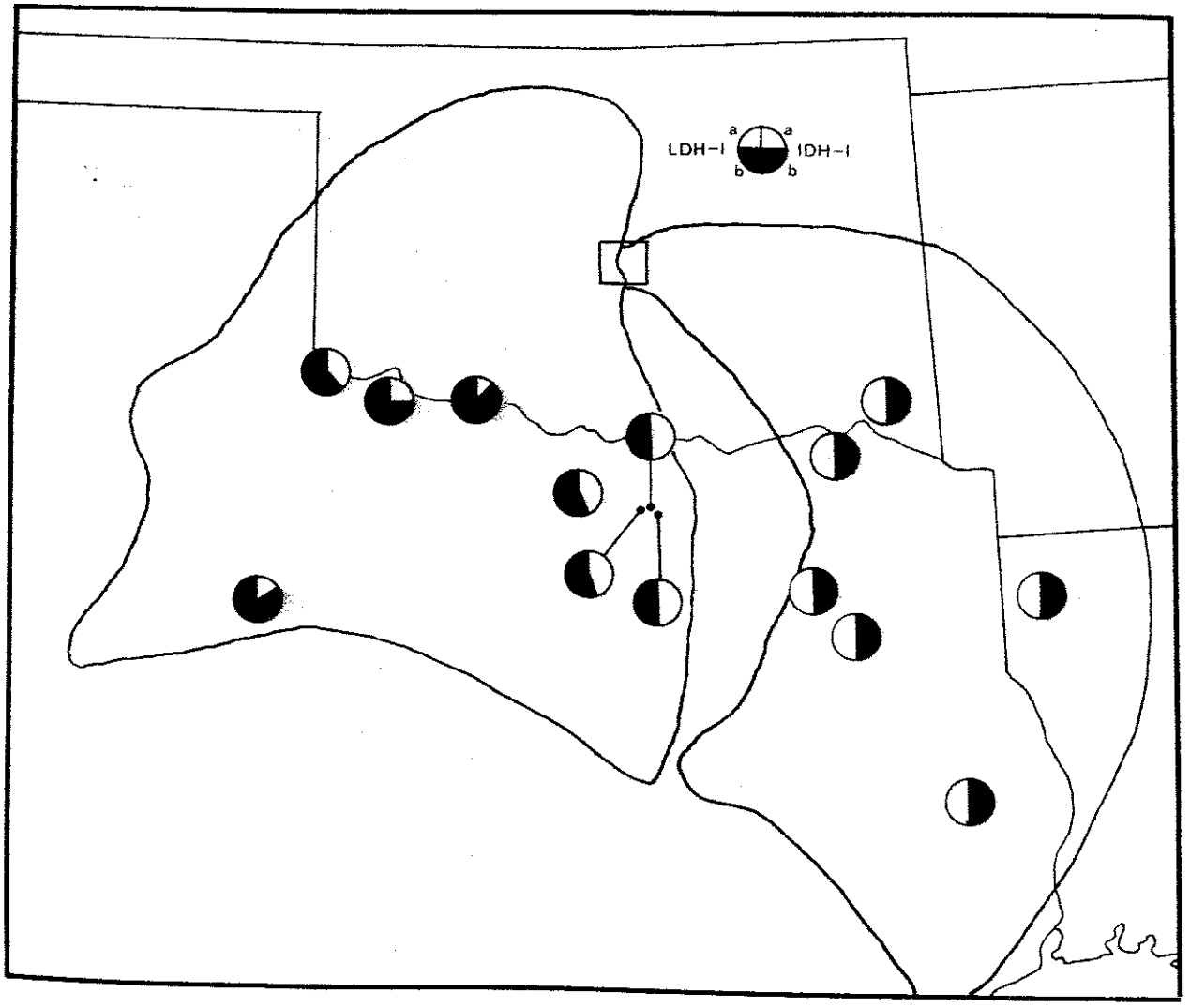

Fig. 5--Allelic frequencies of LDH-1 and IDH-1 for populations 1-8 of the western race and 20-25 of the eastern race.

