TAXONOMIC ANALYSIS OF ELECTROPHORETIC BLOOD SERUM PATTERNS IN THE COTTON RAT, SIGMODON

Thesis for the Degree of M. S.
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PETER L. DALBY
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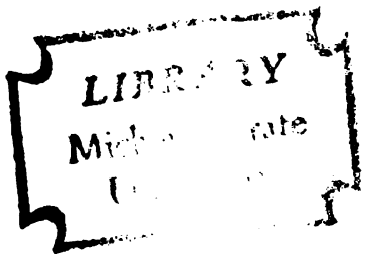
THESIS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ABSTRACT

TAXONOMIC ANALYSIS OF ELECTROPHORETIC BLOOD SERUM PATTERNS IN THE COTTON RAT, SIGMODON.

by Peter L. Dalby

The blood serum protein patterns were analyzed from seven species of cotton rats, Sigmodon alleni, S. fulviventer, S. hispidus, S. leucotis, S. melanotis, S. ochrognathus, and S. planifrons. In all, sera from 156 specimens were studied by means of the acrylamide disc electrophoresis procedure of Ornstein and Davis (1962), Davis (1964) and modified by Wright and Mallmann (1966).

The resulting protein patterns in the acrylamide gel and their densitometric tracings were compared to determine the amount of individual variation (between individuals of the same species from a single locality), geographic variation (between samples of the same species from different localities), and interspecific variation (between samples of different species).

The electrophoretic serum patterns of some population samples within a single species were distinctive. Other samples could not be distinguished either because of the variability within individual population samples or because of close similarity between geographically-separated population samples. One case of transferrin polymorphism within a single population was noted, suggesting the occurrence of a limited gene exchange.

Between certain geographically-separated population samples of S. hispidus, transferrin polymorphism, consisting of five different phenotypes (transferrin patterns A,B,C,D and E) was observed to follow a specific arrangement which allowed for quick regional identification.

No form of a species specific "species curve" or "species pattern" was apparent in the wide ranging ubiquitous S. hispidus, but was present in S. leucotis, a spatially and ecologically restricted cotton rat.

Differences between species were evidenced in the transferrins, some of the prealbumins, and to a lesser degree, in the postalbumin components. On this basis, S. hispidus, S. leucotis and S. ochrognathus could be distinguished from each other. However, Sigmodon fulviventer and S. melanotis appeared indistinguishable from each other and conspecific, as did S. alleni and S. planifrons. A table and key for electrophoretically differentiating the studied species and a diagram of their possible relationships are presented.

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By

Peter L. Dalby

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I. INTRODUCTION

A. Purpose

The objective of this study is to investigate the blood serum patterns of cotton rats (Sigmodon), using acrylamide disc electrophoresis, to determine: (1) non-geographical variation within a single population sample of a species from one locality; (2) geographic variation between population samples of the same species from different localities; and (3) interspecific variation between samples representing currently recognized species.

If detectable differences in the serum protein patterns are observable at these three taxonomic levels, it might then be possible to use these findings to gain a greater knowledge of speciation in Sigmodon, the taxonomy of which is currently based entirely on morphological grounds.

B. Review of Literature

The concept that protein synthesis is dependent on rigid genetic control and is therefore a reflection of the genotype has provided a theoretical basis for utilizing physico-chemical characteristics of proteins in taxonomic studies.

Moore (1945), using the Tiselius apparatus, described distinctive differences in blood serum protein electrophoretic patterns between such species as man, rhesus monkeys, swine, white rats, cats, guinea pigs and hamsters. In a later paper (1959), he reported that the serum patterns in the cotton rat and the guinea pig obtained in 1957 had the same general characteristics as those obtained twelve years previously.

Auernheimer et al. (1960) employed paper strip chromatography and compared the electrophoretic serum protein patterns of the cricetids Peromyscus, Sigmodon, and Neotoma, and concluded that there are generally marked differences between the species. Differences were most evident among the globulins although the albumin content varied over a wide range. He also noted that in several closely related species of Perognathus, no distinguishable characteristics were evident.

Johnson and Wicks (1959) also employed paper strip chromatography and surveyed the serum proteins in eight orders, consisting of 74 species of North American mammals. Although the globulins were quite variable among individuals, these authors concluded that a standard species pattern existed, and that the prime taxonomic significance appeared at the generic and specific levels. In another paper (1964), Johnson and Wicks surveyed 17 mammalian orders consisting of 46 families, 112 genera and 206 species. They concluded that there is a generalized mammalian pattern consisting of albumin and from one to six globulin fractions. After they failed to separate such higher taxa as orders and families, Johnson and Wicks reiterated their former conclusion that the primary usefulness of electrophoresis in taxonomic work is at the generic and specific levels. Johnson (1968), employing paper electrophoresis of blood plasma proteins, studied twenty-five mammalian taxonomic relationships. In nineteen instances, he felt that clarification of relationships were obtained, but in six instances the proteins did not help. Petersen (1966, 1968), employed acrylamide disc electrophoresis to study the serum proteins in eighteen species of Peromyscus from the United States and México. On the

basis of superficial characteristics such as number of bands, symmetry, general appearance, and slope of peaks, he demonstrated taxonomically significant differences at the species-group level.

Since variation in the monor components was recognized in the previous taxonomic studies, the recognition of various species, species groups, genera, and higher taxa has been based on the position of major globulin (e.g. transferrin) and albumin components. However, some workers have demonstrated polymorphism in these also, leaving some doubt as to their taxonomic significance and the reliability of species curves or species patterns. Welser et al. (1965) found albumin polymorphism in five forms of Peromyscus. Vertical starch-gel electrophoresis of the plasma proteins revealed five albumins of slightly different electrophoretic mobilities, two of which frequently appeared in P. maniculatus bairdi. Data indicated that inheritance of these five albumins may be controlled by a single autosomal locus with multiple co-dominant alleles, each allele determining a different albumin. Later, Brown and Welser (1968) made a more detailed study of albumin polymorphism in seven species of laboratory and wild strains of Peromyscus. In P. leucopus populations, a great difference in polymorphism frequency was observed between two natural populations twenty-five miles apart. Albumin polymorphism was also found in natural populations of P. maniculatus, P. leucopus and P. truei. Brown and Welser noted that in P. maniculatus, the long-tailed, forest-dwelling forms of northern and western North America appeared to be monomorphic while the short-tailed, grassland-inhabiting forms were polymorphic.

Transferrin polymorphism was demonstrated by other investigators

such as Ashton and Braden (1961). Using starch-gel electrophoresis, they found three serum β-globulin (transferrin?) types in different strains of laboratory house mice. They also noted that between individual mice of one inbred strain there was considerable variation in the staining intensity, i.e., relative quantity of protein within the β-globulins. Goodman et al. (1965), employing horizontal starch-gel electrophoresis, found 11 molecular forms and 34 phenotypes of transferrin in 372 blood serum samples from six species of macaques. The tendency for polymorphism was observed to vary from species to species and from one local population to another. From this information they concluded that these macaques belong to a single highly-differentiated species rather than several species. Nadler and Hughes (1966) analyzed by two dimensional starch-gel electrophoresis the serum proteins of several species of Spermophilus (=Citellus). Although transferrin polymorphism was noted in one species, they found taxonomic significance at the population, subspecies, and species levels. Ahl (1968), using acrylamide disc electrophoresis, found four transferrin types in the blood plasma of Peromyscus maniculatus nebrascensis from three different altitudes. There were no differences in the plasma proteins between the three altitude groups which could be related to altitude or sex. It appeared that the four types were distributed in accord with geographic proximity of three altitude groups.

Other protein components in blood serum may also vary. Russell and Semeonoff (1957), utilizing starch-gel electrophoresis, reported that evidence has been found for two loci which control the synthesis of separate subunits of a serum esterase isozyme system in Microtus agrestis.

In the natural population studied, both loci appeared to be polymorphic. Russel and Ashton (1958) employed starch-gel electrophoresis and found that horse serum exhibited individual variation in the prealbumins and β-globulins. Popp and Popp (1962) examined the blood serum by starch-gel electrophoresis of 21 strains and three partially inbred stocks of laboratory mice (Mus). A single band of esterase was found in C57BL and C57L mice. Two bands of slightly slower mobility were found in the other strains. Kristjansson (1963) used starch-gel electrophoresis and observed prealbumin polymorphism in domestic pigs. Shaw (1965) called attention to other papers which have demonstrated electrophoretic variations in enzymes and other blood components of Mus, Peromyscus, and larger animals.

In review, a variety of comparative studies on mammalian blood serum, using several electrophoretic techniques, have been conducted at different taxonomic levels. Often laboratory strains which have gone through considerable breeding "bottlenecks" are used. The worker may have studied only a specific serum protein (e.g. albumins, transferrins, esterases) among certain animal groups. The physiological condition of the specimens is frequently overlooked. If field specimens are collected, they are often indiscriminately sampled from a small part of the animals range and/or consist of small sample sizes. However, when properly utilized, valuable information gathered by electrophoretic techniques has proved helpful in animal taxonomy. For this reason, the use of acrylamide disc electrophoresis as a taxonomic tool in studying cotton rat (Sigmodon) systematics is explored.

II. EXPERIMENTAL

A. Collecting Localities

Seven species of Sigmodon (as designated by Hall and Kelson, 1959; Baker and Greer, 1962) were used in this study. Inbred (2-3 generations) laboratory specimens if used, are identified by an asterisk. The number which corresponds to the approximate collecting locality on Figures 1, 2 a and b, the capture locality, and number of specimens are as follows:

Sigmodon hispidus berlandieri Baird

1. Durango: 8 km. SE Zalvalza, 1168 m., 1
2. Jalisco: 2 km. NW La Barca, 1535 m., 7
3. Nuevo León: 37 km. NNE Ciénega de Flores, 555 m., 4
4. San Luis Potosí: 2 km. N Santa María del Río, 1677 m., 3
5. Zacatecas: 3 km. N Santa Rosa, 1174 m., 5
6. Texas: Lubbock Co., Lubbock., 3

Sigmodon hispidus chiriquensis J. A. Allen

7. Canal Zone: Chiva Chiva Road., 2
8. Canal Zone: Empire Range., 1
9. Canal Zone: France Field., 4

Sigmodon hispidus cienegae A. B. Howell

10. Arizona: Pinal Co., Oracle., 6

Sigmodon hispidus furvus Bangs

11. Honduras: Puerto Cortés., 5

Sigmodon hispidus ischyrus Goodwin

12. Guerrero: 7 km. SE Cuajinicuilapa, 91 m., 6

Sigmodon hispidus komareki Gardner

13. Tennessee: Anderson Co., Oak Ridge., 4

14. South Carolina: Aiken Co., Savannah River Project., 3

Sigmodon hispidus littoralis Chapman

15. Florida: Palm Beach Co., Boca Raton., 1

Sigmodon hispidus major V. Bailey

16. Nayarit: 27 km. SE Tuxpan, 146 m., 6

17. Sinaloa: 3 km. N Santa Lucía, 1296 m., 1

Sigmodon hispidus mascotensis J. A. Allen

18. Jalisco: 10 km. SW Autlán, 1342 m., 6

19. Colima: 8 km. NW Santiago, 18 m., 1

Sigmodon hispidus texianus (Audubon and Bachman)

20. Arkansas: Benton Co., 6 km. E Rogers, 422 m., 7

21. Oklahoma: Marshall Co., 2 km. E Willis, 192 m., 6

22. Texas: Colorado Co., Eagle Lake, 52 m., 8

Sigmodon hispidus toltecus (Saussure)

