GENIC DIFFERENTIATION AND EVOLUTION IN THE GROUND SQUIRREL SUBGENUS ICTIDOMYS (SPERMOPHILUS)

THESIS

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By

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The genetic structure of 26 natural populations of three species (S. tridecemlineatus, S. mexicanus, and S. spilosoma) of the Ictidomys subgenus of ground squirrels was analyzed using chromosomal and electrophoretic techniques.

Chromosomal variation was not observed in S. mexicanus, and only slight karyotypic variation was found in the other two species. Chromosomal evidence indicated hybridization between S. tridecemlineatus and S. mexicanus, placing these species within the classical definition of semispecies. Analysis of electrophoretic variation at 29 genetic loci indicated close genetic relationships between these species. Evolution in Ictidomys appears to be linked with Pleistocene events, and speciation appears to have occurred within the last 155,000 years.

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CHAPTER I

INTRODUCTION

Recent development of electrophoretic techniques for demonstrating allelic variation at genetic loci controlling the structure of enzymes and other proteins makes it possible to estimate degrees of genetic variation in natural populations and genetic differentiation of species (Lewontin and Hubby, 1966; Prakash et al., 1969; Selander et al., 1969; Selander and Yang, 1969; 1970; Selander et al., 1969; Selander et al., 1970; Selander et al., 1971). A basic tenet of evolutionary theory is that race formation and speciation occur by the conversion of genetic variation within populations to variation among populations (Lewontin, 1967). Present estimates available on the degrees of genic polymorphism in populations indicate that there is a broad range of variation, both among congeneric species (Selander et al., 1970) and among populations of the same species (Prakash et al., 1969; Selander et al., 1969; Selander et al., 1971, Johnson and Selander, 1971). Studies of geographic variation in allele frequencies of semispecies and sibling species suggest that the amount of genomic modification involved in speciation is highly variable, although generally large (Hubby and Throckmorton, 1965, 1968; Prakash, 1969; Selander et al., 1969; Ayala et al., 1970; Nevo et al., 1974, Penney and Zimmerman, 1975). In using

electrophoresis to examine populations of organisms, Penney and Zimmerman (1975) have emphasized that the following should be investigated to determine if correlations exist between protein variation and the biology of the organism: (1) patterns of protein variation and geographic distribution of an organism; (2) patterns of protein variation and substrate specificity of the protein; (3) patterns of protein variation and adaptive strategy; and (4) patterns of genic divergence, speciation, and evolutionary time. The North American ground squirrels belonging to the subgenus Ictidomys (genus Spermophilus) provide a valuable model for evaluating the above.

The genus Spermophilus is currently comprised of six subgenera and 23 species. The subgenus Ictidomys consists of four species, three of which are found in the United States. The group ranges from the states of Puebla and Tlaxcala in southern Mexico to the north-central regions of the Canadian provinces of Alberta, Saskatchewan, and Manitoba; and from western Ohio to western Montana (Hall and Kelson, 1959). The four species of Ictidomys are morphologically distinct in cranial characteristics and coat color, however, they offer an excellent opportunity for studying the genetics and evolution of a group since there is considerable overlap of the ranges and environmental preferences between the species.

Spermophilus tridecemlineatus is the most widespread member of the group. These squirrels are typically inhabitants of shortgrass prairies, but are also found in dry

meadows, tall grass fields, golf courses, parks, cemetaries, open jack-pine barrens, and even open woodlands (Jackson, 1932). Their food is chiefly green grasses and herbs in early spring, but seeds, flower heads, and insects contribute importantly to their diet as the season advances (Davis, 1966). These squirrels are true hibernators and may spend up to 7 months underground.

Spermophilus mexicanus consists of two disjunct groups of populations corresponding to the two subspecies of this species. Spermophilus mexicanus parvidens is mainly found in west-central Texas and northeastern Mexico, while S. mexicanus mexicanus is found in south-central Mexico. Both inhabit brushy or grassy areas and are frequently associated with mesquite and cactus flats (Howell, 1938). Their burrows are usually constructed in sandy or gravelly soils, but they are not restricted to them and are often found in parks and golf courses. Their food in early spring is chiefly green vegetation, but they also feed on mesquite leaves and beans, agarita leaves and berries, Johnson grass, and insects (Bailey, 1931). These squirrels apparently do not hibernate but go below ground and plug their burrows for long periods during cold weather.

Spermophilus spilosoma are found from south-central Mexico to Nebraska and western Texas to western Arizona. Spotted ground squirrels seem to prefer dry, sandy areas, but are also found in grassy parks open pine forest, scattered brush, and occasionally on rocky mesas (Howell, 1938). Their food is

largely green vegetation, seeds, and some insects. Hibernation probably is not complete in these squirrels, especially in the southern part of their range (Davis, 1966).

In the past, members of this subgenus have been the subject of studies of hibernation (Kayser, 1965), various aspects of reproduction (Wade, 1927; and Zinny, 1965), growth (Zimmerman, 1972), and ecology and population biology (McCarley, 1966). Chromosomal relationships of the subgenus have been investigated by Nadler and Hughes (1966).

Howell (1938) and Bryant (1945), using morphological characteristics, stated that the subgenus Ictidomys can be distinguished from the other subgenera of Spermophilus by cranial, dental, and myological characters. Dental characters in particular led Bryant (1945) to postulate that Ictidomys is most closely related to the subgenus Spermophilus. Black (1963), utilizing paleontologic material, concluded that Ictidomys differentiated from Spermophilus as recently as the late Pliocene and Pleistocene. Black (1963) also stated that these two advanced subgenera of ground squirrels were derived from the Otospermophilus group, probably splitting off in the middle Pliocene.

Within Ictidomys, the forms of the cranium, rostrum, and baculum are specifically variable. Bryant (1945) postulated that the species assinged to this subgenus are not as closely related as are the species in the other subgenera of the genus. The color pattern and baculum of S. spilosoma is markedly

different from that of the other two species of the subgenus, while the bacula of S. mexicanus and S. tridecemlineatus are similar and have a distinctive form. From this, Bryant (1945) concluded that either S. spilosoma is not as closely related to the other two members of the subgenus as has been assumed, or the form of the baculum is not of supraspecific significance in this group of squirrels.

Nadler and Hughes (1966) had similar findings in their study of the chromosomal relationships of Ictidomys. Spermophilus tridecemlineatus and S. mexicanus appear to be the more closely related species with a diploid number of 34 and similar karyotypes. Spermophilus spilosoma is chromosomally divergent in that it lacks a pair of metacentrics found in the other two species, and the Y chromosome is a minute rather than a large acrocentric. However, Nadler concluded that the species of this subgenus, based on karyological characters, were more similar to each other than they are to species of the other subgenera of Spermophilus.

The relationships of Ictidomys based on conventional taxonomic characters and karyological techniques do not correlate. Both methods rank the species in the same way, however the degree of relationship is presently unclear. In addition, S. tridecemlineatus and S. mexicanus show some of the characteristics of semspecies in that they are largely allopatric with limited overlaps in their ranges, and are morphologically rather similar. Although hybrids between the two species have not

been reported, the similarities between the karyotypes (Nadler and Hughes, 1966) and the shape of the baculum (Bryant, 1945) indicate that there could be hybridization in the area where the ranges of these two species overlap.

In this study, 27 proteins controlled by 29 loci were examined electrophoretically for 26 populations of the subgenus Ictidomys to clarify the systematic relationships of the members of this subgenus. In addition, karyological data were utilized to compare chromosomal multiformity with genetic heterogeneity.

CHAPTER II

MATERIALS AND METHODS

Samples

Ground squirrels (n = 216) were obtained from 26 naturally occurring populations (Table 1) in Indiana, Texas, New Mexico, and Chihuahua using Tomahawk live traps and a noosing technique described my McCarley (1966). Squirrels were maintained in the laboratory in plastic cages and provided an ad libitum diet of Purina Laboratory Chow and water until blood samples could be taken and prepared. Representative specimens were deposited in the mammal collection of North Texas State University.

Two S. tridecemlineatus from Denton, Denton County, Texas were used as control animals. One or both of these animals were bled and run on gels with each population sample. The standard banding pattern was then compared with population samples to assess the fast and slow migrating bands.

Chromosomal Techniques

Representative specimens from each species were processed for somatic (bone marrow) metaphase chromosomes using the procedure outlined by Patton (1967) and modified by Lee (1969). In this procedure 1 cc colchicine, a mitotic inhibitor, was injected intraperitoneally with an optimum period of

Table 1, Collecting localities and sample sizes for 26 populations of three species of *Spermophilus*

Population number	Locality	Number of individuals
Spermophilus tridecemlineatus		
1	Denton, Denton Co., Texas	18
2	Wichita Falls, Wichita Co., Texas	17
3	Paris, Lamar Co., Texas	10
4	Lewisville, Denton Co., Texas	7
5	Terre Haute, Vigo Co., Indiana	12
6	Corsicana, Navarro Co., Texas and 2 mi. N.E., Bellmead, McLennan Co., Texas	5
7	Gruver, Hansford Co., Texas	6
8	Lubbock, Lubbock Co., Texas	5
9	Amarillo, Potter Co., Texas	5
Spermophilus mexicanus		
10	Vicinity Sheffield, Pecos Co., Texas	15
11	Austin, Travis Co., Texas	7
12	Carlsbad, Eddy Co., New Mexico	7
13	New Braunfels, Comal Co., Texas	5
14	Hogan Park, Midland, Midland Co., Texas	10
15	Pecos, Reeves Co., Texas	
16	Hobbs, Lea Co., New Mexico	10
17	Harlingen, Cameron Co., Texas	7
18	Corpus Christi, Nueces Co., Texas	9

Table 1 (Continued)

Population number	Locality	Number of individuals
Spermophilus spilosoma		
19	Del Norte Golf Course, El Paso, El Paso Co., Texas	22
20	Ponder Park, El Paso, El Paso Co., Texas	7
21	North East El Paso Co., Texas	7
22	8 mi. W. Borger, Hutchinson Co., Texas	5
23	5 mi. N. Janos, Chihuahua, Mexico	2
24	Deming, Luna Co., New Mexico	6
25	1 mi. E. Carrizozo, Lincoln Co., New Mexico	2
26	South Padre Island, Cameron Co., Texas	5

exposure of 50 to 60 min. After sacrificing the animal, the femur of one leg was removed, all muscle was detached, and the bone was splintered into a centrifuge tube containing 10 cc of 1% sodium citrate solution at 37°C. After vigorous shaking, the tube, containing bone marrow cells and bone chips, was incubated for 12 min at 37°C. The tube was then gently inverted several times to resuspend the cells. The bone chips were allowed to sediment, and the supernatant was decanted into another tube. The suspension of cells was centrifuged for 2 min at 500 rpm, resulting in a button of cells in the bottom of the tube. The supernatant was decanted, 10 cc of new fixative was added, and the button was resuspended. This washing procedure was repeated three times. On the third wash 1 cc of fixative was added, and the button was resuspended. The suspension was then dropped onto chilled, wet slides and flame-dried. Slides were stained for 20 min in a 4% solution of buffered Giemsa stain and washed with deionized water.

Karyograms of the mitotic chromosomes were constructed from photomicrographs and major classes of chromosomes were established as follows: metacentric chromosomes (arm ratio approximately 1:1), submetacentric chromosomes (arm ratio 1:1.1 to 1:1.9), subtelocentric chromosomes (arm ratio 1:2 or greater), and acrocentric chromosomes (second arms minute or absent).

Horizontal Starch Gel Electrophoresis

Preparation of Tissue Extracts

Blood was obtained by inserting a 1.0 x 100 mm capillary tube into the suborbital canthal sinus. For serum samples, approximately two capillary tubes of blood were collected in a dry 6 x 50 mm culture tube and allowed to clot for approximately 20 min. The samples were then centrifuged at 1000 g for 10 min. Serum samples were placed in a clean culture tube and were either used immediately or stored at -20°C until used.

Transferrin samples were prepared by removing one drop of the serum and placing it in a clean 10 x 75 mm culture tube containing one drop of a 0.15% ferric chloride solution. Four drops of a 0.6% rivanol solution (2-ethoxy-6, 9-diaminoacridine lactate) were added, the solution was thoroughly mixed by shaking, and the precipitate was centrifuged at 1000 g for 3 min (Chen and Sutton, 1967). This process removed most of the proteins other than transferrin (Sutton and Karp, 1965). Electrophoresis of the clear yellow supernatant solution was completed within 6 h of sample preparation. The remaining serum was diluted with an equal volume of buffered saline solution. Albumins and plasma esterases were run from dilute serum samples.

For hemoglobin and erythrocyte protein samples, approximately one capillary tube of blood was collected in a 10 x 75 mm test tube containing 0.5 ml of 4% sodium

citrate solution. Samples were centrifuged at 1000 g for three min. Erythrocytes were washed two times in buffered saline solution and hemolysed by the addition of three to four drops of deionized water. Hemolysed erythrocyte preparations were then centrifuged at 1000 g for 10 min. Hemoglobin samples were run within 5 h of bleeding. Storage, even at low temperature, has been reported to result in denaturing of hemoglobin (Jensen, 1970; Selander et al., 1969).

Tissue extracts were prepared by homogenizing samples of liver, kidney and testis in two volumes of buffer (0.1 M tris pH 7.0, 0.001 M EDTA) for approximately 1 min in a cooled, 7-ml glass tissue grinder (Selander et al., 1971). Extracts were centrifuged at 10,000 rpm for 6 min. The supernatant solution was removed and used immediately, or stored at -20°C for as long as a month.

Organs not homogenized immediately after the death of the animal were dissected and frozen in two volumes of homogenizing buffer. Selander et al. (1971) reported that most proteins remain undenatured in intact organs frozen for weeks or months at -20°C, but in solution the activity of many enzymes was soon lost at this temperature.

Electrophoretic Apparatus and Techniques

Horizontal starch gel electrophoresis (Smithies, 1955) was used to fractionate all samples. Gel molds were modified from those described by Kristjansson (1963). The mold consisted of a glass plate (152 mm x 220 mm x 6 mm) and four plexiglass

strips, two 6 mm x 19 mm x 196 mm and two 6 mm x 19 mm x 114 mm. The plexiglass strips were held in place with petroleum jelly. When the liquid gel was poured into the mold, a plexiglass plate (152 mm x 220 mm x 6 mm) was used to cover the mold.

All gels were prepared from a 12% suspension of hydrolysed starch (Sigma Chem. Co., St. Louis, Missouri). Suspended starch was poured into the buffer heated to boiling in a 1,000 ml round-bottom flask and shaken vigorously for 1 min. The mixture was then degassed with an aspirator for approximately 1 min. After degassing, the clear liquid gel was poured into the mold, covered with a plexiglass plate, and allowed to cool at room temperature for a minimum of 90 min.

After the gels had cooled the plexiglass plate and long plexiglass strips were removed. Gles were cut parallel to and 2.0 cm from one of the short sides of the gel to form an insertion line, and the smaller portion of the gel was gently pushed back. All samples were absorbed into no. 3 filter paper (4 mm x 5 mm) with the exception of albumin and plasma esterase samples which were absorbed into no. 1 filter paper. Filter paper inserts were blotted and placed approximately 3 mm apart against the exposed cut surface of the smaller portion of the cut gel. After the samples were placed on the gel, the smaller portion was carefully pushed back in contact with the larger portion of the gel.

Saran Wrap (Dow Chemical Company) was used to cover the surface of the gel during electrophoresis (Kristjansson, 1963).

The edges of the Saran Wrap were folded back to expose approximately 1.7 cm of the gel, to allow contact with the bridge from the electrode chambers of the electrophoresis apparatus.

The electrophoresis chamber consisted of a plexiglass tray (405 mm x 360 mm x 88 mm) which was divided into three compartments. The two outer electrode chambers (405 mm x 101 mm) each contained a 304 mm no. 22 platinum wire. The gel was supported on a glass plate placed across the central compartment and sponge cloths (203 mm x 139 mm x 6 mm) were used as bridges between the gel and the electrode buffer. A glass plate was placed on top of the sponge cloth bridges to hold them flat in contact with the gel. Power was supplied by either a Gelman Electrophoresis Model 38206 or a Heathkit 1P17 H.V. power supply. All electrophoresis was completed in a controlled temperature chamber between 0^{o} and 4^{o}C.

Buffer Systems

Seven buffer systems were used to separate the various proteins examined in this study. Hemoglobins and esterase-1 (erythrocyte esterase) were separated in a discontinuous buffer system consisting of a 0.01 M trishydrochloric acid gel buffer, pH 8.5, and an electrode buffer of 0.3 M sodium borate pH 8.2 (Selander et al., 1971). Most efficient separation was obtained at 25 ma with voltage not exceeding 250 v. Two other erythrocyte esterases and 6-phosphogluconate dehydrogenase were separated from the hemolysate in a continuous buffer system consisting of an electrode buffer of 0.1 M

tris- 0.1 M maleic acid- 0.01 M EDTA -0.01 M magnesium chloride, pH 7.4 and a gel of a 1:9 dilution of the electrode buffer of the same pH (Selander et al., 1971). A potential of 100 v for approximately 6 h was sufficient for separation.