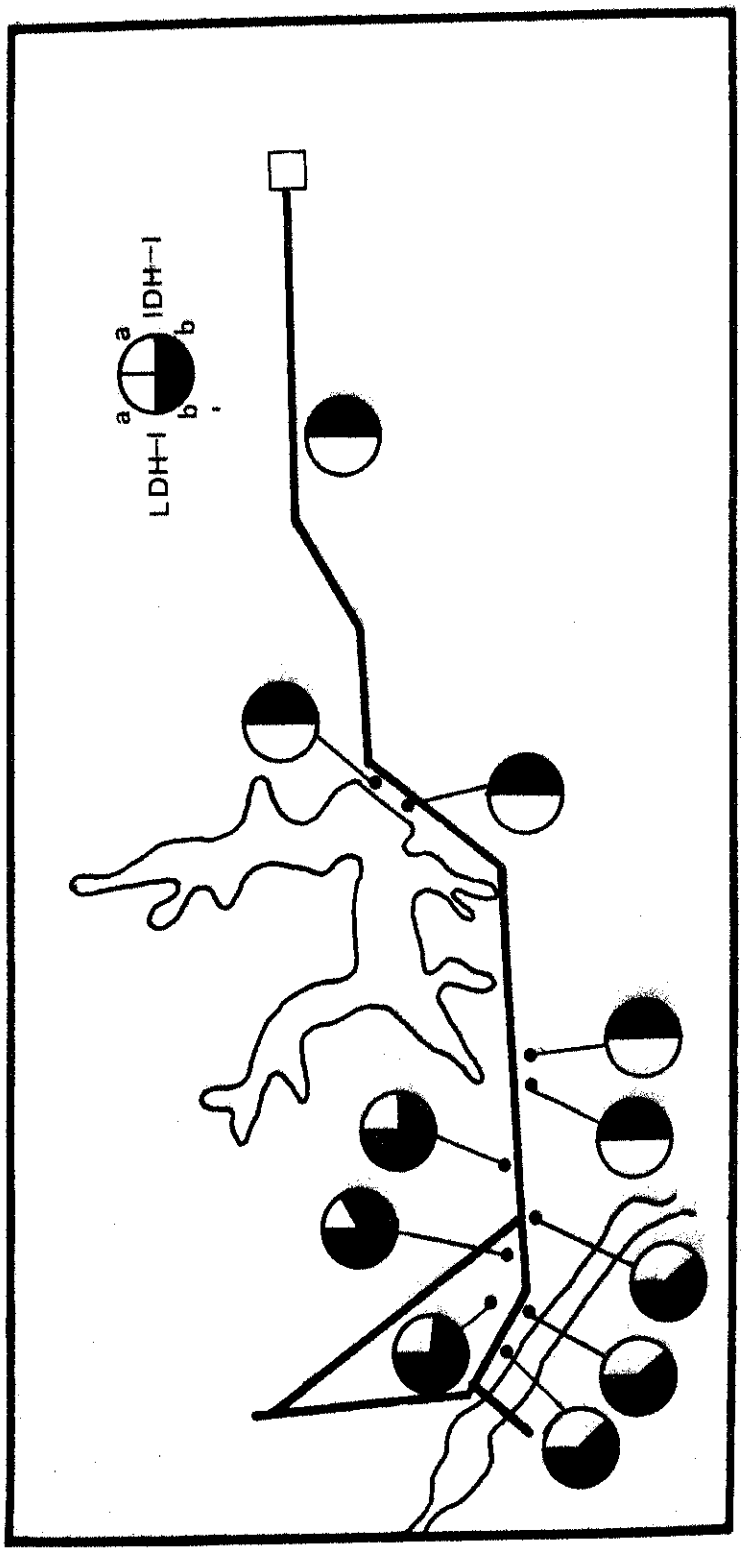

Fig. 6--Allelic frequencies of LDH-1 and IDH-1 for populations 9-19 near Norman, Oklahoma.

to the first three principal components extracted from a matrix of correlations among allelic frequencies (Fig. 7). Loadings, indicating the correlations of characters (= allelic frequencies) with the first three principal components, are given in Table 4. The first three principal components account for 55.1% of the total interlocality variance. This indicates that reducing the 32-dimensional character space to three dimensions results in some distortion of the original distances.

Although all 32 alleles influence each of the three components, Component I was most heavily influenced by the expression of alternate alleles at the ADH-1, MDH-2, LDH-1, and IDH-1 loci. This first component effectively separates the eastern from the western populations along the horizontal axis of the model. The eastern populations are located on the left of the model as a result of fixation or near-fixation for the ADH_b, $MDH\text{-}2_b$, $LDH\text{-}1_a$, and $IDH\text{-}1_b$ alleles. Western populations are clustered on the right, exhibiting fixation or near-fixation for the alternate alleles, $ADH\text{-}1_a$, $MDH\text{-}2_a$, $LDH\text{-}1_b$, and $IDH\text{-}1_a$. Component II was most heavily influenced by frequencies of the $ES\text{-}1_b$ and $ES\text{-}2_c$ alleles. To the rear of the model are populations that are fixed for the $ES\text{-}1_b$ allele. Populations projected to the front of the model demonstrate a low frequency of the $ES\text{-}1_b$ allele, with a concomitant increase in the frequency of the $ES\text{-}2_c$ allele. Component III was mainly influenced by five alleles, $\alpha\text{-}GPD\text{-}1_a$,

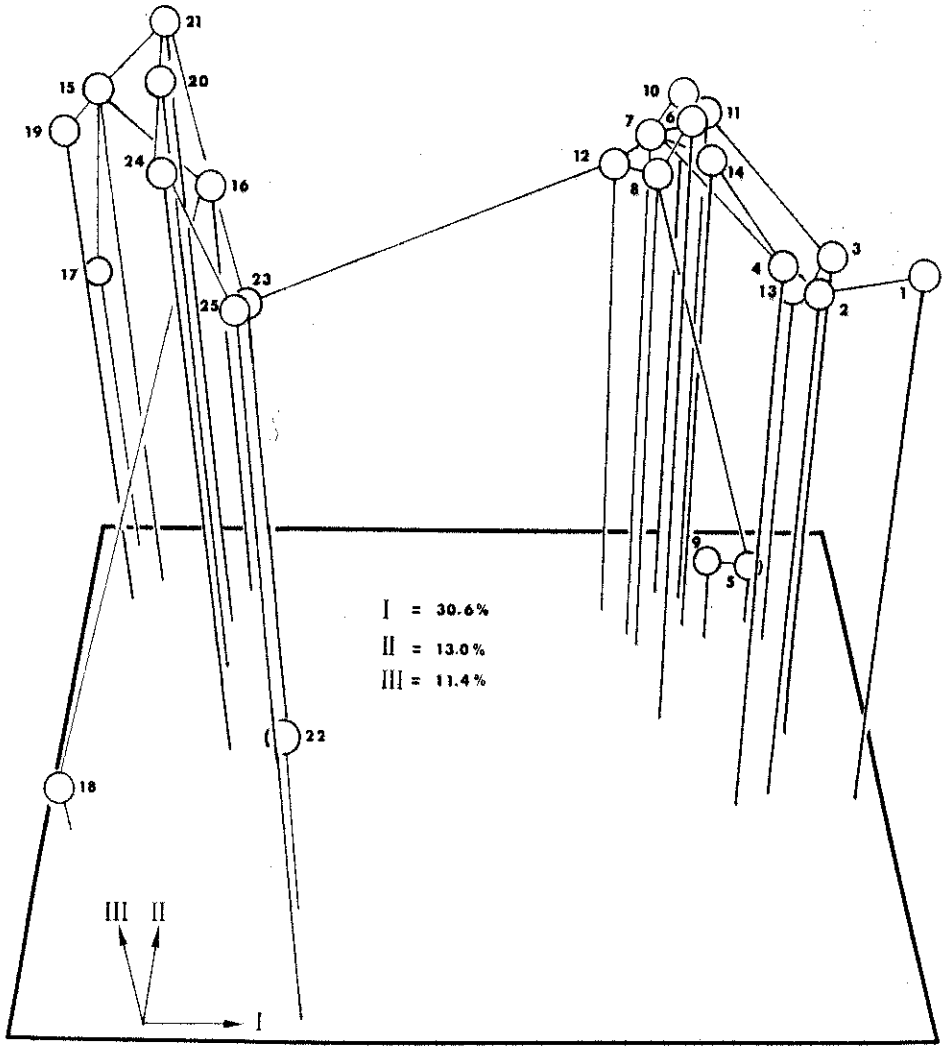

Fig. 7--Projection of 25 populations of Geomys bursarius onto first three principal components of variation in matrix of correlations of allelic frequencies.