23. Tamaulipas: Hda. Acuña, 808 m., 4

24. Veracruz: Isla del Toro, 6 m., 3

Sigmodon alleni V. Bailey

1. Michoacán: 10 km. W Capácuaro, 2059 m., 8

Sigmodon fulviventer fulviventer J. A. Allen

2. Durango: 9 km. NNW Canatlán, 1952 m., 1

3. Durango: Hda. Coyotes, 2475 m., 4

4. Durango: Hda. de Atotonilco, 2037 m., 3

5. Zacatecas: 13 km. S Villanueva, 2090 m., 1

6. Guanajuato: 8 km. SW Ibarra, 2500 m., 6

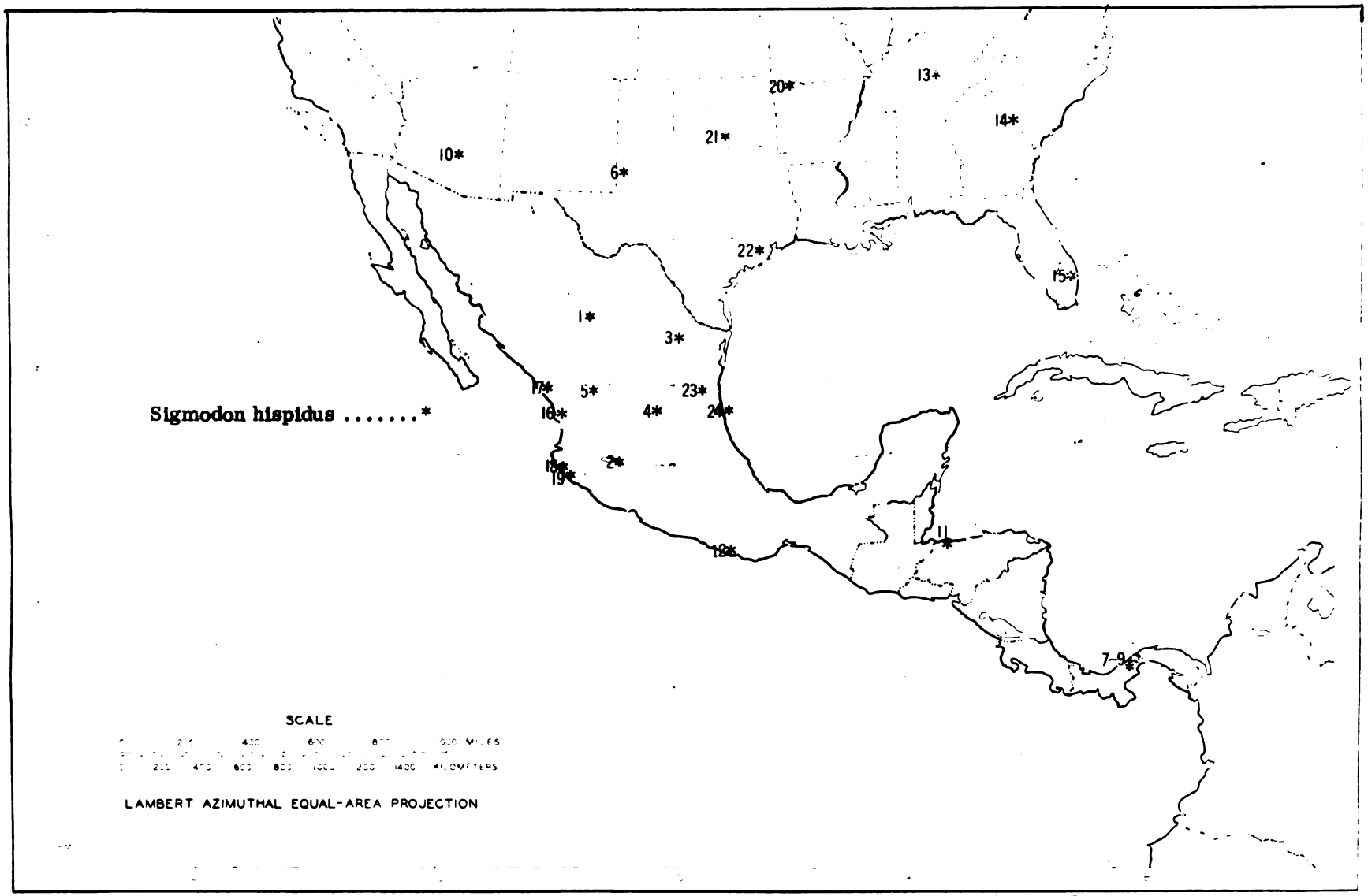


Figure 1. Map showing the approximate geographical locations of Sigmodon hispidus used in this study.

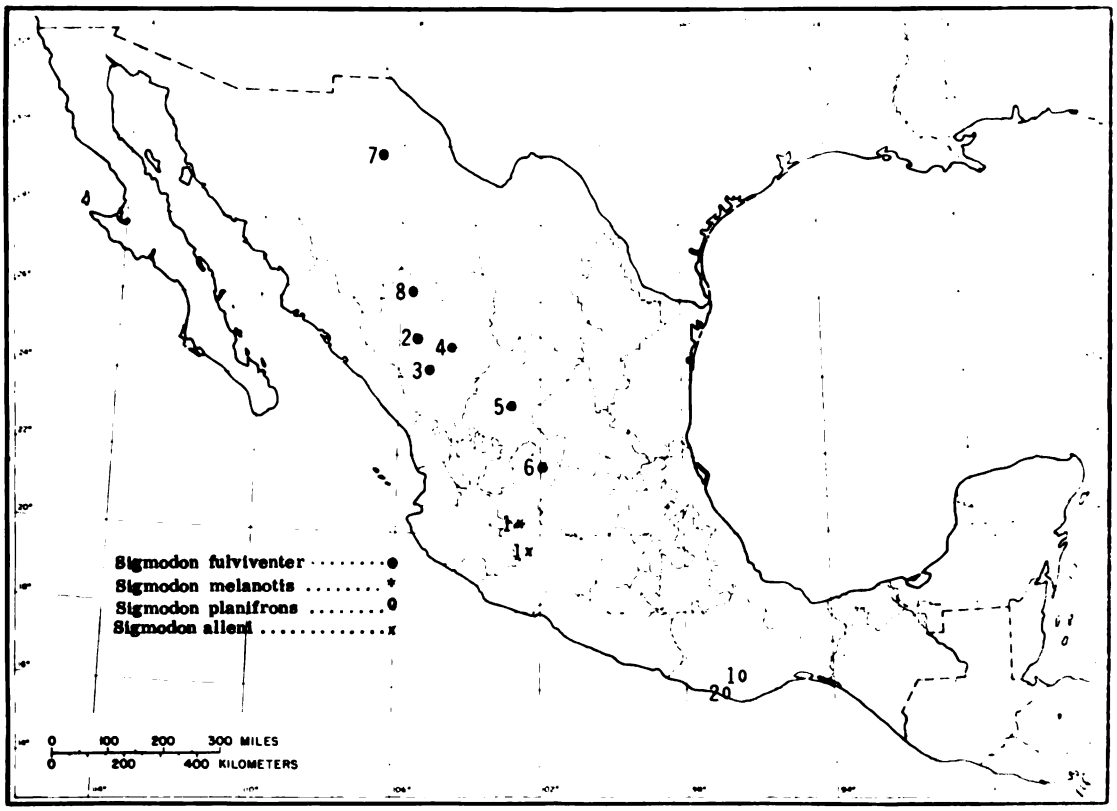


Figure 2a. Map showing the approximate geographical locations of Sigmodon fulviventer, S. melanotis, S. planifrons and S. alleni used in this study.

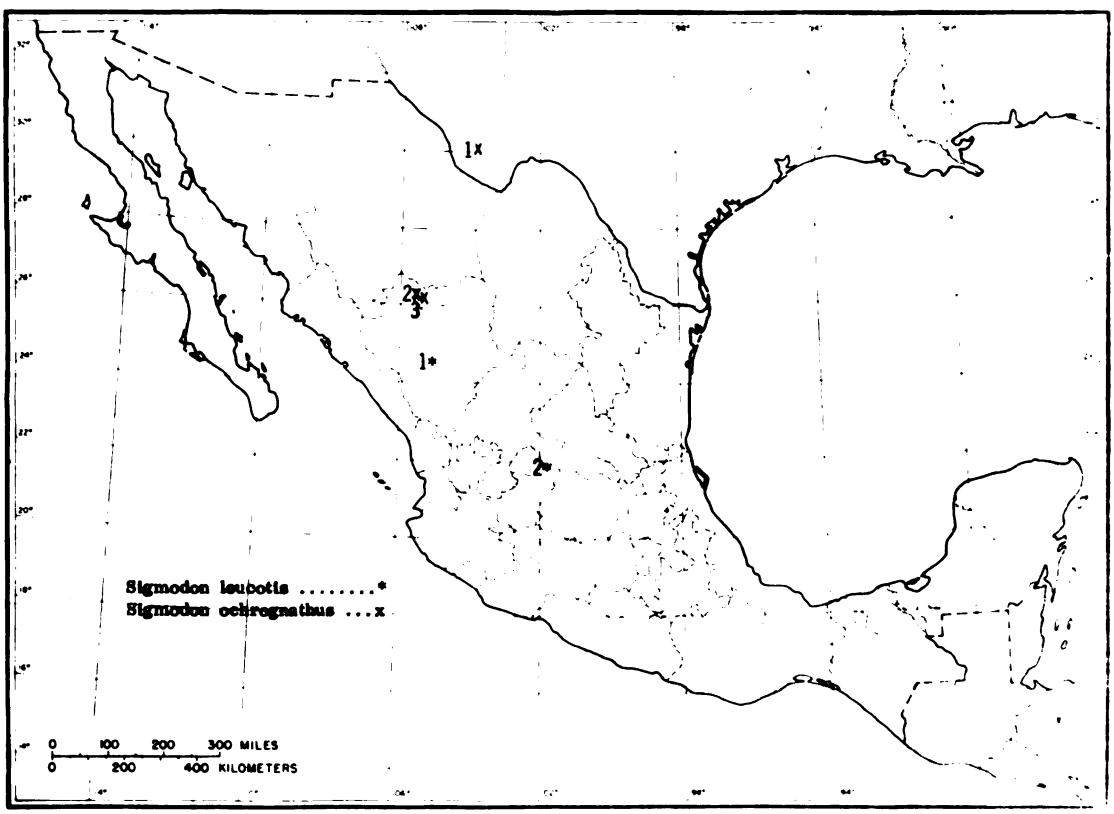


Figure 2b. Map showing the approximate geographical locations of Sigmodon leucotis and S. ochrognathus used in this study.

Sigmodon fulviventer minimus J. A. Allen

7. Chihuahua: Gallego, 1366 m., 3

8. Durango: 11 km. NNE Boquilla, 1952 m., 3

Sigmodon leucotis V. Bailey

1. Durango: Hda. Coyotes, 2477 m., 4

2. Guanajuato; 13 km. SW Ibarra, 2592 m., 5

Sigmodon melanotis V. Bailey

1. Jalisco: 2 km. NW La Barca, 1525 m., 6

Sigmodon ochrognathus baileyi J. A. Allen

1. Texas: Davis Co., 3 km. NW Fort Davis, 1610 m., 2

2. Durango: 3 km. NE Boquilla, 1952 m., 3

3. Durango: 11 km. NE Boquilla, 1952 m., 7

Sigmodon planifrons planifrons Nelson and Goldman

1. Oaxaca: 13 km. SSW Juchatengo, 1921 m., *7

2. Oaxaca: 8 km. ESE Río Grande, 30 m., 2

B. Method of Bleeding

To evaluate properly the taxonomic significance of electrophoretic studies of blood serum proteins, the effects of development and physiological variables, such as age, health, and sexual condition (Engle and Woods, 1960; Petermann, 1960; Moore, 1959) were recognized. To keep such effects at a minimum, all cotton rats used were acclimated to captivity, following standard laboratory procedures, for a minimum of one month. Subsequently, a specimen was bled if it was a breeding adult (non-pregnant, if female) and in good health.