Glutamate oxaloacetic transaminase and α-glycerophosphate dehydrogenase were separated from liver or kidney extracts in a continuous buffer system consisting of a gel buffer of 22.89 mM tris- 5.22 mM citric acid, pH 8.0, and an electrode buffer of 0.687 M tris- 0.157 M citric acid, pH 8.0 (Selander et al., 1971). A potential of 100 v was applied for 4 h for sufficient separation.

Lactate dehydrogenase was separated from kidney and testicular extracts in a discontinuous buffer system consisting of a gel buffer of 0.076 M tris- 0.005 M citric acid, pH 8.7 and an electrode buffer of 0.3 sodium borate, pH 8.2 (Selander et al., 1971). Sufficient separation was obtained at 25 ma per gel with a maximum potential of 250 v for 3 h.

Malate dehydrogenase, malic enzyme, and isocitrate dehydrogenase were separated from kidney extracts in a continuous buffer applied for 5 h to provide sufficient separation.

Most serum proteins (transferrins and esterases) and liver and kidney esterases were separated in a system consisting of a gel of a 1:9 mixture of stock solutions A and B as follows: Stock solution A was a 0.03 M lithium hydroxide- 0.19 M boric acid, pH 8.1, and stock solution B was a 0.05 M tris- 8 mM citric acid, pH 8.4. The electrode buffer consisted

of stock solution A (Selander et al., 1971). Optimum separation was obtained at 25 ma per gel with the voltage not exceeding 350 v for a period of 2.5 h. Albumins were separated from the sera in a discontinuous system consisting of a tris-citrate gel (0.004 M citric acid), pH 6.0. The electrode buffer was a 0.3 M sodium borate solution, pH 6.5 (Jensen and Rasmussen, 1971). Optimum separation was obtained at 25 ma per gel with a maximum potential of 300 v until the borate boundary had migrated 8 cm from the origin. A potential of 170 v was applied for 3 h to produce desired separation.

Phosphglucomutases and phsophoglucose isomerase were separated from liver in a discontinuous phosphate buffer system consisting of an electrode buffer of 0.138 M potassium phosphate--0.062 M sodium hydroxide, pH 6.7, and a gel buffer which was a 1:19 dilution of the electrode buffer. A potential of 120 v for 5 h was applied to obtain adequate separation (Selander et al., 1971).

After electrophoresis, gels were allowed to cool for a few minutes and sliced in 2 mm horizontal sheets with a 0.2 mm wire stretched tightly across a frame. The two halves were then separated onto two glass plates.

Identification of Proteins

Hemoglobins, albumins and tranferrins were stained with a general protein stain of 2% solution of buffalo black NBR (naphtol blue black) for 20 min in a 5:5:1 mixture of methanol, deionized water, and glacial acetic acid.

Enzymes were identified by using specific biochemical staining techniques. Glutamate oxaloacetic transaminase was identified by the staining technique of DeLorenzo and Ruddle (1970) consisting of a stain of 50 ml 0.2 M tris-hydrochloric acid buffer, pH 9.0; 0.5 mg pyridoxal- 5'-phosphate; 200 mg -aspartic acid; 100 mg ketoglutaric acid; and 150 mg Fast Blue RR Salt. Stain was prepared immediately prior to use and gels were stained in the dark for 30 min at 37°C.

Isocitrate dehydrogenase produced two forms, IDH-1 (supernatant) and IDH-2 (mitochondrial). The staining technique of Selander et al. (1971) was used and consisted of 50 ml 0.2 M tris-hydrochloric acid buffer, pH 8.0, 0.2 ml 0.25 M manganese chloride, 3 ml 0.1 trisodium Dl-isocitric acid, 10 mg NADP, 5 mg NBT, and 7 mg PMS. Gles were incubated for 30-60 min., 37°C in the dark. LDH was inhibited on these gels with 1 ml isobutyramide solution per 10 ml stain (4.08 g isobutyramide, 250 ml deionized water, adjust to pH 7.5 with NaOH solution).

Lactate dehydrogenase was detected by a technique modified from Markert and Massaro (1966). The stain consisted of 30 ml of deionized water, 20 ml of 0.2 M tris-hydrochloric acid buffer (pH 8-0), 9 ml 0.5 M sodium DL-lactate, 20 mg β-nicotinamide adenine dinucleotide, 10 mg MTT tetrazolium, and 8 mg phenazine methosulfate. Gels were stained in the dark at 37°C for 1 to 2 h.

Malate dehydrogenase activity was detected by a technique modified from Shows and Ruddle (1968) using a stain

consisting of 30 ml 0.2 M tris-hydrochloric acid buffer, pH 8.0, 5 ml 2.0 M malate solution (pH adjusted to 7.0 with 1.0 M sodium hydroxide), 10 mg β-nicotinamide adenine dinucleotide, 20 mg MTT tetrazolium, and 5 mg phenazine methosulfate. The gels were stained in the dark at 37°C for 1 h. LDH was inhibited on these gels with isobutyramide.

Malic enzyme activity was detected using a stain that was identical to the malate dehydrogenase stain with the exception that NADP was substituted for β-nicotinamide adenine dinucleotide (Kilpatrick and Zimmerman, 1974).

α-Glycerophosphate dehydrogenase was detected with the staining technique of Selander et al. (1971), using 50 ml 0.2 M tris-hydrochloric acid buffer pH 8.0, 1 ml 0.1 M magnesium chloride, 50 mg disodium DL-glycerophosphate, 20 mg NAD, 13 mg NBT, and 4 mg PMS. Gels were incubated in the dark at 37°C for 1 to 2 h. LDH was inhibited on these gels by isobutyramide.

The enzyme 6-phosphogluconate dehydrogenase was detected by staining technique of Carter et al. (1968). The stain consisted of 7 ml 0.2 M tris-hydrochloric acid buffer, pH 8.0, 7 ml 0.1 M magnesium chloride, 3 ml deionized water, 20 mg barium-6-phosphogluconic acid, 1 mg β-nicotinamide adenine dinucleotide phosphate, 4 mg MTT tetrozolium, and 1 mg phenazine methosulfate. Gels were stained in the dark at 20°C for 1 h.

Esterases were detected by and coded according to the methods outlined by Selander et al. (1971). Esterases in

the sera and kidney extracts were stained with a mixture of 1 ml 0.2 M monobasic sodium phosphate, pH 4.4, 1 ml 0.2 M dibasic sodium phosphate, pH 8.7, 47 ml deionized water, 25 mg Fast Blue RR Salt, 1 ml of a solution of 0.1 g α-naphthyl propionate (kidney extracts) or α-naphthyl butyrate (serum) in 10 ml of acetone. Gels were stained at 37°C in the dark for 10 to 30 min. Some esterases of kidney and liver extracts were inhibited by the use of 0.001 M eserine which allowed identification of the non-inhibited esterases. Gels were preincubated at room temperature for 20 min. prior to staining at 37°C in substrate solution.

The phsophoglucomutases were detected using the staining technique of Spender et al. (1964). The stain consisted of 25 ml water, 5 ml 0.2 M tris-hydrochloric acid buffer, pH 8.0 5 ml 0.05 M disodium α-D-glucose-1-phosphate, 5 ml 5 x 10^{-4} M dipotassium α-D-glucose-1, 6-diphosphate, 5 ml 0.1 M magnesium chloride, 4 ml glucose-6-phosphate dehydrogenase (10 units/ml water), 5 mg NADP, 5 mg MTT tetrozolium, and 2 mg phenazine methosulfate. Gels were stained in the dark for 1 h. PGM-3 was the most anodal system while PGM-1 was the least anodal of the three mutase loci (Selander et al., 1971).

The enzyme phosphoglucose isomerase was detected using the staining technique of Carter and Parr (1967). This stain consisted of 30 ml 0.2 M tris-hydrochloric acid buffer, pH 8.0, 10 ml 0.1 M magnesium chloride, 4 ml 0.018 M disodium α-D-fructose-6-phosphate, 2 ml glucose-6-phosphate dehydrogenase

(10 units/ml water), 10 mg NADP, 20 mg MTT tetrozolium, and 10 mg phenazine methosulfate. Gels were incubated in the dark for 30 to 60 min. PGI migrates cathodally.

Erythrocyte esterases were stained with 24 ml 0.2 M monobasic sodium phosphate, pH 4.4, 6 ml 0.2 M dibasic sodium phosphate, pH 8.7, 20 ml deionized water, 25 ml Fast Garnet GBC Salt, and 1 ml of a soltuion of 0.1 g α-naphthyl propionate in 10 ml of acetone. Staining was accomplished at 37°C in the dark for a period of 1 to 2 h. All gels were fixed in the 5:5:1 methanol, deionized water, glacial acetic acid solution for 24 h, scored or photographed, and wrapped in a clear plastic film for storage.

Treatment of Data

Since heterozygous individuals are identifiable, electrophoretic data allowed direct calculation of allelic frequencies. Gene frequencies were calculated by summing the occurrence of a given allele for all loci at which it occurs and dividing by the number of loci times two at which the allele could occur.

The systematic relationships of populations of S. tridecemlineatus, S. mexicanus, and S. spilosoma were analyzed on the basis of genetic similarity and differences of the 29 loci examined. Rogers' (1972) coefficient of genetic similarity (S) was utilized for comparison of populations on the basis of gene frequencies of the populations. Genetic similarity (I), distance (D) and time (T) were calculated for paired

combinations of all populations using Nei's coefficient (1971). From these analyses, genetic similarity dendrograms for populations were drawn and correlated with time.

The genetic similarity (S) between two populations is calculated by summing the probabilites of drawing identical genotypes from the two populations for each genotype of the locus, divided by the sum of the probabilities of drawing identical genotypes from the same population on two successive independent draws from each genotype of the locus as shown below:

$$S_R = 1 - \frac{1}{L} \sum_{i=1}^{L} \left[\tfrac{1}{2} \sum_{j=1}^{A} (P_{ijx} - P_{ijy})2 \right] \tfrac{1}{2}$$

where L is the number of loci, A_1 is the number of alleles at the ith locus and P_{ijx} and P_{ijy} are the frequencies of the jth allele at the ith locus in populations x and y respectively. From a matrix of coefficients of genetic similarity for populations of Ictidomys, cluster analysis was performed by the weighted pair group method of Sokal and Sneath (1963).

Genetic similarity is also calculated for paired combinations for all populations sampled using the coefficients of Nei (1971). The normalized identity of genes between two populations designated as x and y with respect to each locus is defined as:

$$I_j = \frac{\sum j_{xy}}{\sum j_x j_y}$$

where j_x is the probability of identity of two randomly chosen genes in population x and j_y is the probability of identity of randomly chosen genes in population y. Nei's similarity measure has the added advantage of permitting the calculation of an expected divergence time for populations based on biochemical data as defined below:

$$t = \frac{D}{(2cn_t\lambda a)}$$

where D is the geometric mean of genetic similarity ($- \log_e I$), c is the proportion of amino acid substitutions which can be detected by electrophoresis (.40); λa is the rate of amino acid substitutions per polypeptide per site per year (2.1×10^{-9}); and n_t is the total number of amino acids (codons) concerned with synthesis of a protein (731).

Levels of heterozygosity provide provide estimates of genetic variability within populations. Since the proportion of polymorphic loci is strongly dependent upon sample size and is therefore a poor index of the degree of genetic variation within populations, Lewontin and Hubby (1966) proposed an index utilizing the proportion of loci heterozygous per individual. This estimate is calculated by summing the observed frequencies of heterozygotes at each locus, dividing by the total in the population, and then averaging over all loci for each population separately. Effective number of alleles was calculated as the reciprocal of the sum of squares of allelic frequencies for a given locus for each population.

CHAPTER III

RESULTS

Karyological Results

Five distinct chromosomal patterns were observed within the three species of Ictidomys examined in this study. Spermophilus tridecemlineatus and S. mexicanus each had a diploid number of 34 while S. spilosoma had a diploid number of 32.

Spermophilus spilosoma

Two distinct chromosomal patterns were observed in the three subspecies of S. spilosoma examined. These patterns corresponded to those described by Nadler and Hughes (1966) for two subspecies of S. spilosoma. All karyotypes of S. spilosoma contained seven pairs of metacentric and eight pairs of submetacentric autosomes. The X was a medium-sized submetacentric chromosome, and the Y was minute acrocentric chromosome. Spermophilus spilosoma annectens and S. spilosoma marginatus (Fig. 1) differed from S. spilosoma canescens (Fig. 2) in the smallest pair of submetacentric autosomes. In S. spilosoma canescens this pair had centromeres located in a nearly terminal position, and they might be better classified as acrocentrics. Nadler and Hughes (1966) also found this pattern in S. spilosoma marginatus from Otero Co., New Mexico. They felt this karyotypic variation indicated

Figure 1, Karyotype of a female Spermophilus spilosoma annectins from South Padre Island, Texas.

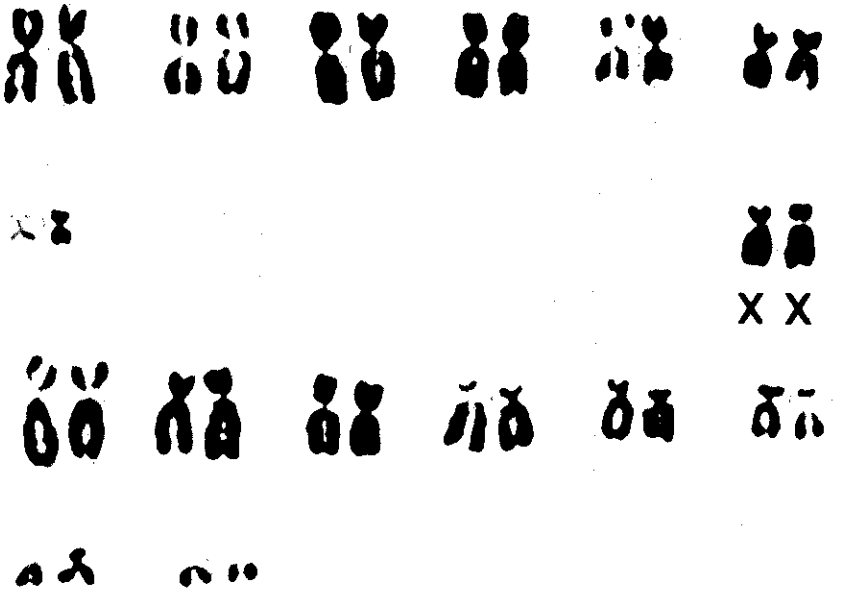
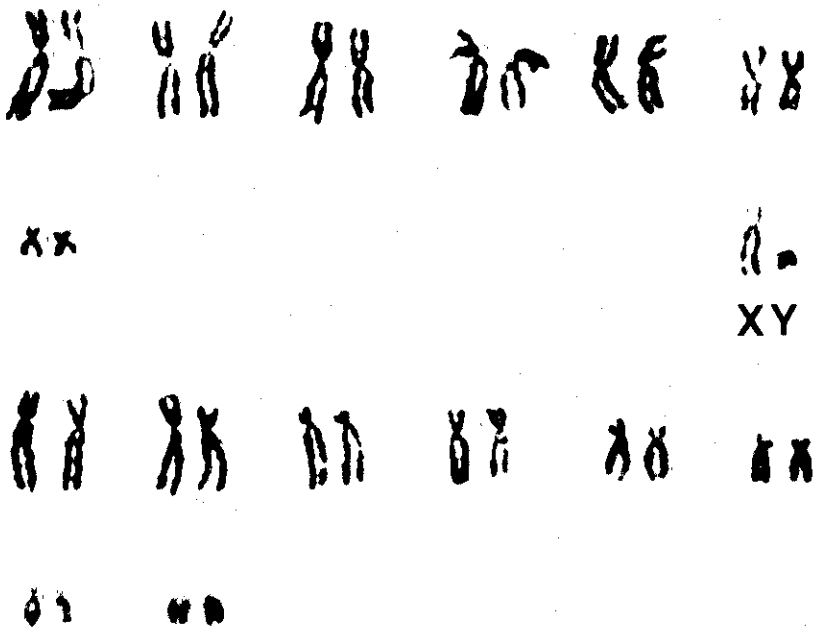


Figure 2, Karyotype of a male Spermophilus spilosoma canescens from Chihuahua, Mexico.



either chromosomal polymorphism or the existence of two distinctive chromosomal populations. Spermophilus spilosoma differs from S. tridecemlineatus and S. mexicanus in that it lacks a pair of metacentrics, and the Y chromosome is a minute rather than a large acrocentric.

Spermophilus tridecemlineatus

Two chromosomal patterns were also found in S. tridecemlineatus. The pattern observed in S. tridecemlineatus arenicola (Fig. 3) was the same as that described by Nadler and Hughes (1966) for S. tridecemlineatus tridecemlineatus and S. tridecemlineatus pallidus. The autosomal compliment of this pattern (Fig. 3) consisted of eight pairs of metacentrics, seven pairs of submetacentrics, and one pair of small acrocentric chromosomes. The X was a submetacentric, and the Y was a medium-sized acrocentric. The second pattern (Fig. 4) was observed in S. tridecemlineatus texensis and also one population of S. tridecemlineatus arenicola. This pattern differed from that of S. tridecemlineatus tridecemlineatus in that the small pair of acrocentric chromosomes was replaced with a small pair of metacentrics.