TABLE 4

CHARACTER LOADINGS ON THE FIRST THREE PRINCIPAL COMPONENTS OF INTERLOCALITY PHENETIC VARIATION IN G. B. MAJOR (W) AND G. B. SAGITALLUS (E)

CHARACTERS	PRINCIPAL COMPONENTS		
	I	II	III
IDH-1a	0.892*	-0.034	0.098
IDH-1b	-0.892*	0.034	0.098
MDH-2a	0.971*	0.141	-0.017
MDH-2b	-0.971*	-0.141	0.017
GOT-1a	0.409	-0.290	0.104
GOT-1b	-0.342	0.456	-0.171
GOT-1c	-0.220	-0.518	0.233
-GPD-a	0.274	0.179	-0.618*
-GPD-b	-0.274	-0.179	0.627*
-GPD-c	0.172	0.109	-0.419
6-PGD-a	0.664	-0.276	0.216
6-PGD-b	-0.664	0.276	-0.216
LDH-1a	-0.969*	-0.168	0.021
LDH-1b	0.969*	0.168	-0.021
ES-1a	0.144	-0.593	0.351
ES-1b	0.077	0.756*	-0.049
ES-1c	0.040	-0.391	-0.298

TABLE 4 Continued

CHARACTERS	PRINCIPAL COMPONENTS		
	I	II	III
ES-2a	0.580	0.264	-0.270
ES-2b	-0.342	0.370	0.669*
ES-2c	-0.231	-0.724*	-0.498
ES-3a	-0.185	-0.542	-0.688*
ES-3b	-0.056	0.364	0.677*
ES-3c	0.290	0.130	-0.143
ES-4a	-0.044	0.562	-0.401
ES-4b	0.044	-0.562	0.401
ADH-a	0.984*	-0.028	-0.048
ADH-b	-0.949*	0.122	0.149
ADH-c	-0.449	-0.364	-0.388
SDH-a	-0.104	0.122	0.074
SDH-b	0.262	-0.250	-0.009
SDH-c	-0.365	0.290	-0.160
SDH-d	-0.349	0.284	-0.175

*CHARACTERS WITH THE GREATEST INFLUENCE ON THE PRINCIPAL COMPONENT

PRINCIPAL COMPONENT I: IDH-1, MDH-2, LDH-1, ADH-a, ADH-b

PRINCIPAL COMPONENT II: ES-1b, ES-2c

PRINCIPAL COMPONENT III: -GPD-a, -GPD-b, ES-2b, ES-3a, and ES-3b

α-GPD-1_b, ES-2_b, ES-3_a, and ES-3_b. Those populations with the greatest vertical projection are characterized by at least one or a combination of the following allelic frequency patterns: fixation of α-GPD-1_b, predominance of the ES-2_b allele, and a high frequency for ES-3_b. Those populations exhibiting the least vertical projection show one or more of the following patterns: presence of α-GPD-1_a, low ES-2_b frequency, and high ES-3_a frequency.

Genetic Similarity and Distance

A matrix of genic identities (I) among all 25 populations is presented in Table 5. Mean intraracial I for the eastern and western chromosome races are 0.932 $\pm$ .005 (S.E.) and 0.967 $\pm$ .002 (S.E.), respectively, while mean interracial genic identity is 0.790. Therefore, the highest values of I are from within group comparisons; the lowest I values are between eastern and western populations. Consequently, the eastern and western races share approximately 79% of their allelic composition at the 19 loci examined. ADH and SDH were excluded due to incomplete data. A dendogram based on genic identities of the 25 populations is illustrated in Figure 8. Collectively these data also reflect a high degree of genic differentiation between the eastern and western races. The mean genetic distance ($\bar{D}$) of .216 between the two races indicates the degree of genomic differentiation at the present time and approximately

TABLE 5

MATRIX OF GENIC IDENTITIES FOR 25 POPULATIONS OF *GEOMYS BURSARIUS* IN TEXAS, OKLAHOMA AND LOUISIANA

WESTERN RACE POPULATION	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	..	.987	.987	.972	.957	.944	.949	.955	.959	.970	.975	.943	.965	.962
2		..	.993	.980	.943	.962	.960	.950	.973	.973	.970	.951	.955	.954
3			..	.980	.941	.956	.968	.954	.970	.983	.983	.957	.949	.967
4				..	.941	.962	.982	.963	.986	.986	.986	.965	.946	.974
5					..	.931	.946	.979	.967	.949	.955	.967	.966	.965
6						..	.969	.960	.963	.976	.963	.965	.946	.949
7							..	.984	.987	.991	.990	.991	.934	.990
8								..	.980	.975	.980	.994	.945	.992
9									..	.979	.980	.984	.946	.979
10										..	.997	.979	.961	.986
11											..	.980	.955	.993
12												..	.935	.990
13													..	.939
14														..