Bleeding was accomplished by using the orbital bleeding technique described by Riley (1960). This technique of rupturing the ophthalmic

venous plexus was varied by slightly etherizing the specimen for better control and by using a 6-inch Pasteur pipette instead of a capillary tube for obtaining blood. The blood was allowed 15-20 minutes to clot, then inserted into an International Clinical Model Centrifuge, and spun at 3200 r.p.m. (1610 x g) for 15 minutes. The serum was drawn off, sealed, and frozen at -12°C.

Infrequently, serum collected for storage by this method would show some hemolysis upon thawing which in extreme cases noticeably affected the protein mobility and resolution. In several other instances, serum samples were whitish after centrifugation. This had little effect on the results when compared with other samples devoid of the whitish substance. When these samples were centrifuged at higher speeds, the white material would gather at the top of the serum, suggesting a lipid material.

C. Development of Procedure

The ease with which pore size can be varied with polyacrylamide gels is a distinct advantage over other gel media. Wright and Mallmann (1966) developed a new procedure by which two consecutive different pore size separating gels gave better separation and resolution of certain animal serums than that of the standard 7.5% separating gel of Ornstein and Davis (1962). The new method was termed "differential disc electrophoresis." In a preliminary investigation of Sigmodon serum proteins, various specimens and gel alterations were tested. The electrophoretic results from employing the standard gel on a representative serum sample from each species are shown in Plate 2a. In Plate 2b, is pictured the result of a serum sample that was electrophoresed using the standard gel and various combinations of differential pore size gels. The results

demonstrated that the "differential disc electrophoresis" method, using 4.75% and 10% separating gels, gave improved separation of the prealbumin and transferrin components over the standard gel.

D. Reagents

The latest modifications (Davis, 1964) in the stock and working solutions were employed. The stock solution, however, was changed to a 4.75% and a 10% acrylamide monomer stock. The reagents are listed in Tables 1 and 2.

E. Equipment

The disk electrophoresis apparatus developed by Lawrence Besaw (per. comm.) was employed. As shown in Plate 1a, each tube has its own upper buffer reservior and current supply. This allowed for independent removal of a gel tube without disturbing the others. The lower common buffer tank holds 1400 ml., the same total volume as for the eight upper buffer reserviors. The electrodes are composed of 20 gauge platinum wire. Current was supplied by a Spinco Duostat Model RD regulated D. C. power supply.

After the electrophoresis procedure, the acrylamide gels were inserted into a plexiglass gel holder (Plate 1b) capable of simutaneously holding 14 gels, and immersed for two hours in a staining tray containing a solution of 1 gm. Amido Black 10B dye to each 100 ml. of 7% acetic acid. The electrolytic destaining unit (Plate 1b) contained 7% acetic acid as the electrolyte and two stainless steel plates as electrodes. Powered by a 12 volt, 1 1/2 amp. battery charger, a normal load of 8 gels was

TABLE 1. Stock solutions

(A)		(B)	
1 N HCL	48 ml	1 N HCL	approx. 48 ml[1]
TRIS	36.6 gm	TRIS	5.98
TEMED	0.23 ml	TEMED	0.46 ml
DHOH	to 100 ml (pH 8.9)	DHOH	to 100 ml (pH 6.7)

(C)	4.75%	10%	(D)	
Acrylamide	19 gm	40 gm	Acrylamide	10.0 gm
BIS	0.4 gm	0.4 gm	BIS	2.5 gm
DHOH	to 100 ml	to 100 ml	DHOH	to 100 ml

(E)		(F)	
Riboflavin	4 mg	Sucrose	40 gm
DHOH	to 100 ml	DHOH	to 100 ml

(G)

Ammonium persulfate	0.14 gm
DHOH	to 100 ml

[1] pH adjusted by titrating with 1 N HCL
TRIS tris(hydroxymethyl)aminomethane
TERMED N, N, N, N - tetramethylethylenediamine
BIS N, N - methylenebisacrylamide
DHOH deionize water

TABLE 2. Working solutions

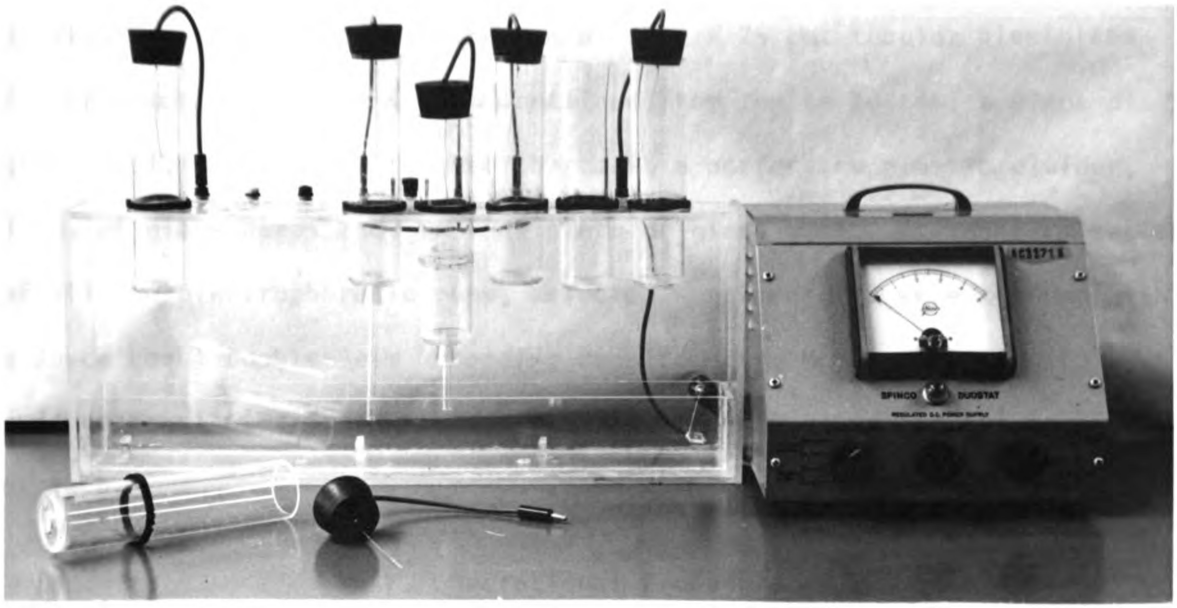
Small-pore solution #1	Small-pore solution #2	Large-pore solution	Stock buffer solution for reservoirs[1]
1 part A	1 part A	1 part B	TRIS
2 parts C 10%	2 parts C 4.75%	2 parts D	Glycine 28.8 gm
1 part DHOH	1 part DHOH	1 part E	DHOH to 1 liter
pH 8.9 (8.8-9.0)	pH 8.9 (8.8-9.0)	4 parts F	pH 8.3
		pH 6.7 (6.6-6.8)	Diluted 1:10 before use

[1] One to three runs were made before the buffer was discarded.

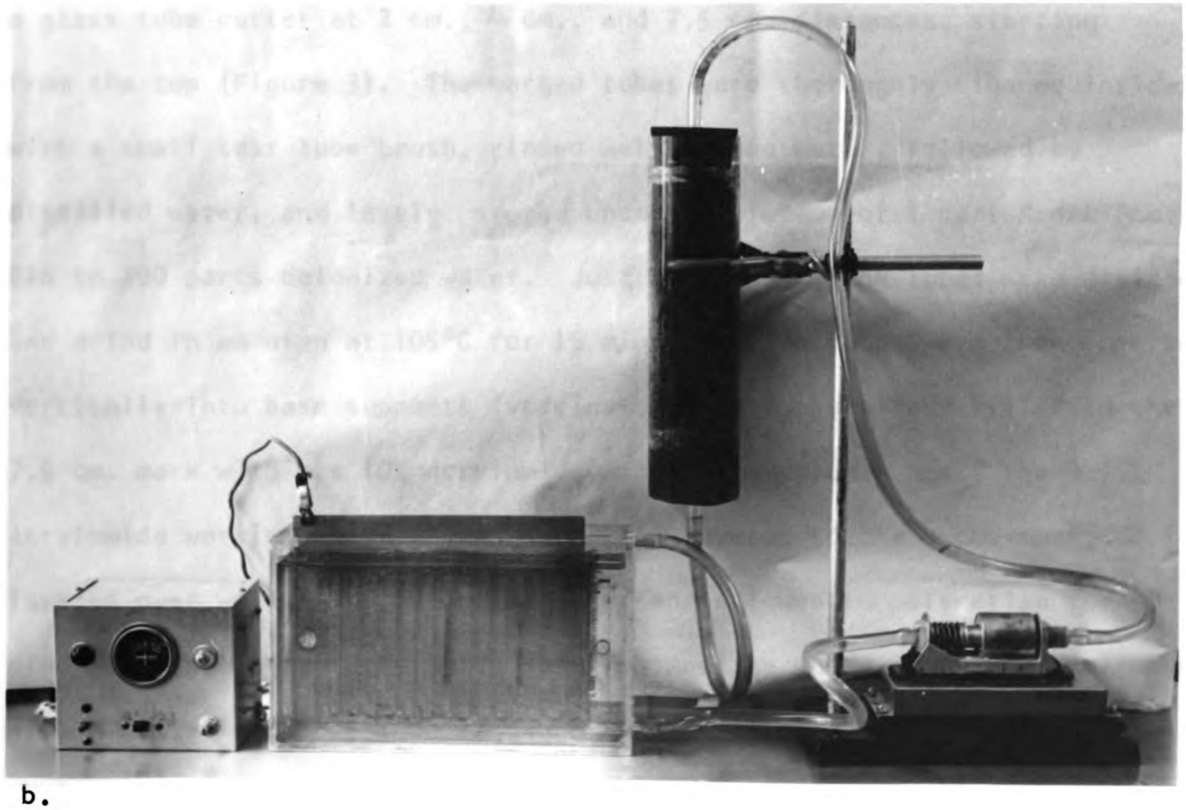
Plate 1a. The electrophoresis apparatus and power supply. This apparatus allows for removal of each electrophoresis tube independently of the others. Different gel lengths may also be electrophoresed concurrently, as shown by adjusting the position of the rubber strip (rubber tubing cut in cross section) circling the upper buffer reservoir.

Plate 1b. Destaining unit, consisting of (l. to r.) battery charger, destaining tank, filter system, and oscillating pump. The destaining tank has one stainless steel electrode removed to show the plexiglass gel-holder with two gels in position.

PLATE 1



a.



b.

destained in 2 hours. The 7% acetic acid, as it accumulated dye from the gels, was continously circulated by a rheostat-controlled Dynalab oscillating pump (Plate 1b) through a 6 cm. x 25 cm. tubular plexiglass filter system. The filter system contained from top to bottom: a piece of glass wool, 16 cm. of activated charcoal, a perforated plastic divider, 3 cm. of glass beads and, another piece of glass wool. At the termination of all the electrophoretic runs, selected specimen gels were scanned with a Joyce Loebl Double-Beam Recording Densitometer, Model MK III C, to produce densitometric curves representing protein zones and the proportions of each.