Spermophilus mexicanus

The single chromosomal pattern observed in S. mexicanus (Fig. 5) was identical to that described by Nadler and Hughes (1966). The only major chromosomal difference observed between S. mexicanus and S. tridecemlineatus is in the size and

Figure 3, Karyotype of a female Spermophilus tridecemlineatus arenicola from Lubbock Co., Texas.

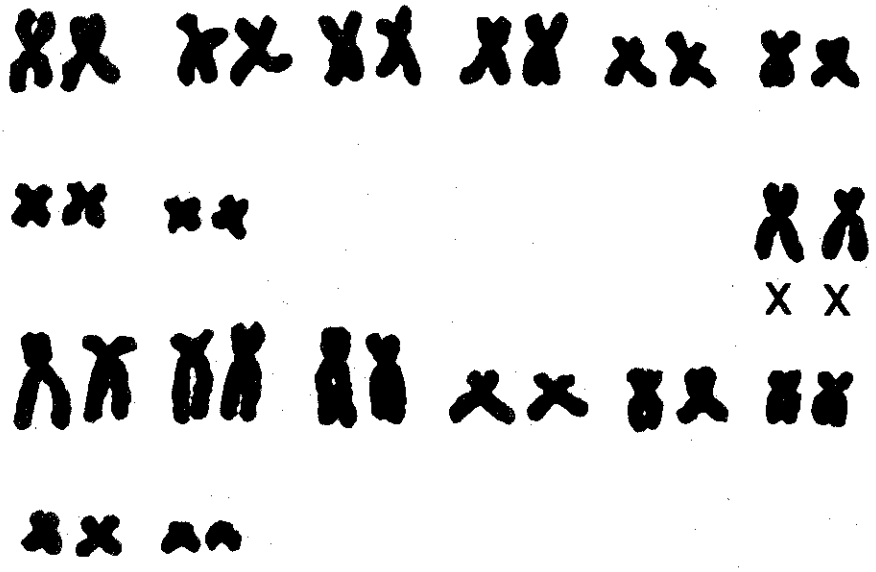


Figure 4, Karyotype of a female Spermophilus tridecemlineatus texensis from Wichita Co., Texas.

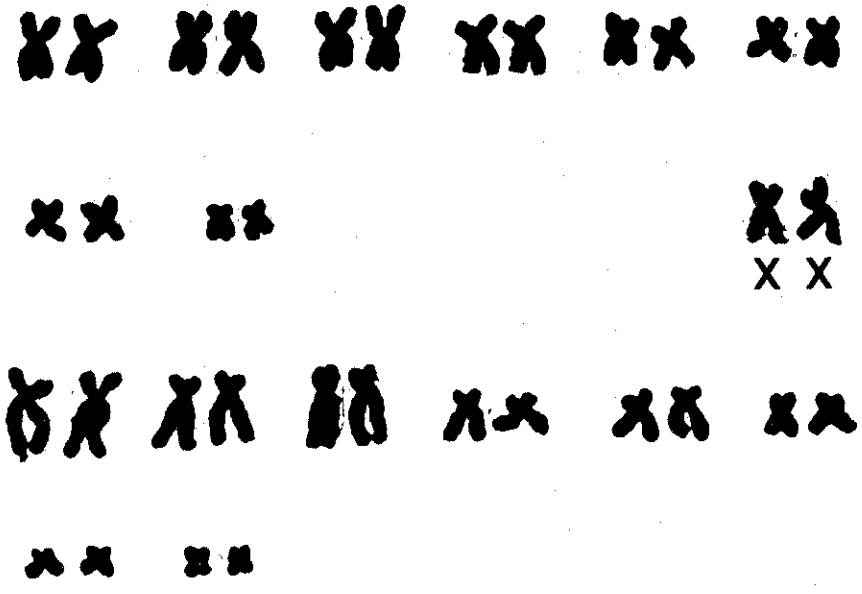
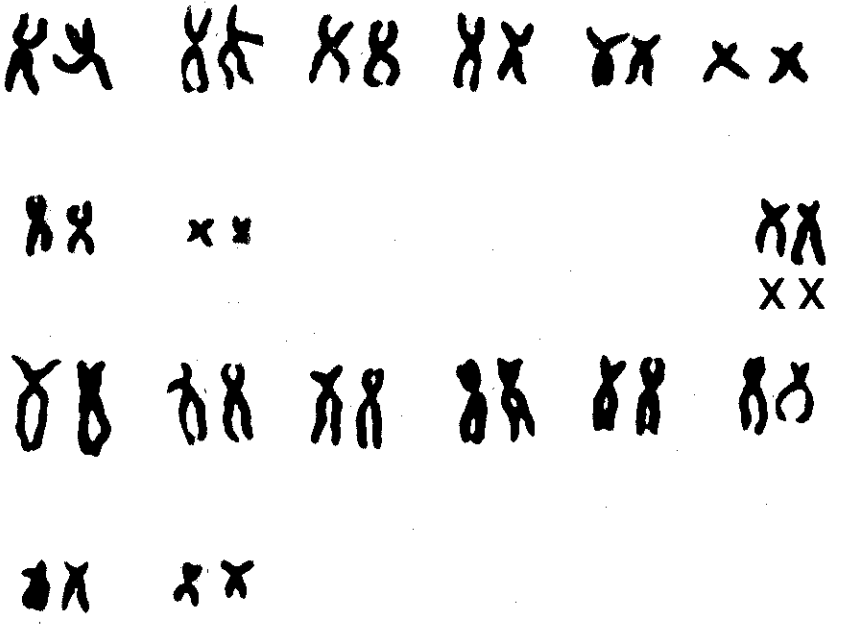


Figure 5, Karyotype of a female Spermophilus mexicanus from Nueces Co., Texas.



centromeric position of one chromosome pair. The last pair of submetacentrics is larger, and the centromere is more nearly metacentric in S. mexicanus. The karyotypic difference may be due to a translocation of a small portion of the fourth longest pair of submetacentric autosomes to another chromosome pair with or without a concomitant pericentric inversion to shift the centromere to a more terminal position (Nadler and Hughes, 1966).

The similarity of the karyotypes of S. tridecemlineatus and S. mexicanus is indicative of their close relationship, and this relationship is exemplified by the discovery of hybrids between these species in population 16 (Lea, Co., New Mexico). Of the 10 individuals examined from this population, four were hybrids (three females, one male). Morphologically, these four individuals were intermediate between the two species. The coat pattern was more nearly like that of S. tridecemlineatus while tail length and length of hind foot were intermediate between the two species. The hybrids were also apparently fertile, as one female was gravid containing five embryos, and one hybrid apparently represented a backcross.

The hybrid karyotypes are represented by three F1-progeny (Fig. 6) and one back-cross (Fig. 7). These hybrid karyotypes show that there are actually three pairs of chromosomes which differ between the two species. The second largest pair of submetacentrics is smaller in S. mexicanus, however, the

Figure 6, Karyotype of a male F1 hybrid (Spermophilus mexicanus X S. tridecemlineatus) from Lea Co., New Mexico.

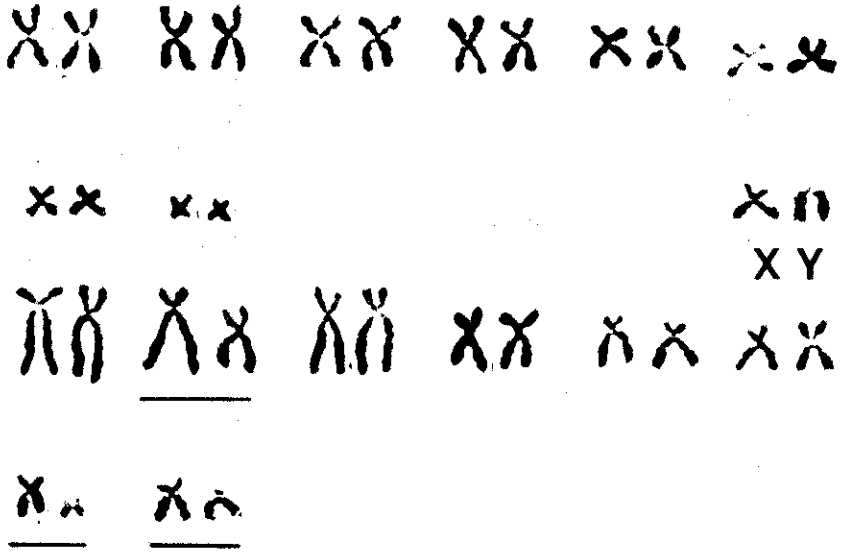
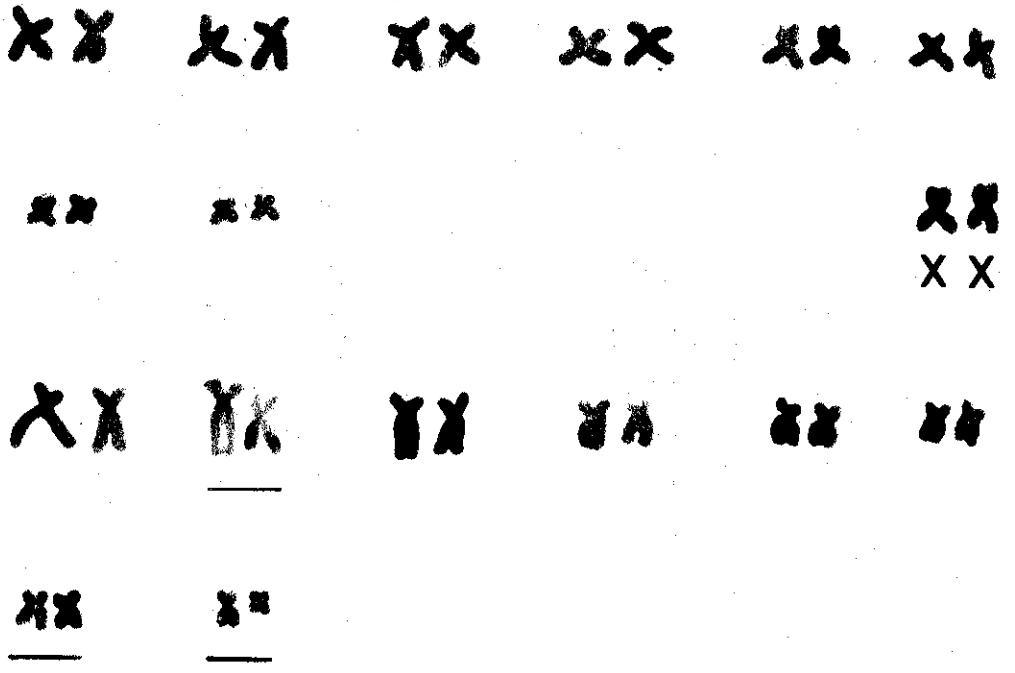


Figure 7, Karyotype of a female backcross between a Spermophilus mexicanus - S. tridecemlineatus hybrid and a S. mexicanus.



centromere is in approximately the same position. In the two smallest pairs of submetacentric autosomes, S. tridecemlineatus has smaller and more nearly acrocentric chromosomes.

Electrophoretic Results

Twenty-nine loci were identified from 216 animals representing 26 populations of the ground squirrel subgenus Ictidomys (Table 2). These samples were represented by 85 S. tridecemlineatus, 75 S. mexicanus, and 56 S. spilosoma from which 15 loci were considered polymorphic. Two loci, Hb-B and α-GPD, were monomorphic but the same allele was not fixed in all species.

No evidence was found to suggest the linkage of the 29 structural loci encoding the 26 proteins examined to the gonosomal complement. Both males and females were heterozygous for all loci for which heterozygous genotypes were found.

Scorable Proteins

Of the 29 loci examined for the three species of Ictidomys 12 were monomorphic with the same allele fixed in all three species. These 12 loci were Ldh-1, Ldh-2, Ldh-3, Idh-2, Mdh-1, Mdh-2, Me, Got-2, Pgi, Es-4, Ipo, and Hb-α-2. In addition two loci were intraspecifically monomorphic. At the α-Gpd locus the a allele was fixed in all populations of S. tridecemlineatus and S. mexicanus. The b allele was fixed in all populations of S. spilosoma except population

Table 2, Allelic variation at 26 loci for three species of Ictidomys (Alleles are listed in order of frequency of occurrence).

Locus	tridecemlineatus	mexicanus	spilosoma	Mean Effective Number of Alleles
Group I Proteins - Glucose Metabolizing Proteins				
LDH-I	a	a	a	1.00
LDH-II	a	a	a	1.00
LDH-III	a	a	a	1.00
IDH-I	b,c,a	b,c,a	b,a,c	1.49
IDH-II	a	a	a	1.00
6-PGD	b,c	b,a	b,a	1.03
MDH-I	a	a	a	1.00
MDH-II	a	a	a	1.00
ME	a	a	a	1.00
α-GPD	b,c	b,a	b,a	1.03
GOT-I	b,a	b,c,a	b	1.24
GOT-II	a	a	a	1.00
PG-I	a	a	a	1.00
PGM-I	b,a	b,a	b,a	1.28
PGM-II	b,a	b,a	b,c,a	1.83
PGM-III	b,a	b,a	b,a	1.07
Group II Proteins - Other Enzymes				
Es-1	b,d,e,f,c,g	c,b,e,f	d,f,b,a	3.26
Es-3	a,b,d,c	b,c,d,a	b,c,a,d	3.35
Es-4	a	a	a	1.00
Es-5	b,c	b,c,e,a	b,d,c	2.19
Es-6	b,a	b,a	b	1.07
Es-8	a,b	b,a	b,a	1.80

Table 2 (Cont.)

Locus	tridecemlineatus	mexicanus	spilosoma	Mean Effective Number of Alleles
Group III Proteins - Nonenzymatic Proteins				
Hb-α1	a	a	b,a	1.31
Hb-α2	a	a	a	1.00
Hb-β	a	a	b	1.00
Alb	a,c,b	b,d,c,a	a,b	1.77
Trf	a	a,b	b,c	1.10
Pt-1	a,b	a	a	1.01

25 (Lincoln, Co., New Mexico) where the a allele was fixed. At the Hb-B locus the a allele was fixed in S. tridecemlineatus and S. mexicanus, and S. spilosoma was monomorpjic for the b allele. The remaining 15 loci were polymorphic with both intra and interspecific variation.

Isocitrate Dehydrogenase-1 (kidney). The Idh-1 locus showed three alleles and was one of the most variable non-esterase proteins (Table 3). Three alleles were found in all species (Fig. 8) with the b allele being most common. The c allele was the next most common allele in S. tridecemlineatus and S. mexicanus, while the a allele was the second most common allele in S. spilosoma. In two populations of S. spilosoma, population 23 (Janos, Chihuahua) and population 24 (Luna, Co., New Mexico), the a allele was the most common allele.

6-Phosphogluconate Dehydrogenase (hemolysate). The 6-Pgd locus was only weakly polymorphic with three alleles (Fig. 10). The b allele was the most common allele in all three species (Table 4). The c allele was a rare allele found in the heterozygous condition in one individual of S. tridecem-lineatus, and the a allele was found in low frequency in both S. mexicanus and S. spilosoma.