TABLE 5 Continued

POPULATION	15	16	17	18	19	20	21	22	23	24	25
1	.739	.758	.765	.706	.725	.769	.770	.778	.833	.792	.791
2	.767	.776	.778	.755	.770	.802	.803	.809	.865	.820	.809
3	.768	.770	.770	.720	.762	.801	.803	.785	.846	.803	.788
4	.780	.780	.777	.748	.771	.828	.828	.818	.873	.834	.831
5	.768	.799	.813	.760	.760	.798	.801	.838	.885	.828	.818
6	.785	.782	.775	.730	.796	.841	.844	.794	.874	.865	.825
7	.819	.811	.802	.746	.807	.867	.810	.818	.891	.861	.842
8	.813	.821	.822	.751	.797	.850	.853	.832	.885	.828	.853
9	.793	.797	.798	.786	.790	.837	.839	.849	.900	.849	.843
10	.797	.796	.789	.716	.787	.844	.846	.792	.870	.842	.813
11	.794	.795	.791	.713	.777	.835	.837	.793	.865	.833	.814
12	.825	.824	.821	.762	.817	.870	.871	.839	.907	.870	.848
13	.736	.771	.776	.707	.733	.775	.778	.779	.846	.805	.776
14	.810	.811	.809	.728	.786	.846	.848	.811	.879	.844	.833
15	..	.991	.980	.904	.991	.948	.943	.874	.910	.927	.905
16		..	.997	.918	.980	.932	.927	.889	.917	.926	.908
17			..	.930	.971	.916	.911	.901	.919	.918	.907
18				..	.923	.846	.842	.933	.906	.871	.887
19					..	.946	.941	.883	.916	.930	.897
20						..	.999	.915	.957	.980	.952
21							..	.915	.959	.982	.952
22								..	.970	.940	.957
23									..	.973	.964
24										..	.980
25											..

EASTERN RACE

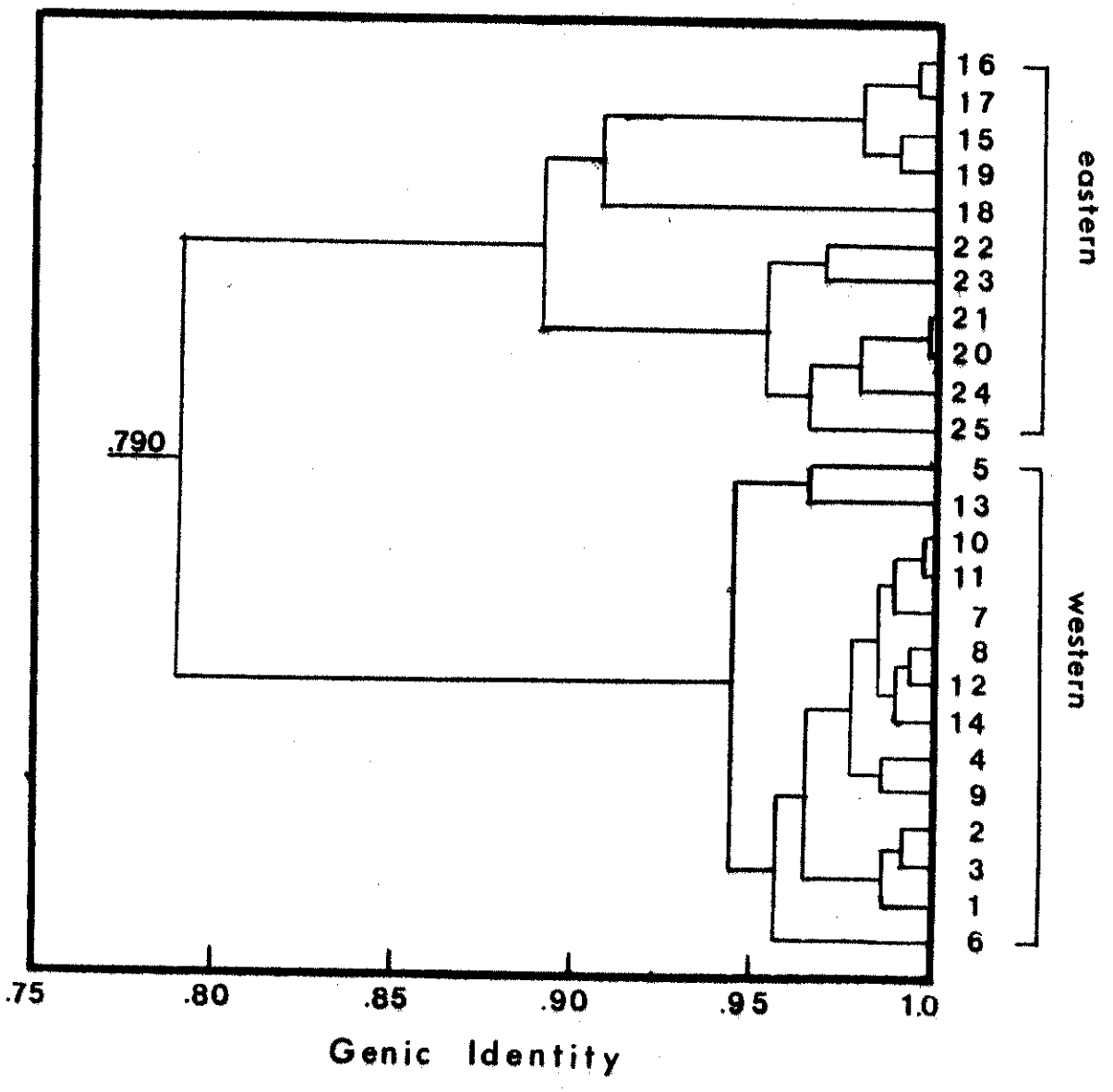

Fig. 8--Dendrogram of genic identities (I) for 25 populations of Geomys bursarius.

21.6 allelic substitutions per 100 loci have occurred since the two races diverged.

Coefficients of Inbreeding

Coefficients of inbreeding (F_{ST}) were calculated for all polymorphic loci in both races (Tables 1 and 2). Zimmerman and Gayden (1979) calculated an effective population size of twelve for local demes of the same two races along the Brazos River. Consequently, only those population samples of twelve or more were used in calculating F_{ST} unless nearly all individuals of a population were collected (these were also then used). A mean F_{ST} of 0.225 and 0.184 was obtained for the eastern and western races, respectively.

Ontogenetic Genic Expression

Results obtained from embryos were variable and difficult to code from gels. Most enzymes showed patterns identical to those of adults. These included IDH, MDH, GOT, IPO, ME, 6-PGD, α-GPD, ADH, SDH, and the esterases. Of these, IDH and MDH proved to be the most consistent. All others showed variable activity, especially when the embryo was 15mm or less in total length. Consequently it was not practical to calculate gene frequencies from embryos.