F. Operational Procedure

Pyrex tubes, (5 mm. i.d. x 12 cm.) were etched circularily with a glass tube cutter at 2 cm., 4 cm., and 7.5 cm. distances, starting from the top (Figure 3). The marked tubes were thoroughly cleaned inside with a small test tube brush, rinsed well in tap water, followed by distilled water, and lastly, stored under a solution of 1 part Kodak Photo Flo to 200 parts deionized water. Just before use, the tubes were drained and dried in an oven at 105°C for 15 minutes. The tubes were then inserted vertically into base supports (vaccine-bottle stoppers) and filled to the 7.5 cm. mark with the 10% acrylamide working solution. Next, the 4.75% acrylamide working solution was carefully layered to the 4 cm. mark, layered over with 5 mm. deionized water and allowed to polymerize for 40 minutes. The latter acrylamide solution and the water layer were introduced with a bent-tipped 3.5 inch 22 gauge needle attached to a 2 ml. plastic syringe barrel topped by a squeeze bulb. After polymerization, spacer gel

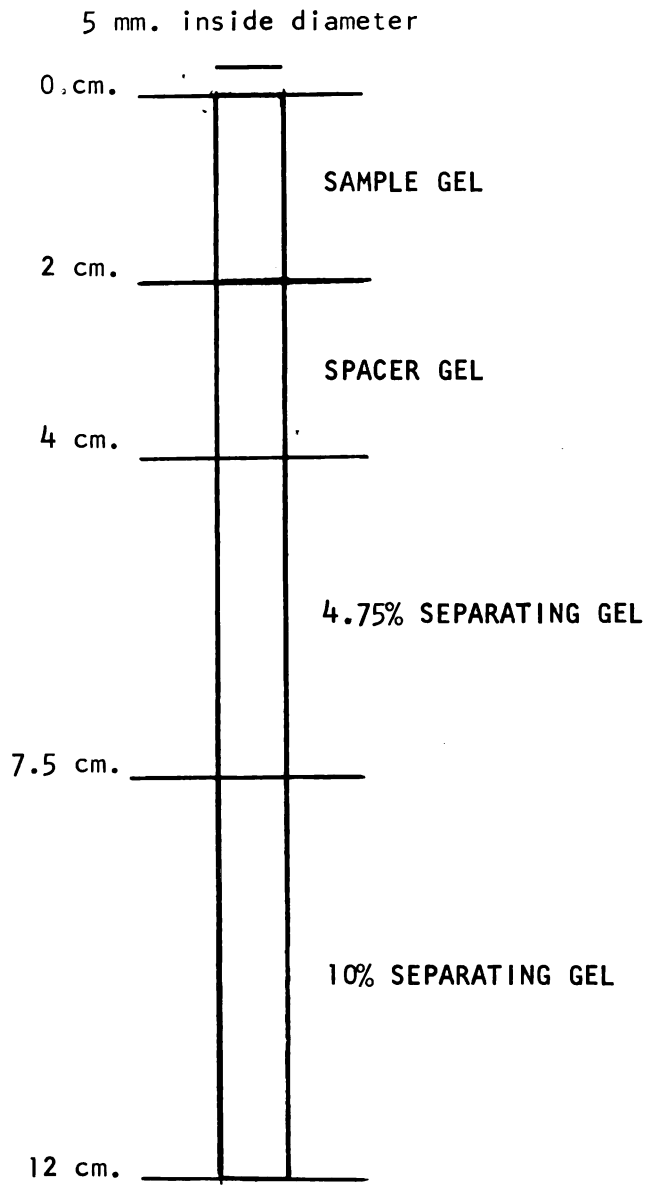


Figure 3. Diagramatic representation of the position of the gels in the differential disc electrophoretic column.

monomer (large-pore working solution) was first added as a rinse, decanted, then re-added to the 2 cm. mark, layered over with 3 mm. of water, and photopolymerized for 15 minutes. During this time each sample gel solution was prepared in a 5 ml. vial by mixing 8 microliters of serum from a 10 microliter syringe with 0.4 ml. of spacer gel monomer. After the spacer gel was polymerized, spacer gel monomer was added as a rinse, and decanted. Sample gel solution was then introduced on top of the spacer gel and photopolymerized for 30 minutes.

Following photopolymerization, the tubes were removed from their bases and the sample gel ends were inserted into the rubber stoppers at the bottom of the empty upper buffer reserviors. The buffer solution, containing a few drops of concentrated bromophenol blue as a tracking dye, was next gently poured into each upper tank.

Electrophoresis was started at 2 mA per gel tube, and continued until the bromophenol blue tracking front, approximately one hour later, reached the separating gel (near 4 cm.).* At this time the current was changed to 4 mA per tube until the tracking front, approximately 3/4 hours later, was flush with the bottom of the gel tube. At that time the current was shut off and the tube removed.

Following electrophoresis, the gels were carefully removed by inserting between the gel and the glass tube wall a blunt-tipped 2-inch, 22 gauge needle mounted on a 10 cc. water-filled syringe to provide constant

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*It should be noted that if two gels tubes are run in parallel the current required for effective separation will be double that required for one tube alone. Likewise, for 3 tubes, three times the current will be required. The voltage will be the same, irrespective of the number of tubes employed (Sargent, 1964).

lubrication. Following their extrusion from the glass tubes, the gels were stained, destained, and analyzed densitometrically as previously mentioned (p.15).

G. Controls

Human blood serum was used as a control throughout the experimental work. In this way any differences or deviations in the electrophoretic technique or the stock solutions were likely to be detected. Of the eight gel tubes which could be run simultaneously, three were used for each of two Sigmodon blood serum samples and the remaining two tubes were for the human serum.

Specimens from one sample or species were not usually electrophoresed at any one time, but randomly inserted throughout the electrophoretic period. From using the human blood serum control and this practice, one could reasonably assume that any distinguishable similarities observed within a population or species were real and perhaps of taxonomic significance. Approximately one-third of all the serum samples, irrespective of the species, were rerun at the termination of the three-month electrophoretic analysis period. This served as a final check and for the purpose of resolving certain questionable observations on protein fractions. Several times during the study, Sigmodon specimens from the laboratory colony were bled by graduate students during the absence of the investigator and the serum was given to him to see if he could determine by gel electrophoresis the species or possibly the original geographical location of the specimen. Several species were also bled again at a later date to confirm previous results.

H. Analysis of Data

The densitometric tracing of a stained gel was magnified by the densitometer approximately 1.5 times on mm. ruled graph paper, and the resulting tracing interpreted the protein components (bands or zones) in the gel as peaks (Figure 4). Although certain mammalian serum proteins such as prealbumins, albumins, and transferrins are commonly found in acrylamide gels (Ornstein and Davis, 1962) at the relative position represented in Figure 4, no chemical tests were performed to confirm these protein identities. It was very possible (as certain electrophoretic runs suggested) that minor protein components were overshadowed by the presence of major components which had migrated the same distance. However, for simplicity the zone identification terminology given in Figure 4 is followed.

Analysis of the densitometric tracing was made by first measuring (in mm.) the x and y coordinates for every peak, using as the origin the boundary between the spacer and the separating gels. Variance and R_m (relative mobility) values, where on the densitometric tracing

$$R_m = \frac{\text{distance in mm. from origin to center of a given peak}}{\text{distance in mm. from origin to center of the albumin peak*}}$$

were calculated for those components which were considered to be of taxonomic importance. The mean relative distance, D_m, within a given number of specimens is equal to $\sum \frac{(R_{m2}-R_{m1})}{n}$ where $(R_{m2}-R_{m1})$ is the difference

*The albumin component was used as a reference point because of its stability within the species of Sigmodon studied.

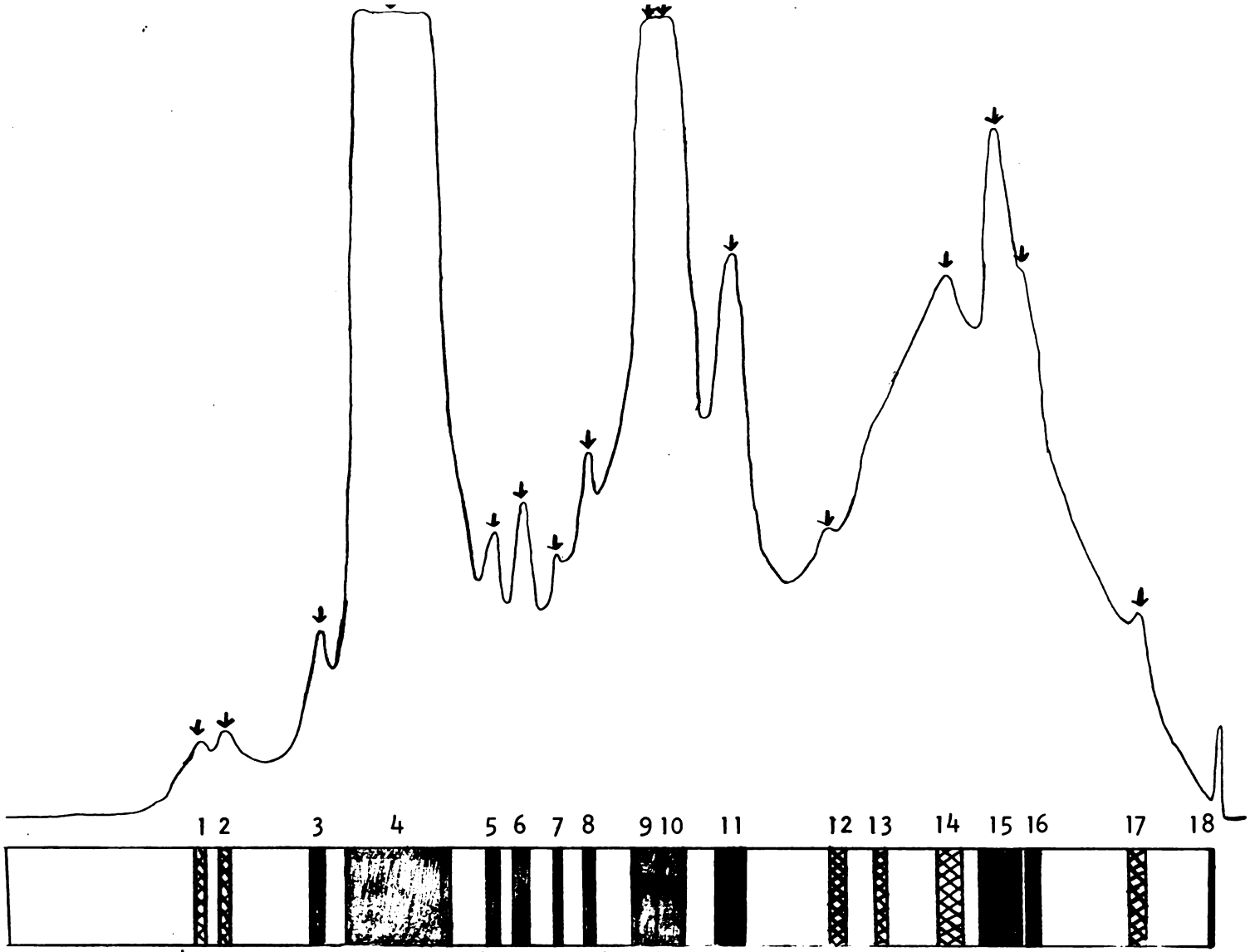


Figure 4. Schematic diagram of a Sigmodon densitometric tracing and accompanying gel and its nomenclature: 1-3-prealbumins; 4-albumin; 5-8-postalbumins; 9-11-transferrins; 12-17-globulins; 18-origin of run. Solid bands, intense staining; cross-hatched, medium or faint staining.

between the relative mobilities of any two peaks (R_{m2} and R_{m1}) within a specimen; $\Sigma(R_{m2}-R_{m1})$ is the sum of the differences in a series of specimens and; n is the number of specimens involved. The height (Y_{max}) of a densitometric peak was assigned to a unit (unit=5 mm.) on the y-axis if it was halfway or more through the unit and still one mm. or more in width. This procedure was followed to reduce error due to background interference. Maximum height of the peaks (e.g. the albumin) was 26 units.