Glutamate Oxaloacetic Transaminase-1 (liver). The Got-1 locus was monomorphic in S. spilosoma and S. tridecemlineatus with the b allele fixed in all populations, except population 7 (Gruver, Texas) of S. tridecemlineatus where the a allele was present at a frequency of 0.17 (Table 5). Three alleles

Table 3, Allelic Frequencies at the Isocitrate dehydrogenase-1 locus for 26 populations of Ictidomys

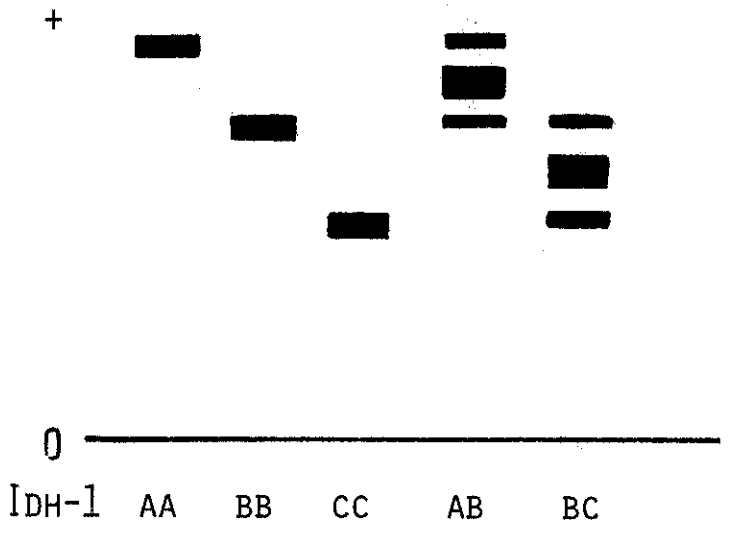
Population	number of individuals	IDH-1 a	IDH-1 b	IDH-1 c
Spermophilus tridecemlineatus				
1 Denton, Texas	18	0.03	0-97	--
2 Wichita Co., Texas	17	0.03	0.97	--
3 Lamar Co., Texas	10	--	1.00	--
4 Lewisville, Texas	7	--	1.00	--
5 Vigo Co., Indiana	12	--	1.00	--
6 Navarro Co., Texas	5	--	0.70	0.30
7 Hansford Co., Texas	6	--	1.00	--
8 Lubbock Co., Texas	5	--	1.00	--
9 Potter Co., Texas	5	--	1.00	--
Spermophilus mexicanus				
10 Pecos Co., Texas	15	--	0.83	0.17
11 Travis Co., Texas	7	--	0.92	0.08
12 Eddy Co., New Mexico	7	--	1.00	--
13 Comal Co., Texas	5	--	1.00	--
14 Midland Co., Texas	10	--	0.65	0.35
15 Reeves Co., Texas	5	--	1.00	--
16 Lea Co., New Mexico	10	--	1.00	--
17 Cameron Co., Texas	7	--	0.86	0.14
18 Nueces Co., Texas	9	0.06	0.61	0.33
Spermophilus spilosoma				
19 Del Norte C.C., El Paso Texas	22	0.36	0.48	0.16

Table 3 (Continued)

Population	number of individuals	IDH-1 a	b	c
20 Ponder Park, El Paso, Texas	7	0.21	0.79	--
21 N.E. El Paso Co., Texas	7	0.21	0.79	--
22 Hutchinson Co., Texas	5	--	1.00	--
23 Janos, Chihuahua, Mexico	2	0.75	0.25	--
24 Luna Co., New Mexico	6	0.67	0.33	--
25 Lincoln Co., New Mexico	2	0.50	0.50	--
26 South Padre Island, Texas	5	--	1.00	--

Figure 8, Electrophoretic variation in isocitrate dehydrogenase (Idh-1).

Figure 9, Electrophoretic variation in glutamate oxaloacetic transaminase (Got-1).


+
0
IDH-1
AA
BB
CC
AB
BC

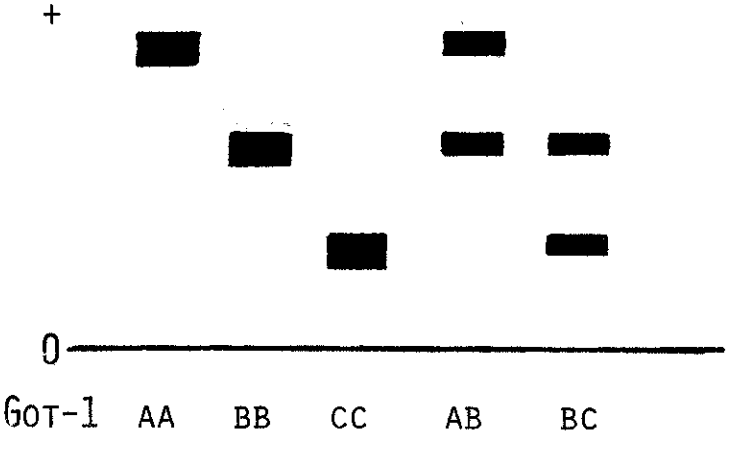

+
0
GOT-1
AA
BB
CC
AB
BC

Table 4, Allelic Frequencies at the 6-Phosphgluconate dehydrogenase locus for 26 populations of Ictidomys

Population	number of individuals	6-PGD a	6-PGD b	6-PGD c
Spermophilus tridecemlineatus				
1 Denton Texas	18	--	1.00	--
2 Wichita Co., Texas	17	--	1.00	--
3 Lamar Co., Texas	10	--	0.95	0.05
4 Lewisville, Texas	7	--	1.00	--
5 Vigo Co., Indiana	12	--	1.00	--
6 Navarro Co., Texas	5	--	1.00	--
7 Hansford Co., Texas	6	--	1.00	--
8 Lubbock Co., Texas	5	--	1.00	--
9 Potter Co., Texas	5	--	1.00	--
Spermophilus mexicanus				
10 Pecos Co., Texas	15	--	1.00	--
11 Travis Co., Texas	7	0.07	0.93	--
12 Eddy, New Mexico	7	--	1.00	--
13 Comal Co., Texas	5	--	1.00	--
14 Midland Co., Texas	10	--	1.00	--
15 Reeves Co., Texas	5	--	1.00	--
16 Lea, New Mexico	10	--	1.00	--
17 Cameron Co., Texas	7	--	1.00	--
18 Nueces Co., Texas	9	--	1.00	--
Spermophilus spilosoma				
19 Del Norte C.C., El Paso Co., Texas	22	0.16	0.84	--

Table 4 (Continued)

Population	number of individuals	6-PGD a	6-PGD b	6-PGD c
20 Ponder Park, El Paso Co., Texas	7	--	1.00	--
21 N.E. El Paso Co., Texas	7	--	1.00	--
22 Hutchinson Co., Texas	5	--	1.00	--
23 Janos, Chihuahua, Mexico	2	--	1.00	--
24 Luna Co., New Mexico	6	--	1.00	--
25 Lincoln Co., New Mexico	2	--	1.00	--
26 South Padre Island, Texas	5	--	1.00	--

Table 5, Allelic Frequencies at the Glutamate Oxaloacetic transaminase-1 locus for 26 populations of *Ictidomys*

Population	number of individuals	GOT-1 a	GOT-1 b	GOT-1 c
Spermophilus tridecemlineatus				
1 Denton, Texas	18	--	1.00	--
2 Wichita Co., Texas	17	--	1.00	--
3 Lamar Co., Texas	10	--	1.00	--
4 Lewisville, Texas	7	--	1.00	--
5 Vigo Co., Indiana	12	--	1.00	--
6 Navarro Co., Texas	5	--	1.00	--
7 Hansford Co., Texas	6	0.17	0.83	--
8 Lubbock Co., Texas	5	--	1.00	--
9 Potter Co., Texas	5	--	1.00	--
Spermophilus mexicanus				
10 Pecos Co., Texas	15	--	0.57	0.43
11 Travis Co., Texas	7	--	1.00	--
12 Eddy Co., New Mexico	7	--	0.29	0.71
13 Comal Co., Texas	5	--	1.00	--
14 Midland Co., Texas	10	0.20	0.45	0.35
15 Reeves Co., Texas	5	0.40	0.30	0.30
16 Lea Co., New Mexico	10	--	0.75	0.25
17 Cameron Co., Texas	7	--	1.00	--
18 Nueces Co., Texas	9	--	1.00	--
Spermophilus spilosoma				
19 Del Norte C.C., El Paso Co., Texas	22	--	1.00	--

Table 5 (Continued)

Population	number of individuals	GOT-1 a	GOT-1 b	GOT-1 c
20 Ponder Park, El Paso Co., Texas	7	--	1.00	--
21 N.E. El Paso Co., Texas	7	--	1.00	--
22 Hutchinson Co., Texas	5	--	1.00	--
23 Janos, Chihuahua, Mexico	2	--	1.00	--
24 Lana Co., New Mexico	6	--	1.00	--
25 Lincoln Co., New Mexico	2	--	1.00	--
26 South Padre Island, Texas	5	--	1.00	--

were present at this locus in S. mexcianus (Fig. 9), with the b allele occurring commonly in most populations.

Phosphaglucomutase-1 (liver). Two alleles were present at the Pgm-1 locus (Fig. 11). The b allele was the most common allele in all species except population 10 (Sheffield, Texas) of S. mexicanus where the a allele was fixed (Table 6).

Phosphaglucomutase-2 (liver). The Pgm-2 locus was the most variable of the nonesterase loci with an effective number of alleles of 1.83 (Fig. 11). The b allele was the most common allele in S. spilosoma and S. tridecemlineatus, while the a allele was most common allele in S. mexicanus. The a allele was also found in S. tridecemlineatus and S. spilosoma, while the c allele was found only in S. spilosoma (Table 7).

Phosphaglucomutase-3 (liver). The Pgm-3 locus was weakly polymorphic, with two alleles present (Fig. 11). The b allele was the most common allele (Table 8), and the a allele was present in all three species.

Esterase-1 (hemolysate). Seven alleles were found at the Es-1 locus (Fig. 12). Six alleles were found in S. tridecemlineatus with the b allele being the most common (Table 9). The g allele was a rare allele found in one individual from population 4 (Lewisville, Texas). Four alleles were present in S. mexicanus. The c allele was the most common allele in this species with the b allele the next most frequent. Four alleles were also found in S. spilosoma. The f allele was the most common allele in S. spilosoma while the d allele was the

Table 6, Allelic Frequencies at the Phosphoglucomutase-1 locus for 26 populations of Ictidomys

Population	number of individuals	PGM-1 a	PGM-1 b
Spermophilus tridecemlineatus			
1 Denton, Texas	18	0.25	0.75
2 Wichita Co., Texas	17	--	1.00
3 Lamar Co., Texas	10	--	1.00
4 Lewisville, Texas	7	--	1.00
5 Vigo Co., Indiana	12	--	1.00
6 Navarro Co., Texas	5	--	1.00
7 Hansford Co., Texas	6	--	1.00
8 Lubbock Co., Texas	5	0.20	0.80
9 Potter Co., Texas	5	--	1.00
Spermophilus mexicanus			
10 Pecos Co., Texas	15	1.00	--
11 Travis Co., Texas	7	--	1.00
12 Eddy Co., New Mexico	7	0.27	0.73
13 Comal Co., Texas	5	0.50	0.50
14 Midland Co., Texas	10	--	1.00
15 Reeves Co., Texas	5	0.20	0.80
16 Lea Co., New Mexico	10	0.05	0.95
17 Cameron Co., Texas	7	0.14	0.86
18 Nueces Co., Texas	9	--	1.00
Spermophilus spilosoma			
19 Del Norte C.C., El Paso Co., Texas	22	--	1.00

Table 6 (Continued)

Population	number of individuals	PGM-1 a	PGM-1 b
20 Ponder Park, El Paso Co., Texas	7	--	1.00
21 N.E. El Paso Co., Texas	7	--	1.00
22 Hutchinson Co., Texas	5	0.30	0.70
23 Janos, Chihuahua, Mexico	2	0.25	0.75
24 Luna Co., New Mexico	6	--	1.00
25 Lincoln Co., New Mexico	2	--	1.00
26 South Padre Island, Texas	5	--	1.00

Table 7, Allelic Frequencies at the Phosphoglucomutase-2 locus for 26 populations of Ictidomys

Population	number of individuals	PGM-2 a	b	c
Spermophilus tridecemlineatus				
1 Denton, Texas	18	--	1.00	--
2 Wichita Co., Texas	17	0.36	0.64	--
3 Lamar Co., Texas	10	--	1.00	--
4 Lewisville, Texas	7	0.50	0.50	--
5 Vigo Co., Indiana	12	--	1.00	--
6 Navarro Co., Texas	5	--	1.00	--
7 Hansford Co., Texas	6	0.75	0.25	--
8 Lubbock Co., Texas	5	1.00	--	--
9 Potter Co., Texas	5	0.40	0.60	--
Spermophilus mexicanus				
10 Pecos Co., Texas	15	0.28	0.72	--
11 Travis Co., Texas	7	--	1.00	--
12 Eddy Co., New Mexico	7	0.86	0.14	--
13 Comal Co., Texas	5	--	1.00	--
14 Midland Co., Texas	10	1.00	--	--
15 Reeves Co., Texas	5	0.50	0.50	--
16 Lea Co., New Mexico	10	0.90	0.10	--
17 Cameron Co., Texas	7	1.00	--	--
18 Nueces Co., Texas	9	--	1.00	--
Spermophilus spilosoma				
19 Del Norte C.C., El Paso Co., Texas	22	--	0.58	0.42

Table 7 (Continued)

Population	number of individuals	PGM-2 a	b	c
20 Ponder Park, El Paso Co., Texas	7	--	0.62	0.38
21 N.E. El Paso Co., Texas	7	--	0.87	0.13
22 Hutchinson Co., Texas	5	--	0.40	0.60
23 Janos, Chihuahua, Mexico	2	--	0.75	0.25
24 Luna Co., New Mexico	6	0.08	0.75	0.17
25 Lincoln Co., New Mexico	2	--	0.75	0.25
26 South Padre Island, Texas	5	--	1.00	--

Table 8, Allelic Frequencies at the Phosphoglucomutase-3 locus for 26 populations of Ictidomys

Population	number of individuals	PGM-3 a	PGM-3 b
Spermophilus tridecemlineatus			
1 Denton, Texas	18	--	1.00
2 Wichita Co., Texas	17	--	1.00
3 Lamar Co., Texas	10	--	1.00
4 Lewisville, Texas	7	0.25	0.75
5 Vigo Co., Indiana	12	--	1.00
6 Navarro Co., Texas	5	--	1.00
7 Hansford Co., Texas	6	--	1.00
8 Lubbock Co., Texas	5	0.20	0.80
9 Potter Co., Texas	5	--	1.00
Spermophilus mexicanus			
10 Pecos Co., Texas	15	0.20	0.80
11 Travis Co., Texas	7	--	1.00
12 Eddy Co., New Mexico	7	--	1.00
13 Comal Co., Texas	5	--	1.00
14 Midland Co., Texas	10	--	1.00
15 Reeves Co., Texas	5	--	1.00
16 Lea Co., New Mexico	10	--	1.00
17 Cameron Co., Texas	7	--	1.00
18 Nueces Co., Texas	9	--	1.00
Spermophilus spilosoma			
19 Del Norte C.C., El Paso Co., Texas	22	0.17	0.83

Table 8 (Continued)

Population	number of individuals	PGM-3 a	PGM-3 b
20 Ponder Park, El Paso, Co., Texas	7	--	1.00
21 N.E. El Paso Co., Texas	7	--	1.00
22 Hutchinson Co., Texas	5	0.30	0.70
23 Janos, Chihuahua, Mexico	2	--	0.00
24 Luna Co., New Mexico	6	--	0.00
25 Lincoln Co., New Mexico	2	--	0.00
26 South Padre Island, Texas	5	--	0.00

Table 9, Allelic Frequencies at the Esterase-1 locus for 26 populations of *Ictidomys*

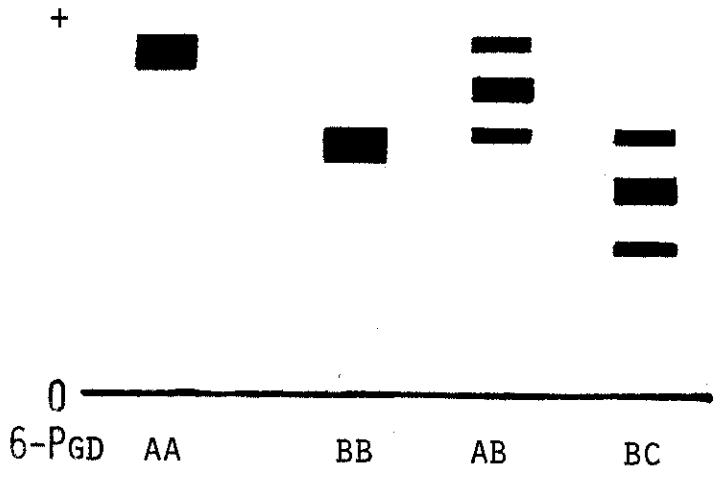
Population	number of individuals	ES-1 a	b	c	d	e	f	g
Spermophilus tridecemlineatus								
1 Denton, Texas	18	--	0.25	--	0.42	0.33	--	--
2 Wichita Co., Texas	17	--	0.47	--	0.53	--	--	--
3 Lamar Co., Texas	10	--	0.40	--	--	0.60	--	--
4 Lewisville, Texas	7	--	--	0.14	--	0.29	0.50	0.07
5 Vigo Co., Indiana	12	--	--	0.13	--	--	0.87	--
6 Navarro Co., Texas	5	--	0.60	0.40	--	--	--	--
7 Hansford Co., Texas	6	--	0.42	0.33	0.25	--	--	--
8 Lubbock Co., Texas	5	--	0.30	--	0.66	0.10	--	--
9 Potter Co., Texas	5	--	0.40	--	0.60	--	--	--
Spermophilus mexicanus								
10 Pecos Co., Texas	15	--	0.20	0.80	--	--	--	--
11 Travis Co., Texas	7	--	0.43	--	--	0.57	--	--
12 Eddy Co., New Mexico	7	--	--	1.00	--	--	--	--
13 Comal Co., Texas	5	--	0.60	--	--	--	0.40	--
14 Midland Co., Texas	10	--	0.25	0.75	--	--	--	--