LDH presented the only unique ontogenetic difference. LDH is encoded from a duplicated locus in mammals (hence LDH-1 and LDH-2), and is a tetramer. There are five possible

combinations of the two differently-charged polypeptides to form the tetramer (AAAA, AAAB, AABB, ABBB, BBBB), producing five isozymes. In one- to two-week embryos (gestation is estimated at four weeks [Wilks, 1963]), only the slower three isozymes appear. In later embryos, only the slower four isozymes are expressed. The faster isozymes therefore appear sequentially through development.

If more than one LDH isozyme is present, then both LDH loci are active. Why, then, should the three slowest isozymes appear in smaller embryos, with the fourth and fifth isozymes appearing sequentially through development? Fine et al. (1963) found similar results from tissue extracts of fetal calves. They concluded that control of the LDH-1 and LDH-2 loci was done within individual cells by unknown factors associated with differentiation and development. They hypothesized that in early development most cells produced only the slower LDH polypeptide (BBBB), with only a few cells producing both. As development progressed, more cells would begin producing both polypeptides, evidenced by increasing activity of the faster isozymes. This would appear to fit my data, because the faster isozymes, when present, consistently showed less activity than the slower isozymes.

CHAPTER IV

DISCUSSION

Genic Variation

It is now clear that there is a definite difference between the genomes of the eastern and western chromosome races of G. bursarius. The main separation occurs at four different loci: LDH-1, IDH-1, MDH-2 and ADH. Previously, Zimmerman and Gayden (1979) had established the possibility of separate gene pools of these two races in a contact zone along the Brazos River in central Texas. Twenty-one localized populations were examined in Falls and McClennan Counties, Texas. Nine populations of the western race were separated by 12 km. from 12 populations of the eastern race. The pattern of alternate fixation of alleles is clearly present in this narrow contact zone. Figure 9 represents the allelic frequencies for LDH-1 and IDH-1, with LDH-1 demonstrating complete fixation for alternate alleles between the races, while IDH-1 exhibits limited polymorphism in both groups. In the western populations the IDH-1_a allele is predominant, and in eastern populations the IDH-1_b allele is predominant. However, the IDH-1_a and IDH-1_c alleles occurred in eastern populations along the Brazos River but were not found in eastern populations of the

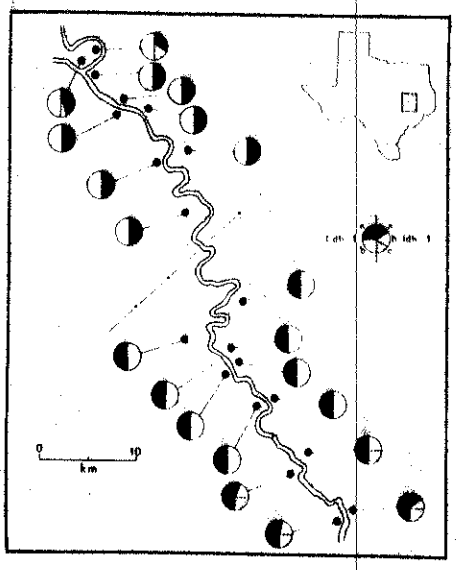

Fig. 9--Allelic frequencies of LDH-1 and IDH-1 for 21 local populations of G. bursarius along the Brazos River in McClennan and Falls Counties, Texas (Zimmerman and Gayden, 1979).

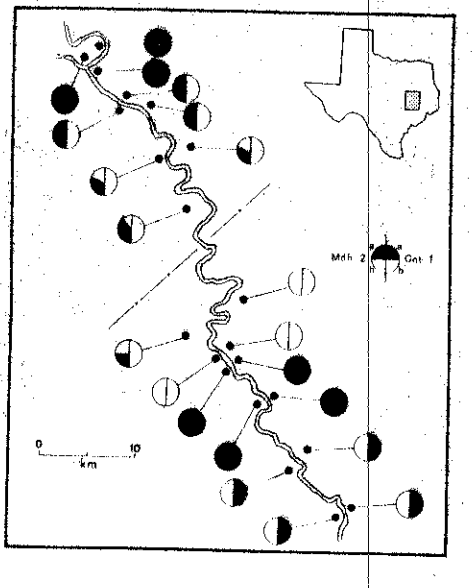

Fig. 10--Allelic frequencies of MDH-2 and GOT-1 for 21 local populations of G. bursarius along the Brazos River in McClennan and Falls Counties, Texas (Zimmerman and Gayden, 1979).

present study. The MDH-2 pattern within this area is not as clear as over the entire geographic distribution (Fig. 10). Both alleles are found in eastern and western races, although the MDH-2_a allele predominates in the western group, and MDH-2_b predominates in the eastern group. This compares favorably with frequencies of the allele found throughout the ranges of both races in this study. Data for ADH were not available from this area.

Gene frequencies from the other known contact zone, near Norman, Oklahoma (Figs. 2, 4, and 6), also indicate a pattern of genomic differentiation by fixation of alternate alleles. In this instance, individuals from the two groups were found within 3.2 kilometers of each other. Here all four loci, LDH-1, IDH-1, MDH-2 and ADH, show identical patterns of genic differentiation as found throughout the respective geographic ranges of both races. IDH-1 polymorphism found in western populations may be indicative of limited gene flow in the past; however, this same pattern of IDH-1_a polymorphism is found in almost all western populations. Also, the IDH-1_a allele does not appear in any eastern population along this transect. In addition, 6-PGD also shows a marked pattern of alternate fixation (Tables 1 and 2). In populations other than those in the Norman area, the 6-PGD_a allele was predominant, with the 6-PGD_b allele occasionally occurring at low frequencies. However, in populations 15 through 19 of the eastern race only the 6-PGD_b

allele was found; while in populations 9 through 14 of the western race only the 6-PGD_a allele occurs. Therefore a total of five loci exhibiting alternate fixation of alleles separates the western populations from the eastern populations in this area. The transition from the western genic pattern to the eastern genic pattern is abrupt, with no evidence of recent gene exchange. Baker and Glass (1951), using morphometric analysis, and Hart (1971), using karyotypic analysis, both indicated intergradation to be taking place in this zone. The presently existing genic patterns, however, do not support this conclusion.