To correlate densitometric peaks with their respective protein bands in the gels, each gel and its corresponding densitometric tracing were viewed together before any measurements were taken. This was especially necessary where 2-4 bands could be visually observed in the destained gel but appeared together in the densitometric tracing.

III. RESULTS

A. Individual Variation Within a Population Sample

1. Sigmodon hispidus

Twenty-four population samples of S. hispidus totaling 97 individuals were available for examination. The samples ranged in size from single specimens from each of six localities to eight specimens from each of two localities (pp.6,7). Electrophoretic results indicate that between individuals within a sample from one locality some blood serum protein components varied in mobility, number, and relative volume (as measured visually and by the height of individual peaks) while other serum components showed little variation.

Minimal individual variation is observable within a sample of six specimens from Oracle, Arizona (Plate 2c, Figure 5). To demonstrate best the general relationships in the sample without losing the identity of each densitometric tracing, four of the six tracings are shown superimposed in Figure 7a. The transferrins, the albumin and the prealbumin adjacent to the albumin (Pa_1) are similar in amplitude and mobility in all specimens. Other blood serum protein components varied as follows:

a. The calculated R_m values in two specimens for several postalbumin peaks overlap with the values calculated for closely neighboring peaks on other specimens. However, these peaks maintain similar respective positions to each other in all the densitometric tracings of this sample.

b. In four specimens (the last four shown in Plate 2c, and Figure 5c,d,e,f), one or two prealbumins are present in addition to the stable prealbumin (Pa_1).

c. In two specimens (the first two shown in Plate 2a and in Figure 5a,b), only one globulin, instead of two, is present.

Although most samples were intermediate in variation, the extreme amount found was observed in the six specimens from Autlán, Jalisco (Plate 2d and Figure 6; with four of the densitometric tracings superimposed in Figure 7b). The prealbumin adjacent to the albumin (Pa_1), the albumin, and two transferrins farthest from the origin are similar in amplitude and mobility in all specimens. Other blood serum protein components varied as follows:

a. In two specimens (Figure 6e,f), five transferrins occurred instead of four. This resulted in a lighter series of three components nearest the origin occupied by two transferrins in the other four specimens. This was the only case in the Sigmodon studied which suggested transferrin polymorphism within a population sample.

b. One specimen (Figure 6a) has three globulins present; the other five specimens have two globulins.

c. The number of postalbumins varied from four to seven components.

d. One specimen (Figure 6b) has one additional prealbumin other than Pa_1.

It is known (Longsworth, 1959) that the presence of nearby protein components may influence the ionic environment. The possibility of a protein-protein interaction may also exist. In both instances this could influence the protein mobilities. However, no extreme changes in

R_m values occurred in this instance or others which might give evidence of the above phenomena affecting the electrophoretic results.

2. Comments on the other species of Sigmodon.

Individual variation within a population sample in the number of prealbumins (excluding the one nearest the albumin) and globulins occurred in S. alleni, S. ochrognathus and S. planifrons. The number of globulins and also the number and position of the postalbumins and prealbumins varied in S. melanotis and S. fulviventer.

B. Geographic or Intraspecific Variation

1. Sigmodon hispidus

A single gel representative of S. hispidus from each of 16 population samples is shown in Plate 3a. Gels 1-7 are from specimens within the United States, gels 8-15 are from México, and gel 16 is from the Canal Zone. Representative gels of cotton rats from South Carolina, Honduras and the Mexican states of Guerrero, Nuevo León, Sinaloa, and San Luis Potosí were not available when the photograph was taken. Geographic or intraspecific variation in S. hispidus is characterized as follows:

a. Prominent in the 97 specimens of S. hispidus is the presence of a prealbumin adjacent to the albumin (Pa_1) where the mean R_m=1.08 and s^2=.0001, mean Y_{max}= 5.6 units, and s^2=1.9.

b. Except for the Lubbock, Texas sample, representing a subspecies which inhabits the open, arid country of the southwestern United States and México's Central Plateau, all the samples from the United States were

characterized by having the transferrin nearest the origin widely separated from the remaining two confluent transferrins by a mean relative distance, $D_m \geq .065$. Termed transferrin pattern A, it is represented in the first six gels of Plate 3a, and in the densitometric tracings of the Oracle sample, shown separately in Figure 5, and superimposed in Figure 7a.

c. The Mexican forms, with two exceptions, are characterized by four transferrins (pattern B), the two nearest the origin separated from each other more than the next two which are slightly confluent. The four transferrin characteristic is represented in gels 7-15 of Plate 3a, and some of the densitometric tracings of the Autlán sample, shown separately in Figure 8b. One exception to this was in the Autlán sample, as mentioned previously. The other exception was in the Guerrero sample, which is characterized by having the transferrin nearest the origin widely separated from the remaining three confluent transferrins by a mean relative distance, $D_m \geq .060$ (pattern C).

d. The Canal Zone samples are characterized by three confluent transferrins (pattern D), represented in gel 16 of Plate 3a.

e. The Honduran sample is characterized by having the transferrin nearest the origin widely separated from

the remaining four confluent transferrins by a mean relative distance, D_m=.070 (pattern E).

The five basic transferrin patterns (A,B,C,D, and E) found in S. hispidus are regionally represented in Figure 9. Using laboratory specimens of S. hispidus, the five regional patterns (A-E) are readily discernible. To identify specimens from any one region to their respective localities is more difficult. Of the population samples studied, approximately one-third could be distinguished with certainty, based on the characteristic postalbumin patterns. For instance, even though there is considerable variability in the postalbumins of the Autlán S. hispidus (Plate 2d and Figures 6,8b), the presence of a very prominent postalbumin identified this population sample from other Mexican population samples. However, specimens from the population samples of S. hispidus from Arkansas, Florida, and Oklahoma (represented in gels 2-4 of Plate 3a) are not readily distinguishable from each other.

2. Comments on the other species of Sigmodon.

Since the electrophoretic results of the S. leucotis, S. ochrognathus, and S. planifrons population samples within each species were essentially identical, differences attributable to geographic or intraspecific variation were not observable. In S. fulviventer, the degree of variation within a sample in the pre- and postalbumins made it impossible to identify a specimen to locality. Because population samples were available from only one locality for S. melanotis and S. alleni, the amount of geographic or intraspecific variation was not determinable.

C. Specific Differences in Sigmodon.

With the intra- and interpopulation information obtained up to this point, the variability and stability of certain protein components in S. hispidus is acknowledged and may be applied to characterize the remaining six species as follows:

1. Sigmodon ochrognathus

Twelve specimens from three localities were studied. Plate 3b and Figure 7b show representative gels and densitometric tracings of three specimens from Boquilla, Durango, since the specimens from the other localities were not available at the time the photograph was taken. This species may be identified by two confluent transferrins (eight specimens have a lesser third confluent component with the transferrin farthest from the origin), and a third near the origin separated from its neighboring transferrin with D_m=.057. A prealbumin (Pa_1) is present next to the albumin with a mean R_m=1.07. Four distinct postalbumins are present.

2. Sigmodon leucotis

Nine specimens from two localities more than 450 km apart were studied. Plate 3c and Figure 7a give representative gels and densitometric tracings. This species may be identified by the presence of four confluent transferrins. The components are separated in gels 3 and 4 for observational purposes. Another more conspicious characteristic of this species is the presence of two distinct prealbumins, with R_m=1.06, and 1.11, respectfully. Four distinct postalbumins are present.

3. Sigmodon alleni

Eight specimens from one locality were studied. Plate 4a and Figure 8c give representative gels and densitometric tracings. This species may be identified by three transferrins. The one nearest the origin is separated from the others by a D_m=.035. The prealbumin nearest the albumin does not differ from that of S. hispidus. A dense component precedes the three transferrins, where R_m=.725 and the mean Y_{max}=10.7 units. Four to five postalbumins are present.

4. Sigmodon planifrons

Nine specimens from two localities were studied. Plate 4c and Figure 8d give representative gels and densitometric tracings of Juchatengo specimens since the specimens from Río Grande were not available at the time the photograph was taken. This species is very similar to S. alleni in relation to the transferrin and prealbumin components. Five to seven postalbumins are present.

5. Sigmodon fulviventer

Twenty-one specimens from seven localities were studied. Plate 4b and Figure 7d give representative gels and densitometric tracings with the exception of specimens from Hda. Altotonilco, which were not available at the time the photographs were taken. This species may be distinguished by its three dense, confluent transferrins and a lesser component confluent with the transferrin farthest from the origin. The prealbumin nearest the albumin, when compared with S. hispidus, has a mean Y_{max}=2.15 cm., and a greater variance, s^2=2.63; also, R_m=1.12, and its variance, s^2=.002, are greater. Two to four distinct postalbumins

are present.

6. Sigmodon melanotis

Six specimens from one locality were studied. Plates 4d and 7c give representative gels and densitometric tracings. This species could not be distinguished from S. fulviventer. Two to three distinct post-albumins are present.

The blood serum electrophoretic characteristics of the seven species of cotton rats are summarized in Table 3.