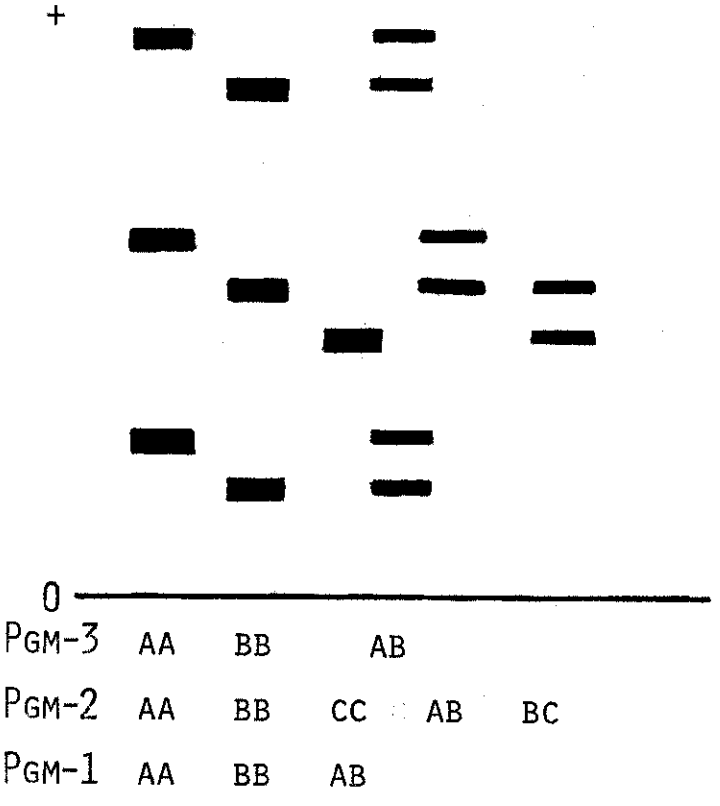
Table 9 (Continued)

Population	number of individuals	ES-1 a	b	c	d	e	f	g
15 Reeves Co., Texas	5	--	0.20	0.80	--	--	--	--
16 Lea Co., New Mexico	10	--	0.30	0.60	--	0.10	--	--
17 Cameron Co., Texas	7	--	1.00	--	--	--	--	--
18 Nueces Co., Texas	9	--	1.00	--	--	--	--	--
Spermophilus spilosoma								
19 Del Norte C.C. El Paso Co., Texas	22	--	--	--	0.14	--	0.86	--
20 Ponder Park, El Paso Co., Texas	7	--	--	--	0.71	--	0.29	--
21 N.E. El Paso Co., Texas	7	--	--	--	0.36	--	0.64	--
22 Hutchinson Co., Texas	5	--	--	--	0.50	--	0.50	--
23 Janos, Chihuahua Mexico	2	--	--	--	1.00	--	--	--
24 Luna Co., New Mexico	6	0.08	--	--	0.92	--	--	--
25 Lincoln Co., New Mexico	2	0.75	--	--	0.25	--	--	--
26 South Padre Island, Texas	5	--	1.00	--	--	--	--	--

Figure 10, Electrophoretic variation in 6-phosphogluconate dehydrogenase (6-Pgd).

Figure 11, Electrophoretic variation in the phosphoglucomutates (Pgm-1, Pgm-2, Pgm-3).


+
0
6-PGD
AA
BB
AB
BC


+
0
PGM-3
AA
BB
AB
PGM-2
AA
BB
CC
AB
BC
PGM-1
AA
BB
AB

next most common. The mean effective number of alleles was 3.26.

Esterase-3 (liver). The Es-3 locus was the most polymorphic locus examined in Ictidomys, with four alleles demonstrated (Fig. 13) and a mean effective number of alleles of 3.35. All four alleles were found in each species (Table 10), and in S. tridecemlineatus, all four were found in nearly equal frequency. The b allele occurred at the highest frequency, while the a allele was the second most common allele in S. mexicanus and S. Spilosoma, while the c allele was the second most common allele in these species.

Esterase-5 (plasma). The Es-5 locus was also highly polymorphic (Table 11). Five alleles were present at this locus (Fig. 14), and the mean effective number of alleles was 2.19. Only the b and e alleles were found in S. tridecemlineatus, with the b allele being the most common, and four alleles were present in S. mexicanus. The b allele was the most common allele in the latter species, with the c ahlele also occurring frequently. The b and c alleles were also frequently observed in S. spilosoma.

Esterase-6 (hemolysate). The Es-6 locus was only weakly polymorphic (Table 12) with two alleles present. Spermophilus spilosoma monomorphic for the b allele. The b allele was also fixed in all but one population of S. tridecemlineatus and most populations of S. mexicanus.

Table 10, Allelic Frequencies at the Esterase-3 locus for 26 populations of *Ictidomys*

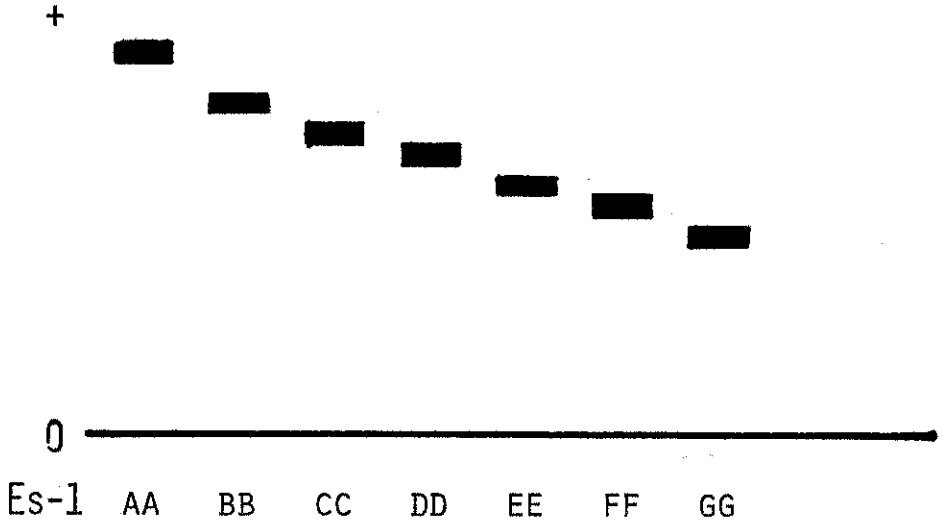
Population	number of individuals	ES-3 a	b	c	d
Spermophilus tridecemlineatus					
1 Denton, Texas	18	0.18	0.41	0.21	0.20
2 Wichita Co., Texas	17	0.44	0.18	0.16	0.22
3 Lamar Co., Texas	10	0.50	--	0.25	0.25
4 Lewisville, Texas	7	0.21	0.36	0.43	--
5 Vigo Co., Indiana	12	0.17	0.83	--	--
6 Navarro Co., Texas	5	0.50	--	0.10	0.40
7 Hansford Co., Texas	6	0.25	0.08	0.17	0.50
8 Lubbock Co., Texas	5	0.30	0.20	0.30	0.20
9 Potter Co., Texas	5	--	0.30	0.30	0.40
Spermophilus mexicanus					
10 Pecos Co., Texas	15	0.23	0.47	0.30	--
11 Travis Co., Texas	7	0.29	0.07	0.64	--
12 Eddy Co., New Mexico	7	0.14	0.72	0.14	--
13 Comal Co., Texas	5	0.10	0.50	0.30	0.10
14 Midland Co., Texas	10	0.15	0.40	0.15	0.30
15 Reeves Co., Texas	5	0.10	0.40	0.10	0.40
16 Lea Co., New Mexico	10	0.25	0.45	0.20	0.10
17 Cameron Co., Texas	7	--	0.28	0.36	0.36
18 Nueces Co., Texas	9	--	0.28	0.50	0.22

Table 10 (Continued)

Population	number of individuals	ES-3 a	b	c	d
Spermophilus spilosoma					
19 Del Norte C.C., El Paso Co., Texas	22	--	0.73	0.27	--
20 Ponder Park, El Paso Co., Texas	7	0.07	0.29	0.14	0.50
21 N.E. El Paso Co., Texas	7	0.14	0.36	0.50	--
22 Hutchinson Co., Texas	5	0.50	0.50	--	--
23 Janos, Chihuahua, Mexico	2	0.25	0.75	--	--
24 Luna Co., New Mexico	6	--	0.58	0.33	0.09
25 Lincoln Co., New Mexico	2	0.50	--	0.50	--
26 South Padre Island, Texas	5	--	1.00	--	--

Figure 12, Electrophoretic variation in the hemolysate esterase-1 (Es-1).

Figure 13, Electrophoretic variation of the tissue esterases (Es-8, Es-3).


+
0
Es-1
AA
BB
CC
DD
EE
FF
GG

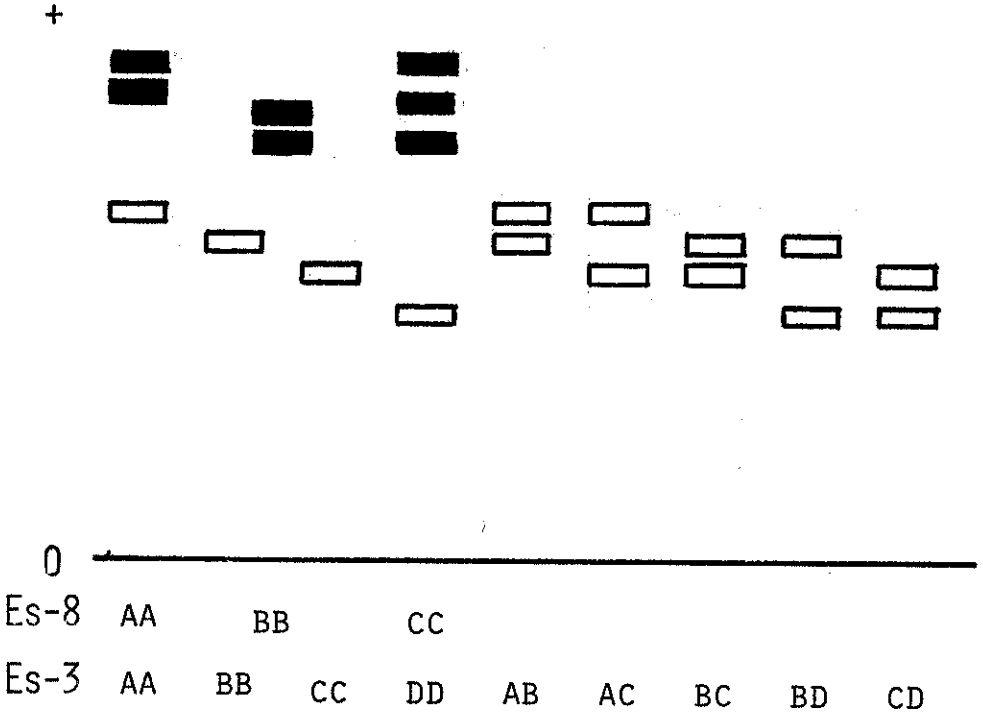

+
0
Es-8
AA
BB
CC
Es-3
AA
BB
CC
DD
AB
AC
BC
BD
CD

Table 11, Allelic Frequencies at the Esterase-5 locus for 26 populations of *Ictidomys*

Population	number of individuals	ES-5 a	b	c	d	e
Spermophilus tridecemlineatus						
1 Denton, Texas	18	--	1.00	--	--	--
2 Wichita Co., Texas	17	--	0.85	--	--	0.15
3 Lamar Co., Texas	10	--	0.50	--	--	0.50
4 Lewisville, Texas	7	--	0.79	--	--	0.21
5 Vigo Co., Indiana	12	--	0.62	--	--	0.38
6 Navarro Co., Texas	5	--	0.37	--	--	0.63
7 Hansford Co., Texas	6	--	0.92	--	--	0.08
8 Lubbock Co., Texas	5	--	0.90	--	--	0.10
9 Potter Co., Texas	5	--	0.80	--	--	0.20
Spermophilus mexicanus						
10 Pecos Co., Texas	15	--	--	0.17	--	0.83
11 Travis Co., Texas	7	--	--	0.57	--	0.43
12 Eddy Co., New Mexico	7	0.21	--	0.65	--	0.14
13 Comal Co., Texas	5	--	1.00	--	--	--

Table 11 (Continued)

Population	number of individuals	ES-5 a	b	c	d	e
14 Midland Co., Texas	10	0.40	0.45	0.15	--	--
15 Reeves Co., Texas	5	0.50	0.50	--	--	--
16 Lea Co., New Mexico	10	0.15	0.40	0.40	--	0.05
17 Cameron Co., Texas	7	--	1.00	--	--	--
18 Nueces Co., Texas	9	--	1.00	--	--	--
Spermophilus spilosoma						
19 Del Norte C.C. El Paso Co., Texas	22	--	0.61	--	0.39	--
20 Ponder Park, El Paso Co., Texas	7	--	0.86	--	0.14	--
21 N.E. El Paso Co., Texas	7	--	0.64	--	0.36	--
22 Hutchinson Co., Texas	5	--	1.00	--	--	--
23 Janos, Chihuahua, Mexico	2	--	0.50	--	0.50	--
24 Luna Co., New Mexico	6	--	0.33	0.25	0.42	--
25 Lincoln Co., New Mexico	2	--	0.50	0.25	0.25	--
26 South Padre Island, Texas	5	--	1.00	--	--	--

Table 12, Allelic Frequencies at the Esterase-6 locus for 26 populations of *Ictidomys*

Population	number of individuals	ES-6 a	ES-6 b
Spermophilus tridecemlineatus			
1 Denton, Texas	18	--	1.00
2 Wichita Co., Texas	17	0.09	0.91
3 Lamar Co., Texas	10	--	1.00
4 Lewisville, Texas	7	--	1.00
5 Vigo Co., Indiana	12	--	1.00
6 Navarro Co., Texas	5	--	1.00
7 Hansford Co., Texas	6	--	1.00
8 Lubbock Co., Texas	5	--	1.00
9 Potter Co., Texas	5	--	1.00
Spermophilus mexicanus			
10 Pecos Co., Texas	15	0.40	0.60
11 Travis Co., Texas	7	--	1.00
12 Eddy Co., New Mexico	7	--	1.00
13 Comal Co., Texas	5	--	1.00
14 Midland Co., Texas	10	--	1.00
15 Reeves Co., Texas	5	--	1.00
16 Lea Co., New Mexico	10	0.30	0.70
17 Cameron Co., Texas	7	0.14	0.86
18 Nueces Co., Texas	9	--	1.00
Spermophilus spilosoma			
19 Del Norte C.C., El Paso Co., Texas	22	--	1.00

Table 12 (Continued)

Population	number of individuals	ES-6 a	ES-6 b
20 Ponder Park, El Paso Co., Texas	7	--	1.00
21 N.E. El Paso Co., Texas	7	--	1.00
22 Hutchinson Co., Texas	5	--	1.00
23 Janos, Chihuahua, Mexico	2	--	1.00
24 Luna Co., New Mexico	6	--	1.00
25 Lincoln Co., New Mexico	2	--	1.00
26 South Padre Island, Texas	5	--	1.00

Esterase-8 (liver). This locus was also highly polymorphic (Table 13) with an effective number of alleles of 1.80. Two alleles were present at the Es-8 locus (Fig. 13), and both alleles were found in all three species. The a allele was the most common allele in S. tridecemlineatus, while the b allele was the most frequent allele in S. mexicanus and S. spilosoma.

Hemoglobin-α1 (hemolysate). Two electrophoretic patterns were demonstrated fro hemoglobin (Fig. 15). Chain separations were run on cellulose acetate electrophoresis to determine whether the variation was in the α or β chains (Fig. 16). The results showed the variation was in the α chain and also indicated duplication of the α locus. Both S. tridecemlineatus and S. mexicanus had the a allele fixed at the Hb-α locus in all populations. Spermophilus spilosoma showed both the a and b alleles in almost equal frequency with the b allele in slightly higher frequency (Table 14).