Additional support for the lack of gene flow between the eastern and western races is gained through multivariate analysis using gene frequencies as characters. Genic differentiation among populations 1 through 25 (Fig. 7) of this study and populations examined by Zimmerman and Gayden (1979) (Fig. 12) is represented by a projection of the populations with respect to the first three principal components extracted from the correlation matrix of gene frequencies. In both three-dimensional plots, the first principal component is most heavily influenced by those loci which effectively separate the eastern and western populations. The multivariate analyses and the resulting three-dimensional models serve to demonstrate numerically the interlocality genomic difference between the eastern and western races already apparent from gene frequencies.

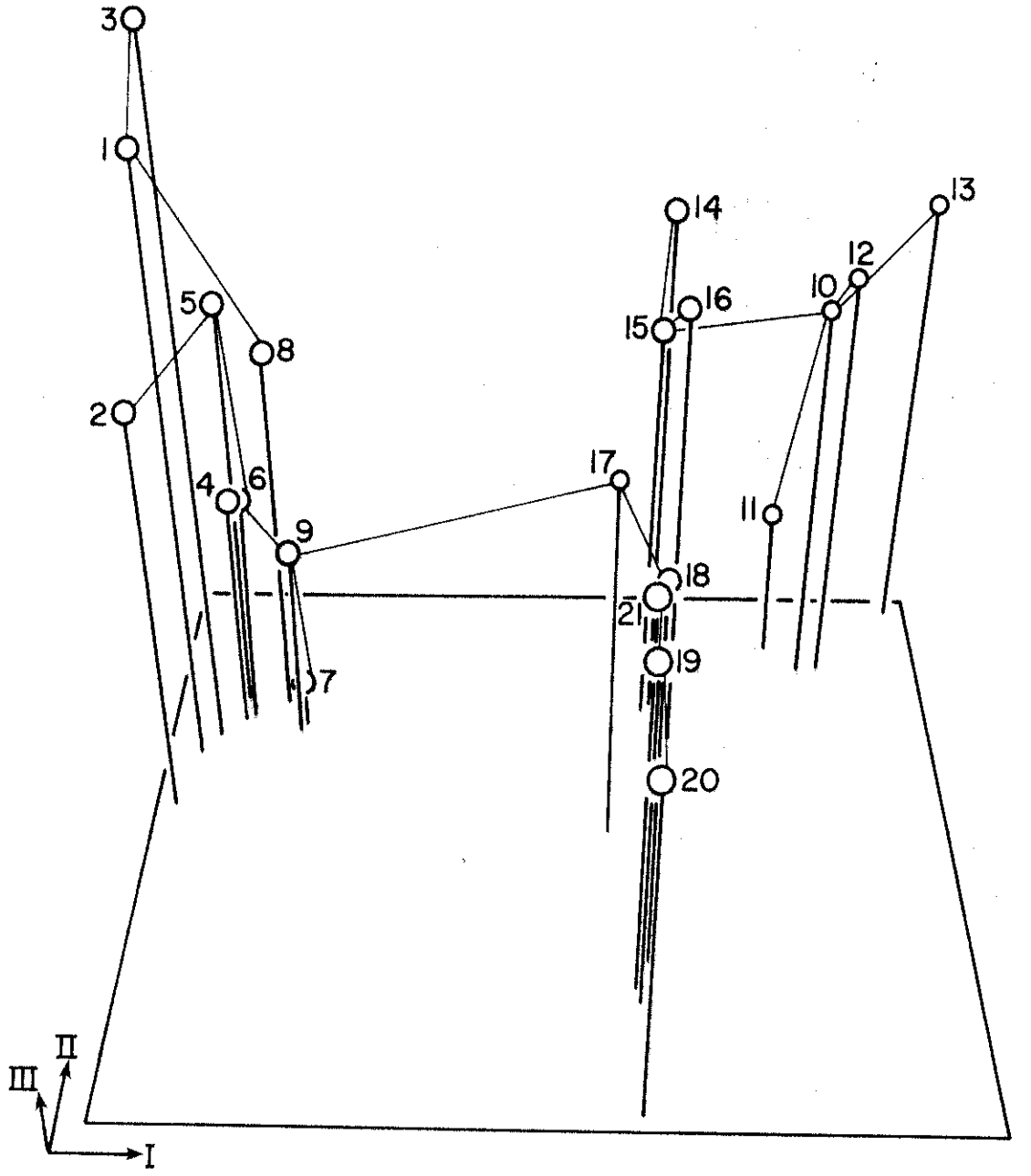

Fig. 11--Projection of 21 populations onto the first three principal components of variation in the matrix of correlation of 32 allelic frequencies of Geomys bursarius.

Genic ($\bar{I}$) identity over 19 of the 21 loci further illustrates the allozymic differences between the two races. Mean genic identity between the two races was .807, with $\bar{D}$ = .216, while mean I values among the eastern and western races were .932 and .967, respectively. Intraracial I values compare well with those for local geographic populations of Peromyscus (.971, Zimmerman et al., 1978). Interracial I values are fairly consistent with those found for semispecies and sibling species of other rodent genera, .837 in Peromyscus (Zimmerman et al., 1978), .881 in Neotoma (Zimmerman and Nejtek, 1977), and .925 in Thomomys (Nevo et al., 1974), but higher than the $\bar{I}$ of .625 between well-differentiated species of Geomys reported by Penney and Zimmerman (1976). The amount of genic differentiation, then, is similar to other taxonomic groups in which reproductive isolation is complete or nearly complete. $\bar{D}$ values also compare favorably in this regard with those obtained for the semispecies, P. leucopus and P. gossypinus, .178 (Zimmerman et al., 1978); three semispecies of Neotoma, .123 (Nejtek and Zimmerman, 1977); the T. talpoides complex, .078 (Nevo et al., 1974); two semispecies of Spermophilus, .036 (Cothran et al., 1977); the T. bottae species complex, .144 (Patton and Yang, 1977), and the T. umbrinus species complex, .181 (Patton and Feder, 1978). However, the $\bar{D}$ of .216 is below that for well-differentiated species of other rodent taxa (Zimmerman et al., 1978).