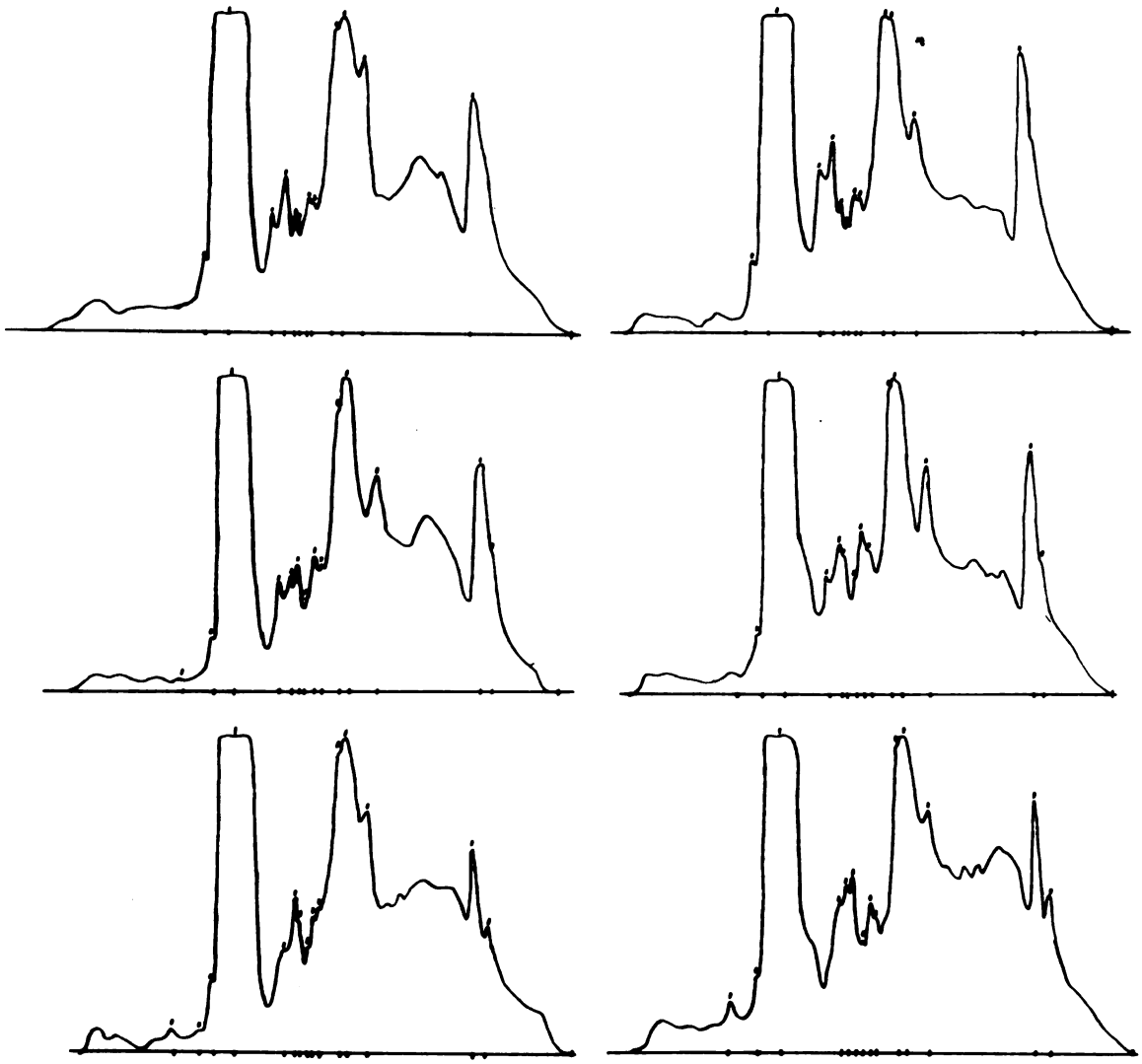


Figure 5. Sketched densitometric tracings of the six Sigmodon hispidus from Oracle, Arizona to demonstrate minimal individual variation within a population sample.

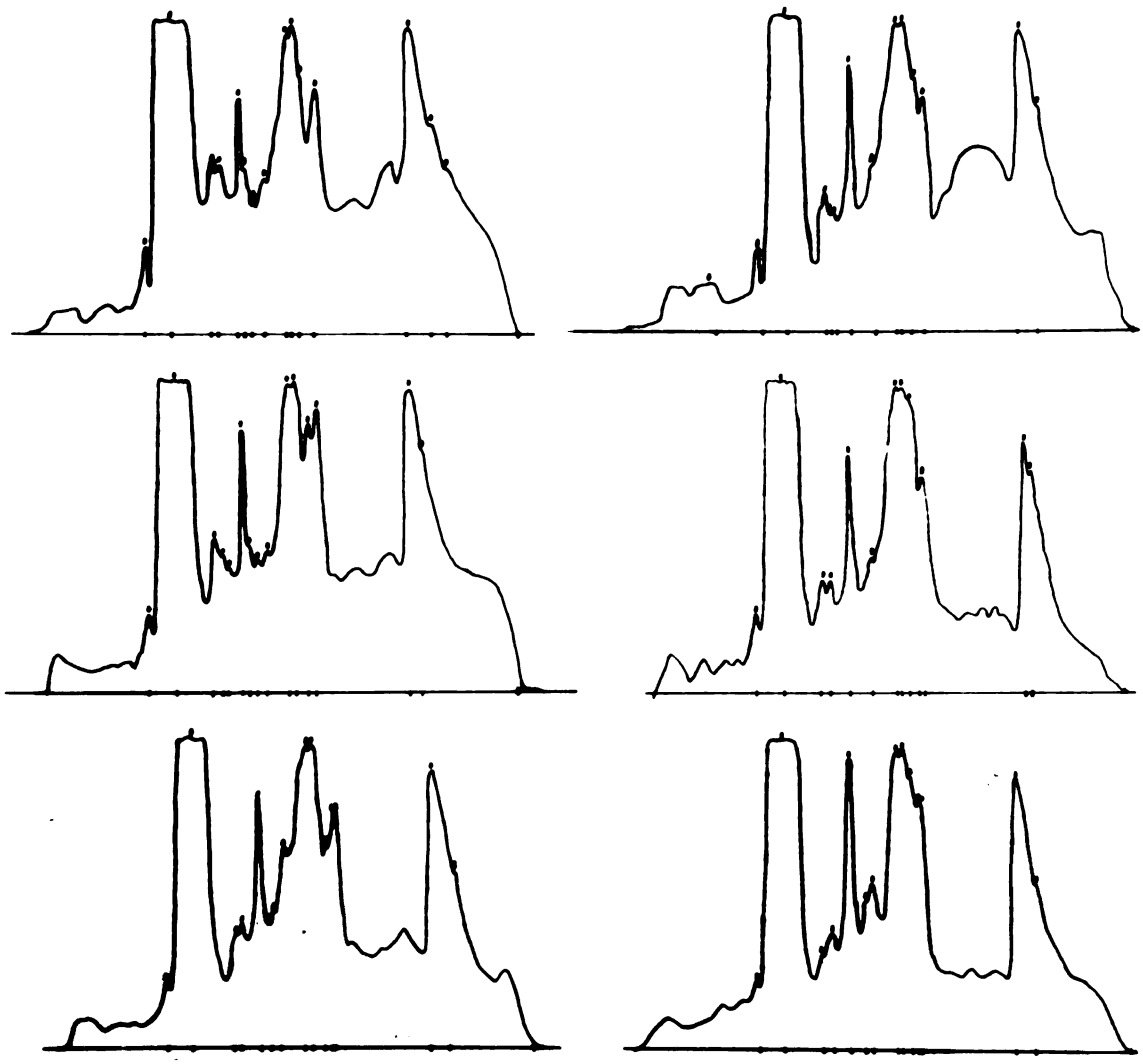


Figure 6. Sketched densitometric tracings of the six Sigmodon hispidus from Autlán, Jalisco, to demonstrate maximal individual variation within a population sample.

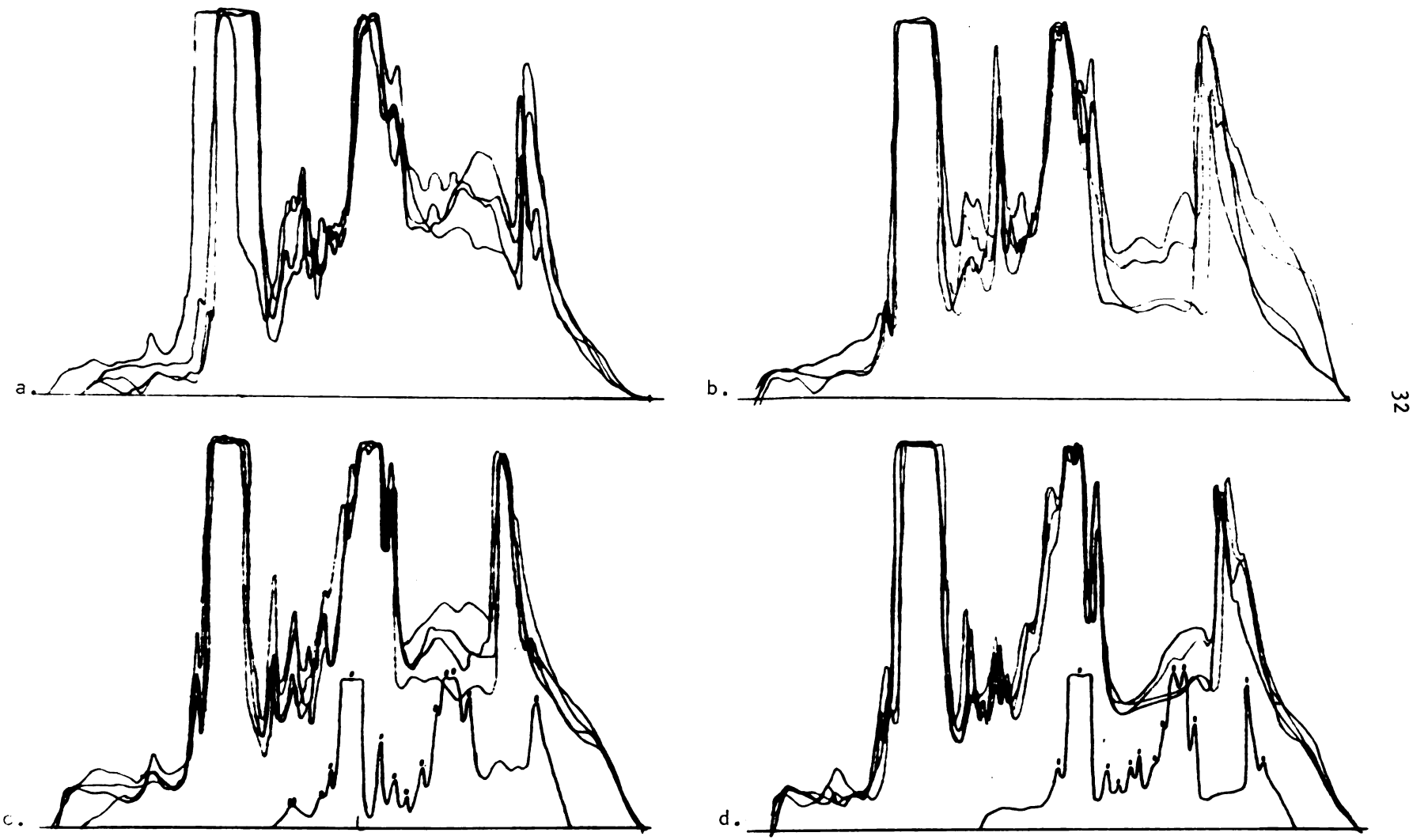


Figure 7. Sketched densitometric tracings from four specimens each of: Sigmodon hispidus from (a) Oracle, Arizona and (b) Autlan, Jalisco are superimposed to demonstrate the general similarity found within a population sample and between populations; S. alleni (c) and S. planifrons (d) are superimposed to demonstrate the similarity within a species. One tracing from the latter two species is further reduced and marked indicating the position of the various blood serum components.

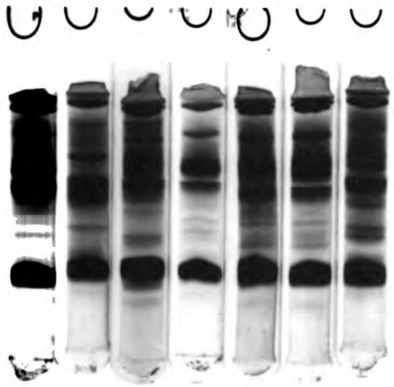
Plate 2a. Davis and Ornstein 7.5% standard gels of (l. to r.) Sigmodon alleni, S. ochrognathus, S. leucotis, S. planifrons, S. melanotis, S. fulviventer, and S. hispidus.

Plate 2b. The resulting electrophoretic serum protein patterns specimen of Sigmodon hispidus, using various pore size separating gels. These are (l. to r.) Davis and Ornstein 7.5% standard gel; 7.5%-10% gel; 10% gel; 4.75% gel; 7.5% gel; 4.75%-10% gel. Electrophoresis was terminated in each gel when the tracking front reached the bottom of the electrophoresis tube.