Albumin (plasma). The Alb locus was the second most variable of the nonesterase proteins (Table 15), with four alleles segregating at this locus (Fig. 17) and an effective number of alleles of 1.77. The a allele was fixed in all populations of S. spilosoma except population 19 (Del Norte Golf Course, El Paso Co., Texas) where one individual was an ab heterozygote. Three alleles were found in S. tridecemlineatus. The a allele was fixed in most populations of this species with the b allele found in low frequency, however, only the b and c alleles were found in population 5 (Terre Haute,

Table 13, Allelic Frequencies at the Esterase-8 locus for 26 populations of Ictidomys

Population	number of individuals	ES-8 a	ES-8 b
Spermophilus tridecemlineatus			
1 Denton, Texas	18	0.47	0.53
2 Wichita Co., Texas	17	0.87	0.13
3 Lamar Co., Texas	10	0.60	0.40
4 Lewisville, Texas	7	0.43	0.57
5 Vigo Co., Indiana	12	1.00	--
6 Navarro Co., Texas	5	0.50	0.50
7 Hansford Co., Texas	6	0.58	0.42
8 Lubbock Co., Texas	5	0.40	0.60
9 Potter Co., Texas	5	0.50	0.50
Spermophilus mexicanus			
10 Pecos Co., Texas	15	0.33	0.67
11 Travis Co., Texas	7	0.29	0.71
12 Eddy Co., New Mexico	7	0.36	0.64
13 Comal Co., Texas	5	0.50	0.50
14 Midland Co., Texas	10	0.30	0.70
15 Reeves Co., Texas	5	--	1.00
16 Lea Co., New Mexico	10	--	1.00
17 Cameron Co., Texas	7	0.50	0.50
18 Nueces Co., Texas	9	0.56	0.44
Spermophilus spilosoma			
19 Del Norte C.C., El Paso Co., Texas	22	0.66	0.34

Table 13 (Continued)

Population	number of individuals	ES-8 a	ES-8 b
20 Ponder Park, El Paso Co., Texas	7	0.14	0.86
21 N.E. El Paso Co., Texas	7	0.28	0.72
22 Hutchinson Co., Texas	5	--	1.00
23 Janos, Chihuahua, Mexico	2	--	1.00
24 Luna Co., New Mexico	6	0.67	0.33
25 Lincoln Co., New Mexico	2	0.50	0.50
26 South Padre Island, Texas	5	0.10	0.90

Table 14, Allelic Frequencies at the Hemoglobin-alpha-1 locus for 26 populations of Ictidomys

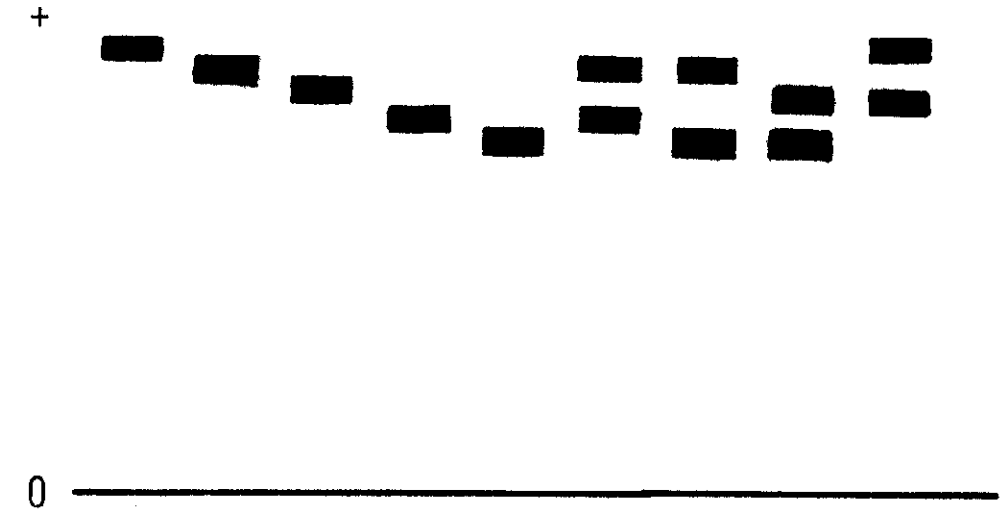
Population	number of individuals	HB-α1 a	b
Spermophilus tridecemlineatus			
1 Denton, Texas	18	1.00	--
2 Wichita Co., Texas	17	1.00	--
3 Lamar Co., Texas	10	1.00	--
4 Lewisville, Texas	7	1.00	--
5 Vigo Co., Indiana	12	1.00	--
6 Navarro Co., Texas	5	1.00	--
7 Hansford Co., Texas	6	1.00	--
8 Lubbock Co., Texas	5	1.00	--
9 Potter Co., Texas	5	1.00	--
Spermophilus mexicanus			
10 Pecos Co., Texas	15	1.00	--
11 Travis Co., Texas	7	1.00	--
12 Eddy Co., New Mexico	7	1.00	--
13 Comal Co., Texas	5	1.00	--
14 Midland Co., Texas	10	1.00	--
15 Reeves Co., Texas	5	1.00	--
16 Lea Co., New Mexico	10	1.00	--
17 Cameron Co., Texas	7	1.00	--
18 Nueces Co., Texas	9	1.00	--
Spermophilus spilosoma			
19 Del Norte C. C., El El Paso Co., Texas	22	0.45	0.55

Table 14 (Continued)

Population	number of individuals	HB-α1 a	HB-α1 b
20 Ponder Park, El Paso Co., Texas	7	0.07	0.93
21 N.E. El Paso Co., Texas	7	0.07	0.93
22 Hutchinson Co., Texas	5	--	1.00
23 Janos, Chihuahua, Mexico	2	1.00	--
24 Luna Co., New Mexico	6	0.67	0.33
25 Lincoln Co., New Mexico	2	1.00	--
26 South Padre Island, Texas	5	--	1.00

Figure 14, Electrophoretic variation in serum esterase (Es-5).

Figure 15, Starch gel electrophoretic variation in hemoglobin.


+
0
Es-5 AA BB CC DD EE BD BE CE AC

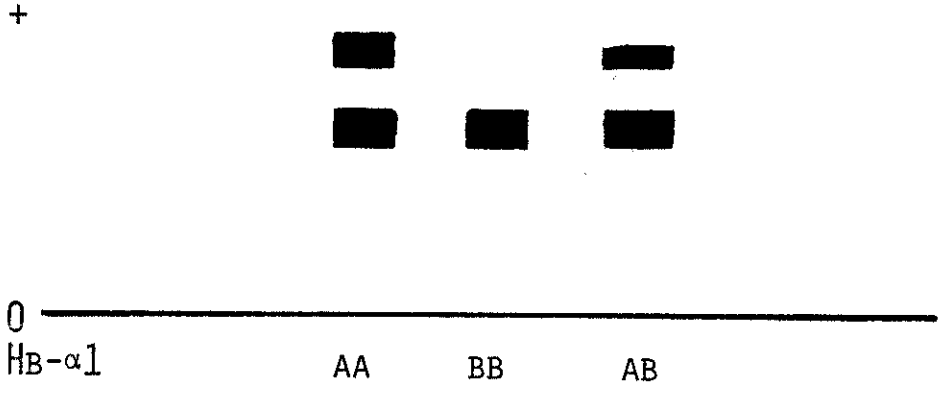
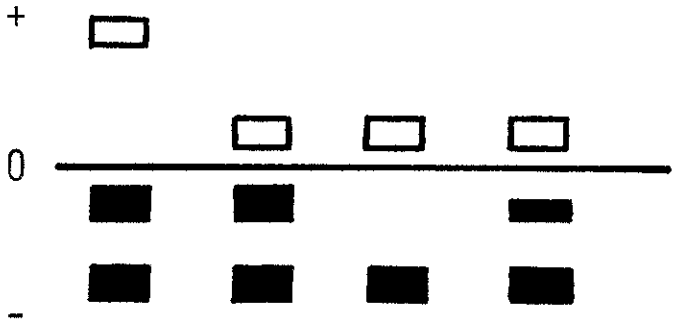

+
0
Hb-α1
AA
BB
AB

Figure 16, Cellulose acetate electrophoretic variation in hemoglobin (Hb-β, Hb-α1, Hb-α2).



Hb-β	AA	BB	BB	BB
Hb-α1	AA	AA	BB	AB
Hb-α2	AA	AA	AA	AA

Table 15, Allelic Frequencies at the Albumin locus for 26 populations of Ictidomys

Population	number of individuals	ALB a	ALB b	ALB c	ALB c
Spermophilus tridecemlineatus					
1 Denton, Texas	18	1.00	--	--	--
2 Wichita Co., Texas	17	0.91	0.08	--	--
3 Lamar Co., Texas	10	1.00	--	--	--
4 Lewisville, Texas	7	0.86	0.14	--	--
5 Vigo Co., Indiana	12	--	0.13	0.87	--
6 Navarro Co., Texas	5	1.00	--	--	--
7 Hansford Co., Texas	6	1.00	--	--	--
8 Lubbock Co., Texas	5	1.00	--	--	--
9 Potter Co., Texas	5	1.00	--	--	--
Spermophilus mexicanus					
10 Pecos Co., Texas	15	--	--	0.43	0.57
11 Travis Co., Texas	7	--	0.71	0.29	--
12 Eddy Co., New Mexico	7	--	0.36	0.36	0.28
13 Comal Co., Texas	5	--	--	0.60	0.40
14 Midland Co., Texas	10	--	0.40	0.35	0.25
15 Reeves Co., Texas	5	--	0.40	0.10	0.50
16 Lea Co., New Mexico	10	0.15	0.45	0.20	0.20
17 Cameron Co., Texas	7	--	0.79	--	0.21
18 Nueces Co., Texas	9	--	0.78	--	0.22

Table 15 (Continued)

Population	number of individuals	ALB a	b	c	d
Spermophilus spilosoma					
19 Del Norte C.C., El Paso Co., Texas	22	0.98	0.02	--	--
20 Ponder Park, El Paso Co., Texas	7	1.00	--	--	--
21 N.E. El Paso Co., Texas	7	1.00	--	--	--
22 Hutchinson Co., Texas	5	1.00	--	--	--
23 Janos, Chihuahua, Mexico	2	1.00	--	--	--
24 Luna Co., New Mexico	6	1.00	--	--	--
25 Lincoln Co., New Mexico	2	1.00	--	--	--
26 South Padre Island, Texas	5	1.00	--	--	--

Indiana). The albumin locus was highly polymorphic in S. mexicanus. The b allele was the most common allele, although the c and d alleles were also found in relatively high frequencies in this species. The a allele, which was the dominant allele in S. tridecemlineatus, was only found in the population of S. mexicanus from Lea, Co., New Mexico. Three individuals were ac heterozygotes, and these were the same individuals which had hybrid karyotypes. The ac genotype for the albumin locus was further indication that these individuals were S. mexicanus-S. tridecemlineatus hybrids as this genotype was a combination of S. mexicanus and S. tridecemlineatus alleles, c and a, respectively, at this locus. The fourth individual with a hybrid karyotype was a bd heterozygote.

Transferrin (plasma). The Trf locus was monomorphic in S. tridecemlineatus, with the a allele fixed in all populations (Table 16). The a allele was also fixed in all populations of of S. mexicanus except population 11 (Travis Co., Texas) where one individual was an ab heterozygote. The b and c alleles were found in S. spilosoma with the b allele being the predominant allele in this species (Fig. 18).

Protein-1 (plasma). The Pt-1 locus was only weakly polymorphic with two alleles present (Table 16). The a allele was fixed in all populations of all species except in population 5 (Terre Haute, Indiana) where the b allele was found at a frequency of 0.17.

Figure 17, Electrophoretic variation in albumin (Alb).

Figure 18, Electrophoretic variation in transferrin (Trf).

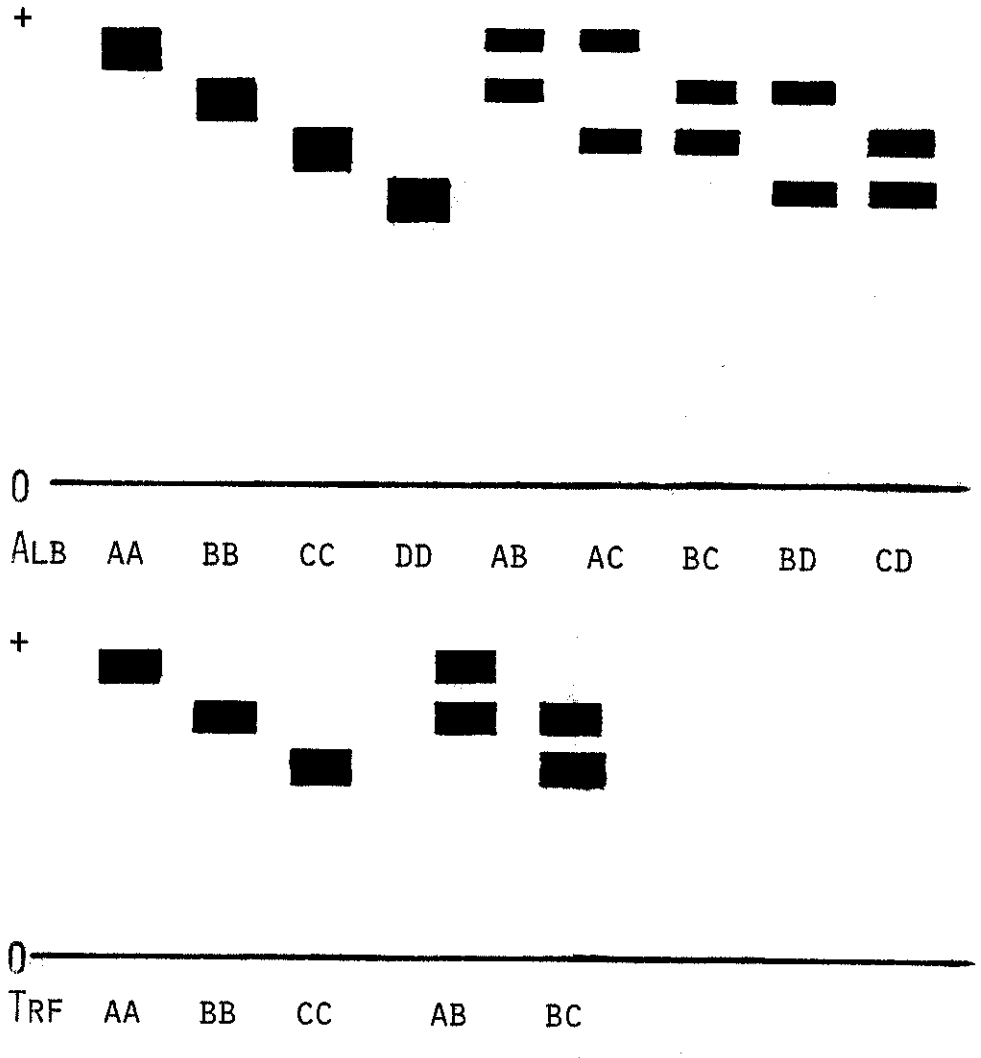

+
0
ALB AA BB CC DD AB AC BC BD CD
+
0
TRF AA BB CC AB BC

Table 16, Allelic Frequencies at the Transferrin locus for 26 populations of Ictidomys

Population	number of individuals	TRF a	TRF b	TRF c
Spermophilus tridecemlineatus				
1 Denton, Texas	18	1.00	--	--
2 Wichita Co., Texas	17	1.00	--	--
3 Lamar Co., Texas	10	1.00	--	--
4 Lewisville, Texas	7	1.00	--	--
5 Vigo Co., Indiana	12	1.00	--	--
6 Navarro Co., Texas	5	1.00	--	--
7 Hansford Co., Texas	6	1.00	--	--
8 Lubbock Co., Texas	5	1.00	--	--
9 Potter Co., Texas	5	1.00	--	--
Spermophilus mexicanus				
10 Pecos Co., Texas	15	1.00	--	--
11 Travis Co., Texas	7	0.93	0.07	--
12 Eddy Co., New Mexico	7	1.00	--	--
13 Comal Co., Texas	5	1.00	--	--
14 Midland Co., Texas	10	1.00	--	--
15 Reeves Co., Texas	5	1.00	--	--
16 Lea Co., New Mexico	10	1.00	--	--
17 Cameron Co., Texas	7	1.00	--	--
18 Nueces Co., Texas	9	1.00	--	--
Spermophilus spilosoma				
19 Del Norte C.C., El Paso Co., Texas	22	--	0.43	0.57

Table 16 (Continued)

Population	number of individuals	TRF a	TRF b	TRF c
20 Ponder Park, El Paso Co., Texas	7	--	0.79	0.21
21 N.E. El Paso Co., Texas	7	--	0.86	0.14
22 Hutchinson Co., Texas	5	--	1.00	--
23 Janos, Chihuahua, Mexico	2	--	1.00	--
24 Luna Co., New Mexico	6	--	1.00	--
25 Lincoln Co., New Mexico	2	--	1.00	--
26 South Padre Island, Texas	5	--	1.00	--

Table 17, Allelic Frequencies at the Protein-1 locus for 26 populations of Ictidomys

Population	number of individuals	PT-1 a	b
Spermophilus tridecemlineatus			
1 Denton, Texas	18	--	1.00
2 Wichita Co., Texas	17	--	1.00
3 Lamar Co., Texas	10	--	1.00
4 Lewisville, Texas	7	--	1.00
5 Vigo Co., Indiana	12	0.83	0.17
6 Navarro Co., Texas	5	--	1.00
7 Hansford Co., Texas	6	--	1.00
8 Lubbock Co., Texas	5	--	1.00
9 Potter Co., Texas	5	--	1.00
Spermophilus mexicanus			
10 Pecos Co., Texas	15	--	1.00
11 Travis Co., Texas	7	--	1.00
12 Eddy Co., New Mexico	7	--	1.00
13 Comal Co., Texas	5	--	1.00
14 Midland Co., Texas	10	--	1.00
15 Reeves Co., Texas	5	--	1.00
16 Lea Co., New Mexico	10	--	1.00
17 Cameron Co., Texas	7	--	1.00
18 Nueces Co., Texas	9	--	1.00
Spermophilus spilosoma			
19 Del Norte C.C., El Paso Co., Texas	22	--	1.00

Table 17 (Continued)

Population	number of individuals	PT-1 a	PT-1 b
20 Ponder Park, El Paso Co., Texas	7	--	1.00
21 N.E. El Paso Co., Texas	7	--	1.00
22 Hutchinson Co., Texas	5	--	1.00
23 Janos, Chihuahua, Mexico	2	--	1.00
24 Luna Co., New Mexico	6	--	1.00
25 Lincoln Co., New Mexico	2	--	1.00
26 South Padre Island, Texas	5	--	1.00

Genetic Variability

Of the 29 structural loci examined, 15 were polymorphic in one or more populations of the three species of the subgenus Ictidomys. Of these 15 polymorphic loci (Table 18), 13 were variable in at least one population of each species. Ten loci were polymorphic in all three species.