Honeycutt's (1978) karyotypic analysis of Geomys bursarius in Texas, as well as others (Hart, 1971; Kim, 1972) questioned the systematic status of the G. bursarius complex due to the extreme amount of chromosomal variation in the species. Hart (1971) and Kim (1972) both concluded that karyotypic differences may be indicative of the occurrence of separate gene pools. However, recent investigations of contact zones between chromosomally distinct populations have indicated that karyotypic differences need not reflect species differences. Baker et al. (1975) and Thaeler (1974) concluded that the effect of chromosomal variation on species differences can only be determined in zones of contact between the chromosomal forms in question. Intensive study of the contact zones between the two races in this investigation are also important due to two additional factors: (1) although local populations of G. bursarius may conform to an island-type distribution, the chromosomal forms are continuously distributed throughout their ranges wherever suitable soils persist, and (2) no differences in soil type preference were found between the eastern and western races of this study, by Honeycutt (1978). Consequently, there are no detectable ecological differences between the two races, although there is a notable size difference (Honeycutt, 1978).

The only known contact zones between the eastern and western races are those contained within this study. The

northern zone of contact is located near Norman, Oklahoma, and the southern zone of contact is near the border of McClennan and Falls Counties in Texas. As previously indicated, there is at present no evidence of hybridization (genic exchange) in the northern contact zone (Figs. 4 and 6), even though the two races have been found within 3.2 km. of each other. Data from Geomys in the southern contact zone also indicate a lack of gene exchange, although not as dramatically as in the northern zone. However, the two races are separated by over 12 km. in this area, and no direct contact has been found. The distinctiveness of the gene pools of these two races, as detected by gel electrophoresis, particularly within zones of contact, is highly indicative of separate species. Final clarification of this problem can only come with further chromosomal data from the two zones of contact. If the eastern and western races do indeed represent separate gene pools, the eastern race is referable to G. breviceps Baird. There are at least two subspecies, G. b. breviceps Baird and G. b. sagitallus Merriam. The western race remains G. bursarius major Davis until further investigation is available in the northern portion of the range of G. bursarius (Hall and Kelson, 1959).

Patterns of Genic Variation in Fossorial Rodents

While the factors involved in the maintenance of

protein polymorphisms in natural populations remain unclear (Lewontin, 1974; Selander, 1976), three general hypotheses have been advanced to explain patterns of genic variation observed in fossorial rodents. First, the relatively low heterozygosities that generally characterize fossorial rodents are thought to be best explained as an adaptive strategy for homozygosity in the relatively homogeneous, subterranean environment (Nevo and Shaw, 1972; Nevo et al., 1974). Second, random genetic drift accounts for a wide range of heterozygosities where large populations occupying broadly contiguous areas are able to maintain higher levels of variability than small, well-isolated populations (Patton and Yang, 1977; Patton and Feder, 1978). Third, a geographic pattern of small effective populations distributed in a mosaic pattern will result in low rates of migration, contributing to high levels of inbreeding (Zimmerman and Gayden, 1979). Founder effect should also play an important role in fossorial rodents.

Selection for homozygosity in a uniform environment may explain genic patterns in Spalax (Nevo and Shaw, 1972) and the "talpoides" group of Thomomys (Nevo et al., 1974), but it has not proved applicable to either T. bottae (Patton and Yang, 1977), T. umbrinus (Patton and Feder, 1978), or species within the genus Geomys (Selander et al., 1974; Penney and Zimmerman, 1976; Zimmerman and Gayden, 1979). Genic variation should be uniformly low over all species, according to

this hypothesis, but heterozygosities for the three genera, Spalax, Thomomys and Geomys are highly variable (.023-.093), although the mean (.049 ± .004, S.E.) is lower than the $\bar{H}$ of 6.0% for non-fossorial rodents (Selander, 1976).

Within T. bottae (Patton and Yang, 1977) and T. umbrinus (Patton and Feder, 1978), random genetic drift determined by the degree of connectedness (gene flow) and population size appears to correlate well with genic data for these two species. Large interconnected populations have much higher heterozygosities than small isolated ones. Individual population heterozygosities may range from 0.022 up to 0.100 for T. bottae and from 0.030 up to 0.169 for T. umbrinus.

High F_{ST} values, characteristic of fossorial rodents, can be explained by the effects of inbreeding (Wright, 1965; Lewontin and Krakauer, 1973; Selander and Kaufman, 1975). Several F_{ST} values for fossorial rodents have been reported: .421 for T. bottae by Patton and Yang (1977); .017-.957 for T. talpoides by Nevo et al. (1974); .465 and .575 for G. bursarius by Zimmerman and Gayden (1979); and .184 and .225 from the present study for G. bursarius. Comparing these values to those of .030 for Drosophila pseudoobscura (Lewontin, 1974), .148 for humans (Cavalli-Sforza, 1966), and .043 and .291 for two species of minnows, genus Campostoma (Zimmerman et al., 1980) indicates a high level of genic heterogeneity among fossorial rodent populations. Zimmerman and Gayden (1979) also calculated a migration rate in G. bursarius

of only .02 migrants per generation, which is indicative of restricted gene flow.

High F_{ST} values particularly reflect upon the demography of pocket gophers, which can be discussed in terms of metapopulations (Levins, 1970). Metapopulations are described as clusters of populations belonging to the same species, spread over a fixed number of patches. Extinction of old patches and colonization of empty patches by immigrants results in a pattern of occupancy which is constantly changing. This could also be described as an island model or mosaic pattern of distribution. This pattern, confirmed by Kennerly (1954) for G. personatus, should result in genic divergence, as has been demonstrated by Selander et al. (1974), Penney and Zimmerman (1976), Zimmerman and Gayden (1979), and the present study. This should also be expected to result in restricted gene flow and high levels of inbreeding as previously demonstrated.

According to Patton and Yang (1977), large interconnected populations should have higher levels of heterozygosity than small isolated populations. The Red River provides an almost continuous avenue for pocket gopher dispersal, due to its sandy banks. The largest and most dense population in this study, population 21, near the Red River, in eastern Texas, had one of the lowest levels of heterozygosity (.017). Heterozygosity for population 20, a smaller and more dispersed population across the river from

21, was only slightly less (.007). Genic identity between the two was .999, indicating a degree of connectedness between the two populations, yet heterozygosities were extremely low. Inbreeding, with low migration rates in and out of that localized patch, more completely accounts for the genomic structure of these two populations in contrast with the variable heterozygosities of the other populations within this study. Although individual population heterozygosities are variable (.000-0.80) as in Thomomys, they are consistently lower, which would also be accounted for by restricted gene flow and inbreeding.