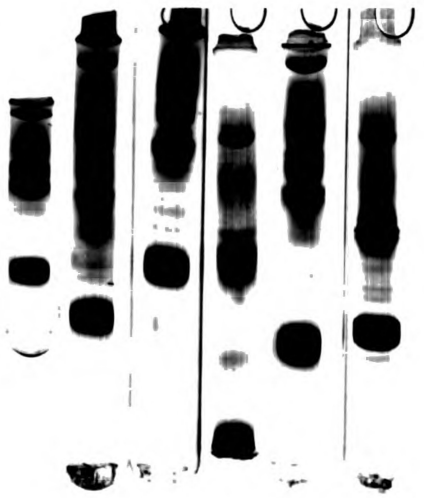
Plate 2c. Electrophoretic gel patterns of six Sigmodon hispidus from the Oracle, Arizona population sample.

Plate 2d. Electrophoretic gel patterns of six Sigmodon hispidus from the Autlán, Jalisco population sample.

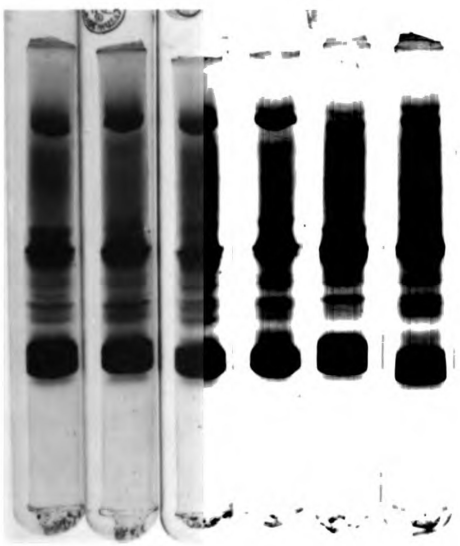
PLATE 2



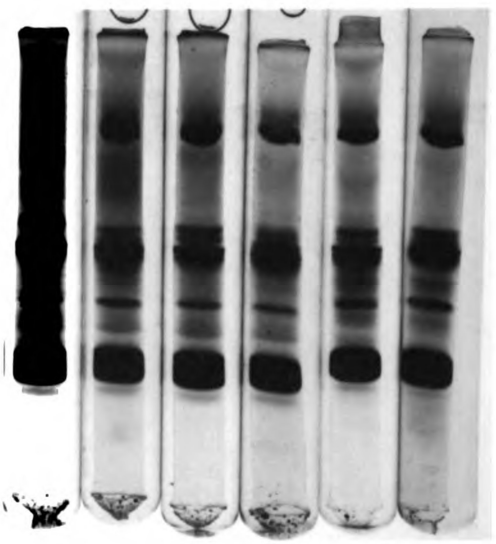
a.



b.



c.



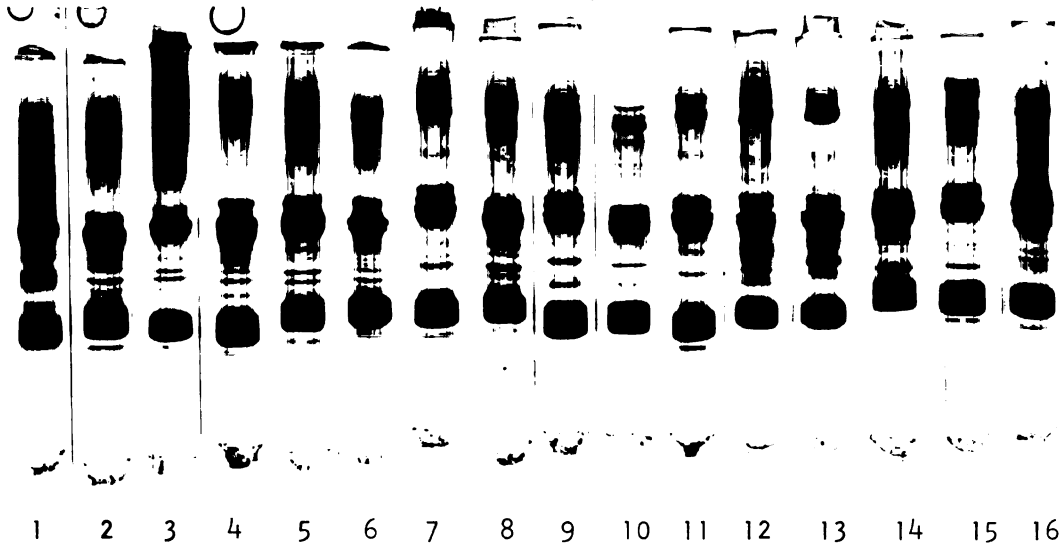
d.

Plate 3a. Electrophoretic serum protein patterns of Sigmodon hispidus from 16 geographic localities. Gels 1-7 are from the United States, gels 8-15 are from México, gel 16 is from the Canal Zone.

Plate 3b. Electrophoretic serum protein patterns of the three Sigmodon ochrognathus from Boquilla, Durango.

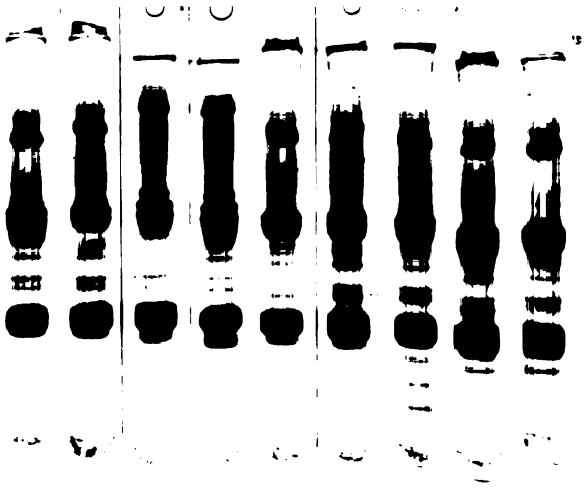
Plate 3c. Electrophoretic serum protein patterns of Sigmodon leucotis, with (l. to r.) first four specimens from Hda. Coyotes, Durango, and last five specimens from Ibarra, Guanajuato. The last two gels from Hda. Coyotes were stopped before the tracking front reached the bottom of the electrophoresis tube. Their normal patterns were like the remaining gels.

PLATE 3



a.

b.



c.

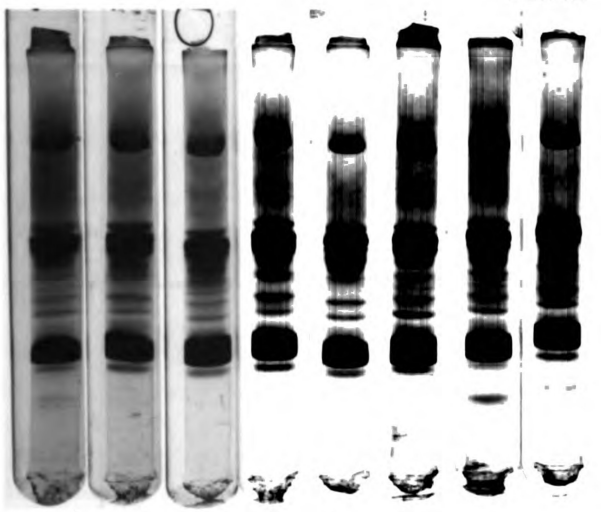
Plate 4a. Electrophoretic serum protein patterns of the eight Sigmodon allenj from Capácuaro, Michoacan.

Plate 4b. Electrophoretic serum protein patterns of six Sigmodon fulviventer. From (l. to r.) Hda. Coyotes, Durango (first two gels); Boquilla, Durango; Canatlán, Durango; Gallego, Chihuahua; Ibarra, Guanajuato.

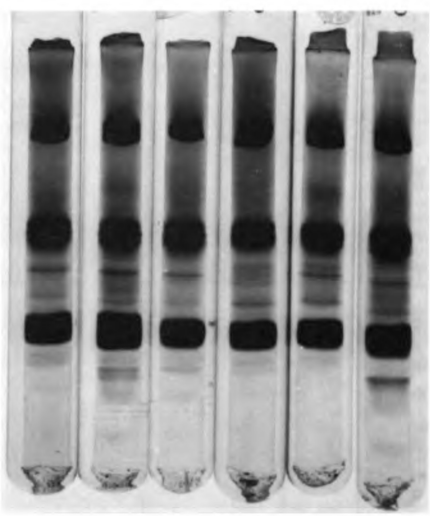
Plate 4c. Electrophoretic serum protein patterns of the seven Sigmodon planifrons from Juchatengo, Oaxaca.

Plate 4d. Electrophoretic serum protein patterns of the six Sigmodon melanotis from La Barca, Jalisco.

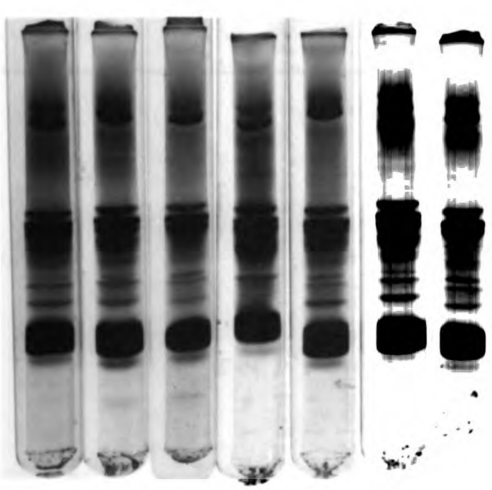
PLATE 4



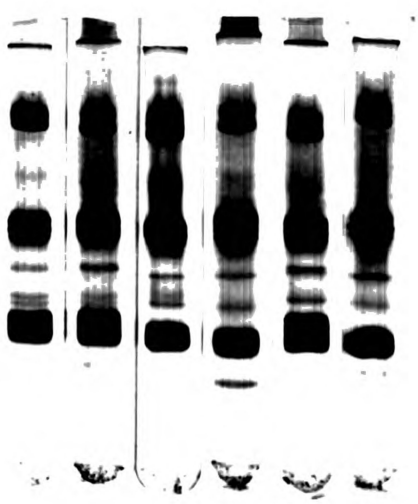
a.



b.



c.



d.

TABLE 3. Electrophoretic characteristics of Sigmodon blood serum proteins.

COMPONENTS	Transferrins*	Stable Prealbumins (Pa)	Unstable Prealbumins	Postalbumins
SPECIES				
S. hispidus				
United States.....	1 + 2; D_m=.065			
México.....	1 + 1 + 2; except Autlán Jalisco specimens may be: 1 + 1 + 1 + 2; or Guerrero specimens are: 1 + 3; D_m=.060	Pa_1: R_m=1.08 (1.07-1.10)	0-5	4-7
Canal Zone.....	3			
Honduras.....	1 + 4; D_m=.070			
S. alleni-planifrons.....	1 + 2; D_m=.035	Pa_1: Same as S. hispidus	0-3	4-7
S. ochrognathus...	1 + 2; D_m=.057 a lesser 3rd component may be present and confluent with transferrin farthest from origin.	Pa_1: R_m=1.07 (1.06-1.08)	1-2	4
S. fulviventer-melanotis.....	3; a lesser 4th component confluent with transferrin farthest from origin.	Pa_1: R_m=1.10 (1.08-1.14)	0-4	2-4
S. leucotis.....	4	Pa_1: R_m=1.06 (1.05-1.08) Pa_1: R_m=1.11 (1.09-1.15)	1-5	4

*Notations are interpreted as in the following example: 1 + 2; D_m=.065, means "One transferrin nearest the origin is separated from two confluent transferrins by a mean relative distance of .065."