Only the Hb-β locus showed unique fixed alleles that were representative of a given species, but variation in seven of the loci examined provided the major differences between the three taxa. Spermophilus tridecemlineatus and S. mexicanus shared a common or fixed allele at 86% of their loci. The loci that distinguishes these two species were the Es-1, Es-3, Es-8, and Alb loci (Table 2). Spermophilus tridecemlineatus and S. spilosoma shared common or fixed alleles at 76% of the loci examined. These two species were separated primarily by the α-Gpd, Pgm-2, Es-1, Es-3, Hb-α1, Hb-β, and Trf loci. S. mexicanus and S. spilosoma shared common or fixed alleles at 79% of their loci and were distinguished by the Pgm-2, α-Gpd, Es-1, Hb-α1, Hb-β, Alb, and Trf loci. In addition, the 6-Pgd, Es-6, and Pt-1 loci showed interspecific allelic variation but contributed little to the total genetic variation.

One method of expressing genetic variability is by calculation of the proportion of loci polymorphic per population (P). The values of P for the three species (Table 19) ranged from 0.03 in population 26 (South Padre Island, Texas) of

Table 18, Polymorphic proteins with proportion of populations polymorphic for each locus and the effective number of alleles at each locus for 26 populations and three species of Ictidomys

	S. tridecemlineatus	S. mexicanus	S. spilosoma	no. of alleles at each locus	ave. effective no. alleles at
Isocitrate dehydrogenase					
IDH-1	0.33	0.56	0.75	3	1.49
6-Phosphogluconate dehydrogenase					
6-PGD	0.11	0.11	0.11	3	1.03
Glutamate Oxalo-acetic Transaminase					
GOT-1	0.11	0.56	---	3	1.24
Phosphogluco-mutase					
PGM-1	0.22	0.56	0.25	2	1.28
PGM-2	0.45	0.45	0.88	3	1.83
PGM-3	0.22	0.11	0.25	2	1.07
Esterases					
Es-1	1.00	0.67	0.75	7	3.26
Es-3	1.00	1.00	0.88	4	3.35
Es-5	0.89	0.67	0.75	5	2.19
Es-6	0.11	0.33	---	2	1.07
Es-8	0.89	0.78	0.75	2	1.80
Hemoglobin					
Hb- 1	---	---	0.50	2	131
Albumin					
Alb	0.33	1.00	0.13	4	1.77
Transferin					
Trf	---	0.11	0.38	3	1.10
Protein-1					
Pt-1	0.11	---	---	2	1.01

Table 19, Proportion of loci polymorphic per population (P), Proportion of loci polymorphic per individual ($\bar{H}$) in 26 populations and three species of Ictidomys

	Population	(P)	All proteins ($\bar{H}$)	Nonesterase proteins ($\bar{H}$)
S. tridecemlineatus				
1	Denton, Texas	0.173	0.049	0.009
2	Wichita Co., Texas	0.281	0.051	0.018
3	Lamar Co., Texas	0.172	0.068	0.011
4	Lewisville, Texas	0.242	0.047	0.033
5	Terre Haute, Indiana	0.176	0.033	0.014
6	Navarro Co., Texas	0.172	0.069	0.008
7	Hansford Co., Texas	0.213	0.068	0.007
8	Lubbock Co., Texas	0.211	0.057	0.029
9	Potter Co., Texas	0.171	0.057	0.000
		$\bar{X}$ 0.200	$\bar{X}$ 0.055	$\bar{X}$ 0.011
S. mexicanus				
10	Pecos Co., Texas	0.343	0.089	0.048
11	Travis Co., Texas	0.281	0.060	0.033
12	Eddy Co., New Mexico	0.244	0.064	0.039
13	Comal Co., Texas	0.178	0.063	0.010
14	Midland Co., Texas	0.312	0.081	0.052
15	Reeves Co., Texas	0.241	0.091	0.070
16	Lea Co., New Mexico	0.282	0.079	0.049
17	Cameron Co., Texas	0.215	0.068	0.041
18	Nueces Co., Texas	0.143	0.073	0.050
		$\bar{X}$ 0.253	$\bar{X}$ 0.078	$\bar{X}$ 0.047
S. spilosoma				
19	Del Norte C. C., El Paso Co., Texas	0.381	0.087	0.049
20	Ponder Park, El Paso Co., Texas	0.284	0.062	0.042
21	N. E. El Paso Co., Texas	0.283	0.058	0.031
22	Hutchinson Co., Texas	0.173	0.049	0.029
23	Janos, Chihuahua, Mexico	0.212	0.093	0.088
24	Luna Co., New Mexico	0.247	0.103	0.068
25	Lincoln Co., New Mexico	0.219	0.101	0.024
26	South Padre Island, Texas	0.034	0.009	0.000
		$\bar{X}$ 0.234	$\bar{X}$ 0.067	$\bar{X}$ 0.040
		$\bar{X}$ 0.227	$\bar{X}$ 0.067	$\bar{X}$ 0.033

S. spilosoma to 0.38 in population 19 (Del Norte C.C., El Paso, Co., Texas) also in S. spilosoma (mean P = 0.23). The mean values of P for each species ranged from 0.20 in S. tridecemlineatus to 0.25 in S. mexicanus. The proportion of polymorphic loci in S. tridecemlineatus ranged 0.171 to 0.281 while P ranged from 0.143 to 0.343 in S. mexicanus.

The proportion of loci polymorphic per individual ($\overline{H}$) was also calculated for Ictidomys (Table 19). The mean value of $\overline{H}$ for the three species was 0.067. The highest mean percentage of loci polymorphic per individual was 0.078 in S. mexicanus, and $\overline{H}$ in S. mexicanus ranged from 0.060 in population 11 (Travis Co., Texas) to 0.091 in population 15 (Reeves Co., Texas). S. tridecemlineatus had the lowest number of loci polymorphic per individual with a mean value of 0.055. The highest value of $\overline{H}$ for this species was 0.069 found in population 6 (Navarro Co., Texas). The least variable population of this species was population 5 (Vigo Co., Indiana) with an $\overline{H}$ value of 0.033. Spermophilus spilosoma from Padre Island were the least variable of the populations studied with an $\overline{H}$ value of 0.009. This species also had the population with the highest value of $\overline{H}$, population 24 (Luna Co., New Mexico), with a value of 0.103.

The greatest contribution to polymorphism in Ictidomys came from the esterase loci. The mean nonesterase value of $\overline{H}$ for the three species was 0.033, and the highest mean value of $\overline{H}$ was 0.047 found in S. mexicanus. The lowest mean value

of nonesterase polymorphism was 0.011 found in S. tridecemlineatus. The values ranged from 0.00 in population 26 (South Padre Island, Texas) of S. spilosoma to 0.088 in population 23 (Janos, Chihuahua, Mexico) also of S. spilosoma.

Effective Number of Alleles

Mean heterozygosity is only one way to examine the amount of variation in a species, and this measure provides an estimate of variation within and between populations. To measure the variation at specific loci and to determine the relationship between genic variation and protein specificity, the effective number of alleles is appropriate and provides an estimate of the amount of variation contributed by the alleles present at any polymorphic locus. For a monomorphic locus the value of the effective number would be 1.00.

The percentage of populations of each species polymorphic at each locus and the mean effective number of alleles at each polymorphic locus are shown in Table 18. The Group II, proteins, were, in general, the most polymorphic loci, with a mean effective number of alleles of 1.95. The Es-3 and Es-1 loci showed the highest effective numbers with values of 3.35 and 3.26 respectively. Similarly, a value of 2.19 was found at the Es-5 locus. The loci with the lowest levels of variability were the Group I, glucose metabolizing proteins, with a mean effective number of 1.12, however, some Group I proteins that were polymorphic showed relatively high effective numbers. In fact, the Pgm-2 locus was the most polymorphic of the

nonesterase proteins with an effective number of 1.83. The Idh-1 locus was also highly polymorphic, with an effective number of 1.49. The mean effective number of alleles for the Group III, nonenzymatic proteins, was 1.20. The most polymorphic locus of the Group III proteins was the Alb locus, with an effective number of 1.77. The effective number of alleles observed in Ictidomys indicates that the proteins with the lowest substrate specificity are the most polymorphic.

Genetic Similarity

Mean coefficients of genetic similarity (S) based on Rogers' (1972) coefficient and percentage of predominant alleles shared by species are shown in Table 20. Nei's coefficient (I) of similarity (Table 20) grouped populations in the same order but gave higher values of similarity. Genically, S. spilosoma was the most divergent member of the subgenus and showed the lowest levels of S between paired combinations of populations of the other two species. S. spilosoma was most similar to S. tridecemlineatus with a mean S value of 0.781. The mean S between S. spilosoma and S. mexicanus was 0.730. No more than 79% of predominant alleles were shared by this species and the other members of the subgenus, and seven loci in S. spilosoma demonstrated alleles that were not found in the other two species. These included the α-Gpd^b, Pgm-2^c, Es-1^a, Es-5^d, Hb-α1^b, Hb-β^b, and Trf^c alleles. Spermophilus mexicanus and S. tridecemlineatus were highly similar with a mean S value of 0.868. These two

Table 20, Mean coefficients of genetic similarity S and I, in parenthesis, (ABOVE) and percent of common alleles shared (BELOW) between three species of the ground squirrel subgenus Ictidomys.

	tridecemlineatus	mexicanus	spilosoma
tridecemlineatus	----	.868 (.911)	.781 (.840)
mexicanus	.864	----	.730 (.812)
spilosoma	.763	.790	----

species shared predominant alleles at 86% of their loci. Spermophilus mexicanus had three loci with unique alleles, including the Got-1^{c}, Es-5^{a}, and Alb^{d} alleles. Spermophilus tridecemlineatus showed three unique, but rare alleles, and these were the 6-Pgd^{c}, Es-1^{g} and Pt-1^{b} alleles.

CHAPTER IV

DISCUSSION

Genetic Variation

Estimates of genetic heterozygosity for 26 populations and three species of the ground squrrel subgenus Ictidomys can be compared to those obtained for other vertebrates. Heterozygosity values for tetrapods are fairly consistent at 5-6%, while those for invertebrates are much higher, averaging 15%. The mean values of $\bar{H}$ observed in rodents is 5.4% with a range of 1.0% to 9.0% (Selander and Kaufman, 1973). Selander et al. (1971), reported $\bar{H}$ values of 5% to 8.6% in mainland populations of Peromyscus polionotus, with a mean value of 6.9%. This value for P. polionotus excluded isolated populations found on small barrier islands and peninsulas on the Florida Gulf Coast. The mean $\bar{H}$ in Ictidomys is also 6.9% if the Padre Island population with a low $\bar{H}$ is excluded.

In general, most heterozygosity values reported for other rodents are lower than those observed in Ictidomys. Kilpatrick and Zimmerman (1975) reported a mean $\bar{H}$ value of 4.4% for four species of the Peromyscus bolyii species group. In this group heterozygosity varied from a low of 0% to 3.9% in P. attwateri, to a maximum of 8.2% in P. polius. Smith et al. (1973) also reported a low mean $\bar{H}$ value in P. floridanus a Pleistocene relict, of 5.3%. Johnson et al. (1972) reported mean $\bar{H}$ values

of 2.1% for Sigmodon hispidus and 2.9% for S. arizonae. Johnson and Selander (1971) found a mean $\bar{H}$ of 2.1% in 11 species of Dipodomys with a range of 0% to 5.1%. They considered these values low for rodents, although esterase loci were not examined. Many of the low levels of heterozygosity for rodents are reported for fossorial rodents. The average $\bar{H}$ value for nine species of fossorial rodents is 4.5%. The highest $\bar{H}$ value of 7% is in Thomomys bottae (Patton et al., 1972), however most fossorial rodents; five species of Geomys (Penney and Zimmerman, 1975), Thomomys talpoides (Nevo et al., 1974), T. unbrinus (Patton et al., 1972) and Spalax ehrenbergi (Nevo and Shaw, 1972; Nevo and Cleve, 1974), have ranges between 3.3% to 4.7%. Nevo et al., (1974) contended that the low $\bar{H}$'s found in fossorial rodents is indicative of selection for low levels of heterozygosity in a uniform environment. This contention was supported for Geomys by Penney and Zimmerman (1975).

In their study of the possible factors affecting the levels of heterozygosity in Dipodomys, Johnson and Selander (1971) observed that the degree of genetic variability is relatively uniform within species. They also observed that the major contributions to heterozyogisty in a species come from one or two loci, and therefore, sampling error may significantly affect estimates of genetic variability. However, they assumed that much of the observed variation was real and reflected some significant degree of interspecific variation in levels of heterozygosity. Johnson and Selander (1971) also observed that no relationship existed between degree of genetic

variability and the extent of geographic range. Of the seven species of Dipodomys exhibiting low levels of heterozygosity, three have small ranges and four have extensive ranges, including D. ordii the most widely distributed member of the genus. A similar pattern was observed in Geomys by Penney and Zimmerman (1975). Geomys bursarius, the species with the most extensive range of the genus, is no more variable than the other members of the genus with more restricted ranges. This is also the pattern observed in Ictidomys. Spermophilus tridecemlineatus with the largest geographic range is the least variable member of the group, while S. mexicanus and S. spilosoma, with more restricted ranges, show higher levels of genetic variability.

The pattern of geographic variation in $\bar{H}$ demonstrated by Ictidomys is quite uniform. Of the nine populations of S. tridecemlineatus, all have $\bar{H}$'s from 3% to 7% and eight of the populations ranged between 5% and 7%. Also, those populations of S. tridecemlineatus from the same geographic area have similar levels of $\bar{H}$. All the populations from north-central Texas have $\bar{H}$'s of approximately 5%, while all the populations from northeastern Texas exhibit values around 7%. Those populations from western Texas have $\bar{H}$'s ranging from 6% to 7%.

Spermophilus mexicanus exhibits a similar pattern, although to a lesser degree. The $\bar{H}$ in this species ranges between 6% and 9%. The two populations from central Texas have $\bar{H}$'s of 6%, while two populations from the Texas coast have $\bar{H}$'s of

7% and 8%. The remaining five populations from western Texas and southeastern New Mexico show more variation, but the $\overline{H}$ values are similar.

Spermophilus spilosoma exhibits more geographic variation in $\overline{H}$. Seven of the eight populations of this species examined have $\overline{H}$'s ranging between 5% and 10%, however, there was no geographic pattern in the variation of this species. The lowest level of genetic variability was found in population 26 (South Padre Island, Texas) of S. spilosoma, with a $\overline{H}$ of 0.9%. The only polymorphic locus in this population was the Es-8 locus. This pattern of low heterozygosity for island or isolated populations is not uncommon. Selander et al., (1971) reported low values of $\overline{H}$ in island and isolated populations of Peromyscus polionotus, with $\overline{H}$'s ranging from 1.8% on islands to 3.8% on peninsulas, while the mean in populations on the mainland was consistently higher and averaged 6.9%. Certain species of lizards of the genus Anolis inhabiting a small island in the Bahamas also have an absence or reduction in heterozygosity (Webster et al., 1973). Similarly, low genetic variability was found in isolated troglobitic populations of the Mexican fish Astyanax mexicanus (Avise and Selander, 1972). The levels of variation observed in these species was attributed to random genetic drift. Several factors are important when considering genetic drift as a process in the genetics of a population, such as, effective population size, severe bottlenecking in the population, and the initial size of the founding population.

The present population of S. spilosoma on Padre Island is probably large, as squirrels of this species can be found along most of the island, and the low level of heterozygosity observed in this population has probably resulted from the founder effect, with low genetic variabilities in the original population isolated on Padre Island.

There are two conflicting explanations for the maintenance of protein polymorphism. Kimura and Crow (1964) have suggested protein variation is adaptively neutral, i.e., that alternative genotypes have effectively identical fitness. If this were the case, allelic frequencies would be determined exclusively by the random process of sampling from generation to generation. Kimura and Crow (1964) also suggested that, on a theoretical basis, heterozygosity of the magnitude revealed by electrophoretic studies should create an excessive genetic load. However, studies of natural and experimental populations indicate that genetically controlled protein variation is not adaptively neutral, but is maintained by balancing natural selection, and the levels of polymorphism are far higher than could exist under conditions of excessive genetic load (Prakash et al., 1969; Ayala et al., 1971; Ayala, Powell, and Tracey, 1972; Ayala et al., 1972; Ayala, 1972; Ayala and Anderson, 1973; Tracey and Ayala, 1974; Dobzhansky and Ayala, 1973; Kojima and Yarbrough, 1967; Zouros and Krimbas, 1973; and Selander and Johnson, 1973).