At present, a more reasonable hypothesis to explain the variation in genomic structuring of fossorial rodent populations would be that different distributional and demographic parameters contribute to this variation among each of these three genera of fossorial rodents. All three hypotheses may be factors, but to differing degrees within the three taxa. This would be expected if adaptation for a fossorial habitat were not the only major factor affecting the population dynamics of fossorial rodents. For instance, Zimmerman and Gayden (1979) indicated that, while Geomys occupies habitats comprised of primarily sandy soils, Thomomys may occur in a wide variety of habitats from sandy soils to those that are indurate. This would lead to greater gene flow in Thomomys, due to a greater tolerance of soil types. Geomys are more restricted by soil type and

show greater effects of inbreeding resulting from a lack of gene flow between populations.

Patterns of Speciation in Fossorial Rodents

The biological species concept of outcrossing, sexual organisms as described by Mayr (1970), consists of groups of interbreeding, natural populations that are reproductively isolated from other such groups. Although this concept is nearly universally accepted, there are shades of gray where reproductive isolation is difficult to prove (Dobzhansky, 1970, 1976). More often than not, this occurs in situations where the process of speciation is not yet complete. For sexually reproducing organisms, speciation is usually initiated through isolation by geographic barriers which interrupt gene flow between two populations. As the populations accumulate genetic differences, they may become recognizable as races, and, if they remain allopatric, sufficient divergence may accumulate to establish reproductive isolating mechanisms. A major question concerning speciation (and in this case for fossorial rodents) is just how much genetic differentiation accompanies the development of reproductive isolation (Avise, 1976). Also, of special concern in reference to fossorial rodents is the question, does the allopatric speciation model exhibit the best fit in regard to the chromosomal and genic data presently available?

In his review of modes of animal speciation, Bush (1975) indicated a number of biological properties that are most pertinent to the speciation process, and attempted to show that the ways in which various groups of animals differ in these properties determine, to a great extent, the mode of speciation they are most likely to follow. Bush outlined three broad patterns of speciation in sexually reproducing organisms: allopatric, parapatric, and sympatric. Allopatric speciation is broken down into two categories, speciation by subdivision and speciation by founder effect. Speciation by subdivision is essentially classical allopatric speciation as discussed above, and is primarily characterized by organisms which are K-strategists (Pianka, 1978), with high vagility, large population size, fine-grained environmental utilization, and little or no chromosomal rearrangement. Speciation by founder effect is a form of geographic speciation which occurs by way of the establishment of a new colony by a small number of founders, as in Hawaiian Drosophila (Carson, 1973, 1975). This form of speciation mainly occurs in organisms which are r-strategists (Pianka, 1978) with high vagility, small population size, selection for homozygosity, and high levels of inbreeding. Speciation may be rapid in these cases.

Parapatric speciation is found whenever species occur as contiguous populations in a continuous cline (Bush, 1975; White, 1973, 1978). Parapatric speciation does not require

spatial isolation and is characteristic of organisms which are r-strategists with extremely low vagility, high potential for inbreeding, selection for homozygosity, and frequent chromosomal rearrangements. Parapatric speciation may also occur rapidly with little structural genic divergence.

Sympatric speciation, defined as the origin of isolating mechanisms within the dispersal area of the offspring of a single cline (Mayr, 1963), is considered by many to be a special case of parapatric speciation (Bush, 1975). Therefore many of the organismal characteristics are similar except that there is rarely any chromosomal rearrangement, and it is usually restricted to parasitic organisms (Bush, 1975).

Fossorial rodents would appear to fit the parapatric model, due primarily to the high degree of karyotypic variation in all species, extremely low vagility, and high levels of inbreeding. Other factors would have more or less effect, depending on the species. It would be difficult to classify pocket gophers as strict r-strategists, for instance. Wilks (1963) summarized that, within G. bursarius, one to two litters were produced per year, averaging two to three offspring per litter. Although pocket gophers reach sexual maturity early, at three months, the average life span is between two to four years. Pocket gophers are also solitary and extremely territorial, which will tend to limit population growth and density. Parapatric speciation is most

clearly indicated by the high degree of karyotypic polymorphism. G. bursarius exhibits eight distinct chromosomal races within Texas alone (Honeycutt, 1978). That the distribution of these chromosomal races is continuous, despite the mosaic distribution pattern, and that they are currently in contact (Honeycutt, 1978; Pembleton and Baker, 1978) are also indicative of parapatric speciation. Spalax (Nevo and Shaw, 1972) and Thomomys (Patton et al., 1972; Thaeler, 1974) also exhibit a high degree of karyotypic polymorphism with zones of contact showing limited hybridization.

If allopatric speciation were operating, there should be more stable karyotypes within species with more restrictive zones of contact. Also the high levels of inbreeding, low vagility, and apparent selection for homozygosity in Spalax, are not indicative of species undergoing allopatric speciation. Large amounts of genic divergence are concomitant with allopatric speciation. This is definitely not the case in Spalax (Nevo and Shaw, 1972) and the Thomomys talpoides group (Nevo et al., 1974) where mean genic identities were .978 and .925 respectively, indicating little structural genic change during speciation. It has been suggested that changes in regulatory genes may play a larger role in such cases where genic differentiation is not detectable by gel electrophoresis (Bush, 1975; Avise, 1976; Wilson, 1976). This is indeed reasonable, due to the high degree of karyotypic polymorphism, increasing the probability

for new gene combinations with the formation of supergenes.

In summary, speciation events for fossorial rodents are most likely to occur through rapid, parapatric, and initially non-adaptive means. This is primarily due to the propensity toward karyotypic divergence. This would also lead to a larger role for regulatory genes in achieving reproductive isolation, resulting in little structural genic change at first, due to the relative rapidity of the event.

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