Based on the hypothesis that protein polymorphism is influenced by selection, protein variation should correlate

with physiological variation and environmental conditions. Gillespie and Kojima (1968) proposed that levels of enzyme polymorphism may reflect environmental variation in substrates. They pointed out that, in laboratory cultures of Drosophila ananasse, far less heterozygosity was observed at the loci of enzymes involved in energy production than at other enzymes loci. Their later, more detailed, examination of natural populations (Kojima and Gillespie, 1970) confirmed their original observation, and they suggested that the greater variability in the Group II, non-glucose-metabolizing enzymes, might reflect greater variability in their substrates, as many of these substrates originate in the external environment.

This pattern of higher variability in the Group II enzymes was demonstrated in Ictidomys, and the esterases were, by far, the most variable proteins. The mean heterozygosity for the polymorphic esterases was 33.3% compared to 12.1% and 7.4% for the polymorphic Group I and Group III proteins, respectively. Values of $\overline{H}$ for nonesterase proteins, alghough considerably lower than those for all proteins, show the same relationships of genetic variability between members of the subgenus. Spermophilus mexicanus was still the most variable member of the subgenus with a mean $\overline{H}$ for nonesterase proteins of 4.7%. A greater range of variability in non-esterase proteins was found in S. spilosoma. The mean for this species was 4%, while the mean for S. tridecemlineatus was 1.1%.

The overall higher values of heterozygosity observed in Ictidomys, especially in S. mexicanus and S. spilosoma, are not easily explained. Although some of the factors relating to maintenance of polymorphism in populations have previously been discussed, there are probably many factors that contribute to the polymorphism in Ictidomys. The present distribution of species of Ictidomys (Hall and Kelson, 1959) indicates the possibility of gene flow between conspecific populations from various parts of the range, resulting in increased variability due to mixing of genomes adapted to slightly different environments. In addition, based on current theories of genetic variability, much of the variation seen in the esterase loci in Ictidomys is a reflection of substrate variability, as these species encounter several substrates resulting from their variable food habits (Davis, 1966; Whitaker, 1972; Flake, 1973).

Genetic Similarity

Coefficients of genetic similarity (S) were calculated for paired combinations of all populations. Values of S for continental populations of conspecifics are generally in the high 0.80's to the low 0.90's and tend to decrease with distance between populations (Avise and Selander, 1971; Avise, 1974). Mean values of S and I obtained for members of Ictidomys are comparable to those obtained for other vertebrates. The highest value obtained is 0.965 found in S. tridecemlineatus, while the lowest value is 0.812 in S. mexicanus.

Mean S values reported for conspecifics ranged from 0.920 in S. tridecemlineatus to 0.874 in S. mexicanus. Mean S values reported for other rodents range from 0.980 for conspecifics of Sigmodon (Johnson and Selander, 1972) to 0.88 for populations of Mus musculus (Selander et al., 1969; Rogers, 1972). The lowest values of S observed for rodents are found in fossorial rodents. Nevo et al. (1974) reported a mean S value of 0.84 for conspecifics of the Thomomys talpoides complex. However, this group represented six chromosomal forms, possibly representing five species. Penney and Zimmerman (1975) reported extremely low coefficients of similarity for populations of Geomys bursarius, with a mean S value of 12 populations of 0.720. They attributed these low values to random fixation of alternate alleles as a result of isolation and the island-type distribution of populations of Geomys.

A pattern of decreasing similarity with increasing geographic distance between populations is also demonstrated by the three species in Ictidomys. The lowest value of S (0.838) in S. tridecemlineatus is between the two most geographically distant populations, Lubbock Co., Texas and the Vigo Co., Indiana. In fact, the Indiana population shows the lowest similarity values to other conspecific populations of S. tridecemlineatus. The least similar populations of S. mexicanus are the Pecos Co., Texas and Nueces Co., Texas populations, with an S value of 0.812, while the least similar populations of S. spilosoma are the Lincoln Co., New Mexico and South Padre Island, Texas populations with an S value

of 0.816. It is obvious that populations from the extremes of the range encounter different environmental conditions and are under differing selective pressures, resulting in decreased genetic similarity.

A wide range of similarity values for species of a genus is expected, since species differences reflect not only the amount of genetic modification that occurs in the process of speciation but also changes accumulating since speciation was effected (Johnson and Selander, 1971). Hubby and Throckmorton (1965) discussed the factors influencing the extent of genetic divergence among species. For nine species of the Drosophila virilis group they obtained similarity values ranging from 0.28 to 0.79. S values for other congeneric species range from 0.94 for Spalax ehrenbergi (Nevo and Shaw, 1972; Nevo and Cleve, 1974) to 0.21 for species of Anolis (Webster et al., 1972). The mean S values obtained between the members of the subgenus Ictidomys are relatively high compared to those reported for other rodents (Table 20). Spermophilus mexicanus and S. spilosoma are the least genetically similar with a mean S value of 0.730, while the highest level of similarity is between S. tridecemlineatus and S. mexicanus with a mean S value of 0.868.

Bryant (1945) using conventional taxonomic methods described the systematic relationships of the members of Ictidomys. He concluded that S. mexicanus and S. tridecemlineatus were the most closely related, while S. spilosoma was the most divergent. Bryant concluded that the members of this subgenus

were not as closely related to each other as are the species in the other subgenera of Spermophilus. However, based on biochemical evidence, the members of this subgenus are quite similar (Fig. 19). Spermophilus spilosoma is the most divergent species in the group, while S. mexicanus and S. tridecemlineatus are closely related. Indeed, based on the evidence now available, S. mexicanus and S. tridecemlineatus fit the classical definition of semispecies.

Semispecies, as defined by Mayr (1963), are populations which have partially completed the process of speciation. Geographical isolates sometimes acquire various biological "pecularities" and partial reproductive isolation. When such populations establish secondary contact with each other, they interbreed to a limited extent and retain some of their "pecularities" (Mayr, 1963). Spermophilus mexicanus and S. tridecemlineatus are largely allopatric with limited sympatry in their ranges in southeastern New Mexico and in several of the counties in the adjoining region of Texas (Hall and Kelson, 1959; Davis, 1966). This is the area where hybrids between these two species were discovered.

Little genetic differentiation seems to take place in the second stage of speciation, i.e., semispecies (Ayala et al. 1974). Ayala et al., in their examination of six semispecies of the Drosphila paulistorum group, suggested that even the development of complete reproductive isolation may not require allelic changes in a large fraction of the genome. In fact,

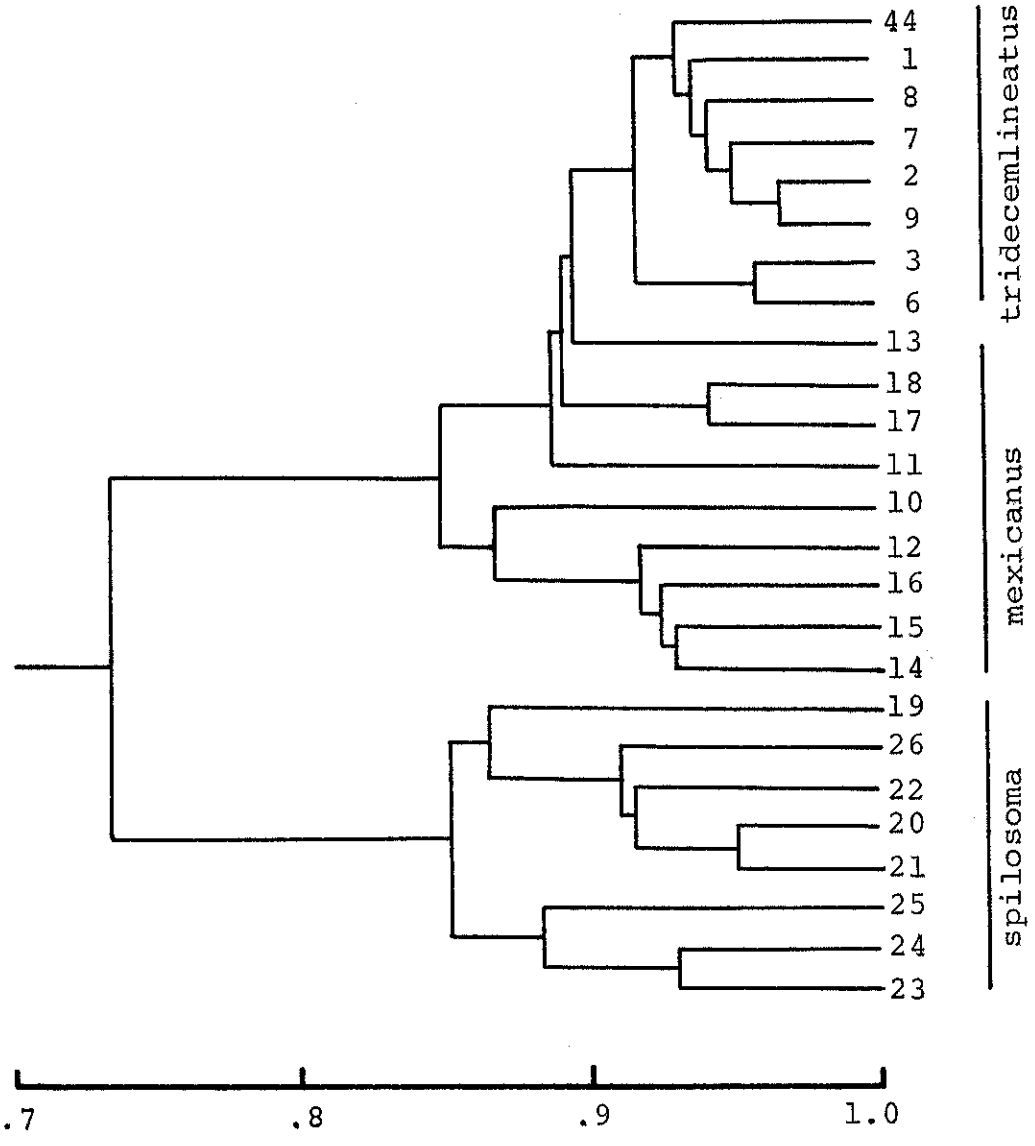


Fig. 19. Dendrogram based on Rogers' measure of genetic similarity for 25 populations of Spermophilus

they found higher coefficients of genetic similarity, based on Nei's (1972) coefficient (I), between the semispecies of D. paulistorum than between subspecies of D. willistoni. The mean value of I reported for semispecies of Drosophila by Ayala et al. (1974) was 0.798, while the mean value of I between S. mexicanus and S. tridecemlineatus is 0.911.

The hybrids from the Lea Co., New Mexico population are the first interspecific hybrids reported for the genus Spermophilus. Due to the small number of individuals collected from this population it was impossible to determine the level of introgression as a result of this hybridization. However, the presence of an individual representing a probable backcross between parental and hybrid forms and a gravid F_1 hybrid female indicate that the degree of introgression could be fairly high in this population.

Groups of actually or potentially interbreeding natural populations which are reproductively isolated from other such groups are recognized as species (Mayr, 1963). Breakdown of the usually effective mechanisms that preserve reproductive isolation may lead to hybridization between species, a phenomenon that takes place less commonly in nature than under laboratory conditions. Hybridization has been widely reported among many animal species (Gray, 1954; White, 1954). McCarley (1954) reported hybridization for the white-footed mice Peromyscus leucopus and P. gossypinus in the southern United States. Lay and Nadler (1969) described hybridization between

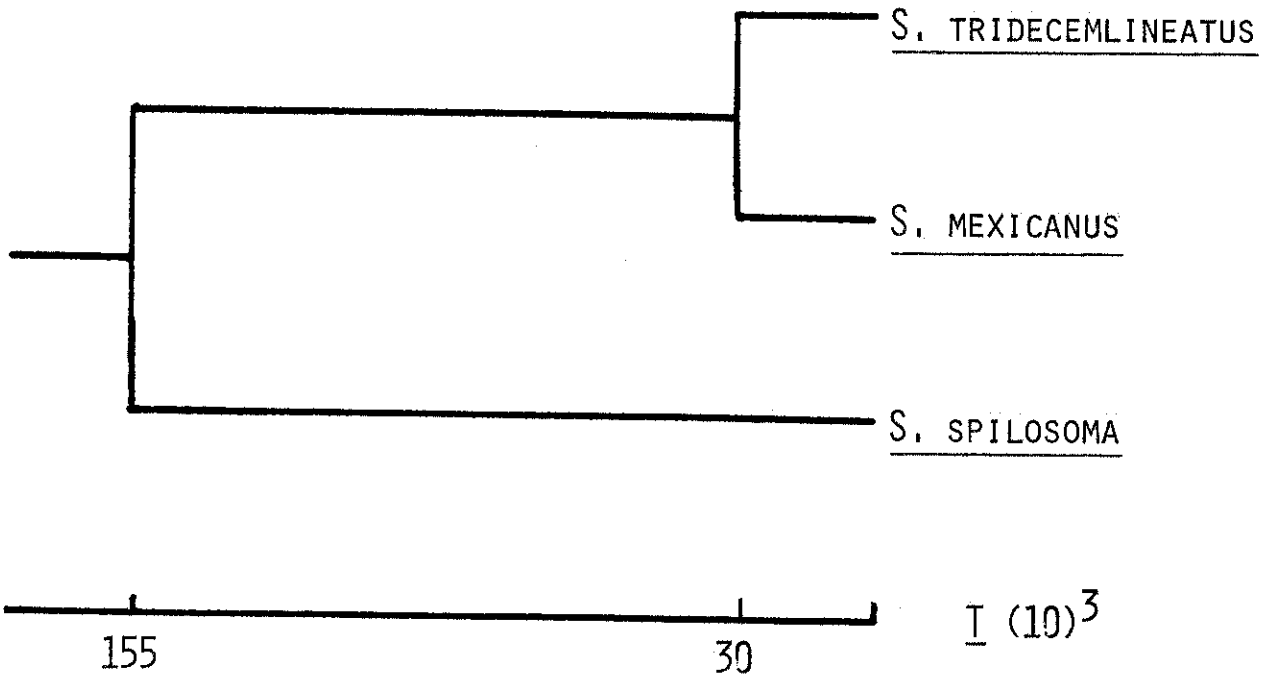
two species of the rodent genus Meriones, M. shawi and M. libycus. There are no previous reports of hybridization in the ground squirrel genus Spermophilus; however, Nadler et al., (1971) reported interbreeding between two chromosomally different subspecies of Spermophilus richardsonii.

One of the primary reasons that hybrids are reproductively inferior is that their genotype is not adjusted to the ecological niche of either parental type (Mayr, 1963), and several examples exist where hybrids are forced to invade a new habitat. Wright and Lowe (1968), reporting on all-female lizards of the genus Cnemidophorus, stated that the evolution of this parthenogenetic species initially resulted from hybridization of two diploid, bisexual species and the concomitent invasion and capture of a new habitat for survival. They described the habitat of the hybrid species as "disclimax, marginal, ecotonal, transient, and perpetually disturbed," all of which are definitions of a "weak habitat." Jackson (1973) considered three hybrid zones between parapatric populations of the iguanid lizards Sceloporus woodi and S. undulatus as ecotone habitats. Hybridization of karyotypically differentiated populations in the lizard Sceloporus grammicus was reported by Hall and Selander (1973). They indicated that the hybrid zone was a transitional region between humid forest and pine woodland habitats. Crenshaw (1965) investigated hybrids between two species of turtles (Pseudemys floridana X P. rubriventris) in North Carolina and postulated that survival of these hybrids was fostered by

the hybrid-habitat ecological characteristics of the lake in which they were found. Since S. mexicanus and S. tridecemlineatus are capable of existing in the same environments in portions of their range affording potential for hybridization, the likelihood that hybrids would be inferior in the parental environment is reduced. Thus, the probability that hybrids can compete successfully is increased, thereby enhancing the possibility for introgression.

Evolutionary divergence time for a pair of species can be estimated from electrophoretic data (Nei, 1971). Nei's index yields only crude estimates; however, fossil evidence of pocket gophers of the genus Thomomys and deer mice, genus Peromyscus, has been shown to correspond remarkedly well with estimates of divergence time from biochemical evidence (Nevo et al., 1974; Zimmerman et al., 1975). Based on fossil evidence, Black (1963) concluded that Ictidomys differentiated from the subgenus Spermophilus as recently as the late Pliocene and Pleistocene. Unfortunately, adequate fossil records for species of Ictidomys are lacking. Biochemical data place the divergence of S. spilosoma from the other two species at approximately 155,000 yr BP (Fig. 20), roughly during the Sangamon interglacial or early Wisconsin glaciation. The close genetic similarity between S. tridecemlineatus and S. mexicanus would indicate that their divergence was a more recent phenomenon, and Nei's index places the divergence of these two species at approximately 30,000 yr BP, in the

Figure 20, Divergence times for three species of Ictidomys based on Nei's measure of genetic similarity.



late Wisconsin. Based on biochemical data, without corroboratory fossil evidence, evolution in this subgenus is linked with the dynamic physical and biotic changes that have taken place in the Pleistocene. In addition, the high interspecific levels of genetic similarity would indicate that speciation in this group has taken place with few genomic changes, and continues as a dynamic process in this group.

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