DISCUSSION

The electrophoretic serum protein patterns observed in Sigmodon indicate that like other taxonomic parameters so often used--cranial measurements, ear length, body weight--there is a limit to their usefulness. Sigmodon hispidus was employed to assess the extent of serum protein variation between specimens within a population sample and also between specimens from different geographic localities.

It was demonstrated in S. hispidus that of the five major protein groups, three, the albumin, one prealbumin (Pa_1) and the transferrins varied little, while three others, the globulins, postalbumins and the variable prealbumins (i.e. excluding Pa_1) varied considerably. The globulins varied from 1-3 main components, but this variation is thought to be a technical problem and not a real difference. Some specimens of S. hispidus have 0-5 variable prealbumins, while others from the same locality may have none, several, or the same number but at different R_m values. The postalbumins also varied in number and mobility, but have some taxonomic value at the population level.

The same protein serum components mentioned as being variable in S. hispidus are also the most variable in the other species of Sigmodon. However, the postalbumins do follow a general number and pattern which appear to differ in each species. The prealbumin next to the albumin (Pa_1), the albumin and the transferrin components were the most stable serum proteins in S. hispidus.

The appearance of transferrin polymorphism within the population sample from Autlán, Jalisco, may indicate a limited gene exchange within local populations. Rasmussen (1964), used two erythrocytic antigens

as genetic markers and found that the zygotic frequencies of the antigenetic phenotypes indicated that association of gametes occur randomly within the deer mouse populations. From this data Rasmussen suggested that a limited gene exchange did occur within natural populations of Peromyscus maniculatus. A limited gene exchange was also suggested by Brown and Welser (1966), who demonstrated albumin polymorphism in Peromyscus, and reported that three of six individuals in a natural population of P. leucopus were heterozygous for two albumins. In this study, the frequency of transferrin polymorphism within a population sample of S. hispidus appears to be low since it was observed only once.

With the exception of the above instance, the five different transferrin patterns (A,B,C,D and E, Figure 9) in S. hispidus followed a specific arrangement which allowed for quick regional identification. The population samples from the United States (except for the Lubbock, Texas sample) and the Canal Zone have their own respective three-transferrin patterns. The five-transferrin pattern from Honduras is distinctive as are the two kinds of four-transferrin patterns found in Mexico: one in samples from localities north of the Río Balsas and one from southern México (Guerrero). The specimens from Guerrero were the only samples available for S. hispidus south of the Río Balsas, whose valley is prominant as a north-south barrier to mammal movements (Baker, 1963). Although it is possible that the distinctive transferrin type found might be a local variation, it seems more probable that the Guerreran sample represents a condition found in other hispid cotton rats in southern México. The relation between this transferrin type and those that are

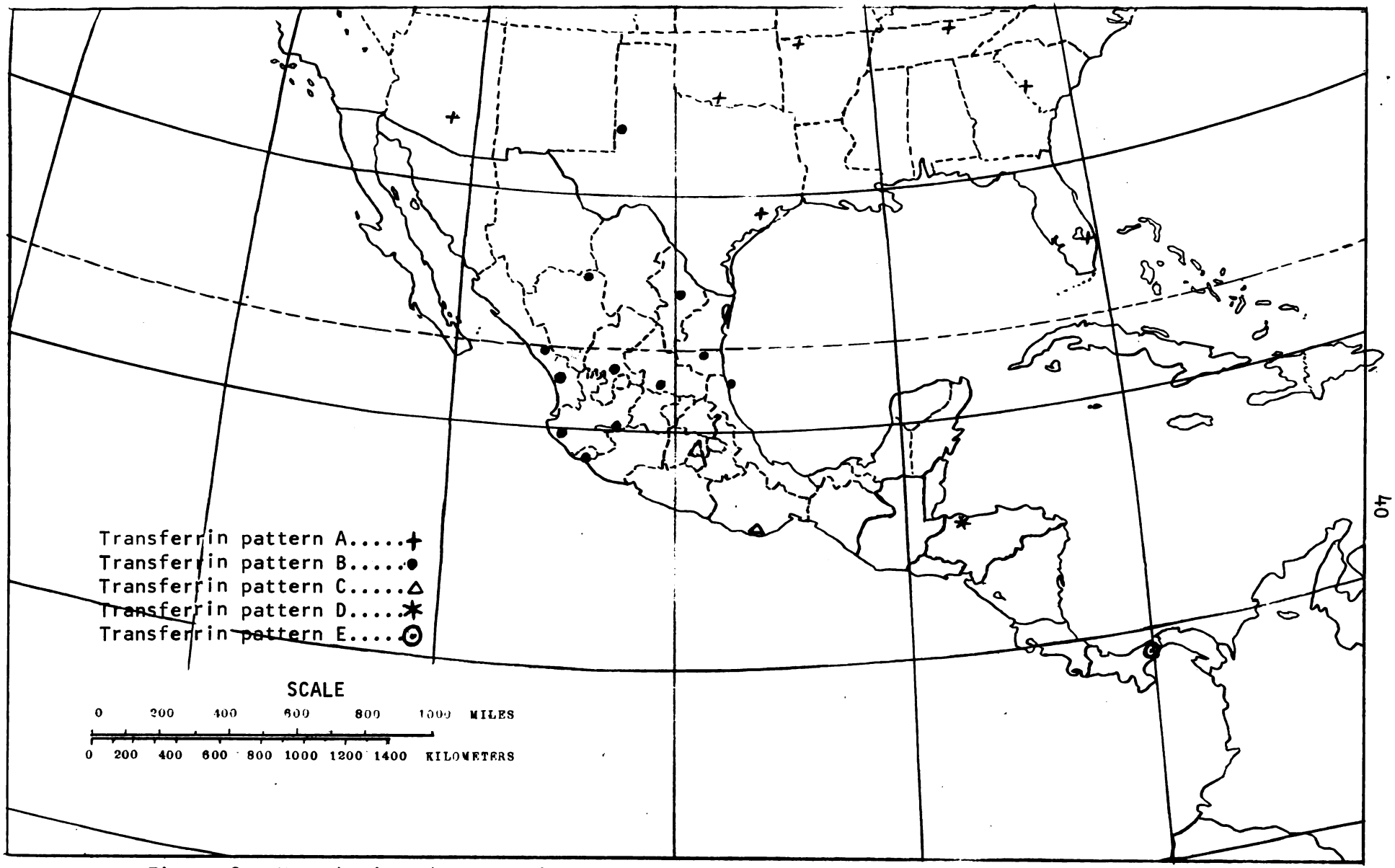


Figure 9. Map showing the approximate geographical distribution of the five transferrin types (A through E) found in Sigmodon hispidus.

found in the samples from Honduras and the Canal Zone cannot be known until sera from animals in the intervening areas are studied.

With such variable forms, no evidence of a "species curve" or a "species pattern" referred to in many electrophoretic-taxonomic studies was apparent in S. hispidus. Populations, especially those from such a ubiquitous wide-ranging species, have had their gene pool considerably altered through various selective pressures. This has resulted in considerable phenotypic variation in the blood serum proteins, negating the possibility of establishing a pattern common to all S. hispidus. Other species of Sigmodon which are more restricted in range and habitat logically demonstrate less geographic variation. For example, the serum proteins of two widely separated (approx. 450 km.) population samples of S. leucotis, a species restricted to a narrow, elongated, montane habitat, present a high degree of similarity in electrophoretic blood serum patterns. The remaining species from different localities are intermediate between S. hispidus and S. leucotis in the degree of variability shown in their blood sera. In this case, a "species pattern" or "species curve" does not consist of all or a majority of the protein components, but only those few which are common throughout the species.

The presence of four transferrins (five in two Autlán specimens) characterizes the population samples from México and western Texas (Lubbock). According to Hooper (1949), Sigmodon is presumed to be primarily a tropical American form that has lately, Pliocene to Recent, moved into both North and South America. A continuing northward spread of the cotton rat into Kansas and Nebraska in this century was noted by Cockrum (1948) and Jones (1964). The geological record shows that in

the upper Pliocene and Lower Pleistocene the genus extended as far northward as Kansas (Hibbard, 1960:17). In the Wisconsin glaciation cotton rats are presumed to have been displaced southward, perhaps into two refugia: peninsula Florida and the American Southwest (see Blair, 1958:460). After the retreat of the last glacier, allowing the southern plains sector of the United States to become suitable again for occupancy by cotton rats, evidence from the serological relationships indicates that the related Floridan and Southwestern populations may not have rejoined each other in this central region. Instead, less-related hispid cotton rats from the Mexican Plateau (now called S. h. berlandieri) moved northward (at least as far as Lubbock, Texas) into this intervening space. This suggested means of repopulation has subsequently led to the establishment of the present-day west-Texas hiatus in the range of the United States transferrin type.

The status of several species of Sigmodon is questionable, and as Hall and Kelson (1959) pointed out, many of the named kinds that now stand as species will prove to be only subspecies of more wide-ranging species. However, they followed the classification as given by Bailey (1902) and Nelson and Goldman (1933). This study suggests, on the basis of the serum protein patterns, that S. fulviventer and S. melanotis are closely related, perhaps conspecific. Also, S. alleni and S. planifrons are indistinguishable, and it is suggested that these two forms may also be conspecific. Based on the blood serum protein electrophoretic characteristics of Sigmodon, an artificial key to separate the different species is as follows:

1. Two stable prealbumins (Pa_1, Pa_2) with R_m=1.06 and 1.11, respectfully; 4 confluent transferrins. . . S. leucotis.

1' One stable prealbumin (Pa_1), transferrins not as above 2

2. Stable prealbumin (Pa_1) with R_m=1.08; transferrins in several combinations, but not 1 + 2 or 1 + 3 with D_m= .057, or 3 confluent transferrins with a lesser 4th component confluent with transferrin farthest from origin. 3

2' Stable prealbumin (Pa_1) with R_m not 1.08; transferrins appearing as 1 + 2 or possibly 1 + 3, or 3 confluent transferrins plus a lesser component, as described above. 4

3. Three transferrins, 1 + 2 combination with D_m=.035. . . . . .S. alleni-planifrons.

3' If three transferrins, not as above. . . . .S. hispidus.

4. Stable prealbumin (Pa_1) with R_m=1.07; 1 + 2 transferrins with D_m=.057, a lesser 3rd component may be present and confluent with transferrin farthest from origin . . . . . S. ochrognathus.

4' Stable prealbumin (Pa_1) with R_m=1.10; 3 confluent transferrins, a lesser 4th component confluent with transferrin farthest from origin. . . . . .S. fulviventer-melanotis.

Sigmodon is first reported from the Blancan of the Upper Pliocene (Hibbard, op. cit.). The modern species, S. hispidus is

recognized as early as Sangamon times (third interglacial) in the Pleistocene Moore Pit local fauna (Slaughter, 1966). Using S. hispidus as the ancestral stock a diagramatic representation of Sigmodon relationships based on the electrophoretic results of the blood serum proteins is shown in Figure 10. Sigmodon leucotis, with two stable prealbumins (Pa_1 and Pa_2) and a lower number of postalbumins (4), appears to be the most specialized of the cotton rats. Sigmodon ochrognathus and S. fulviventer-melanotis are intermediate with one stable prealbumin (Pa_1) and a lower number of postalbumins (4, 2-4, respectfully). The closest relationships to S. hispidus are found in S. alleni-planifrons, since the one stable prealbumin (Pa_1) and also the number of postalbumins appear to be identical to that found in the former.

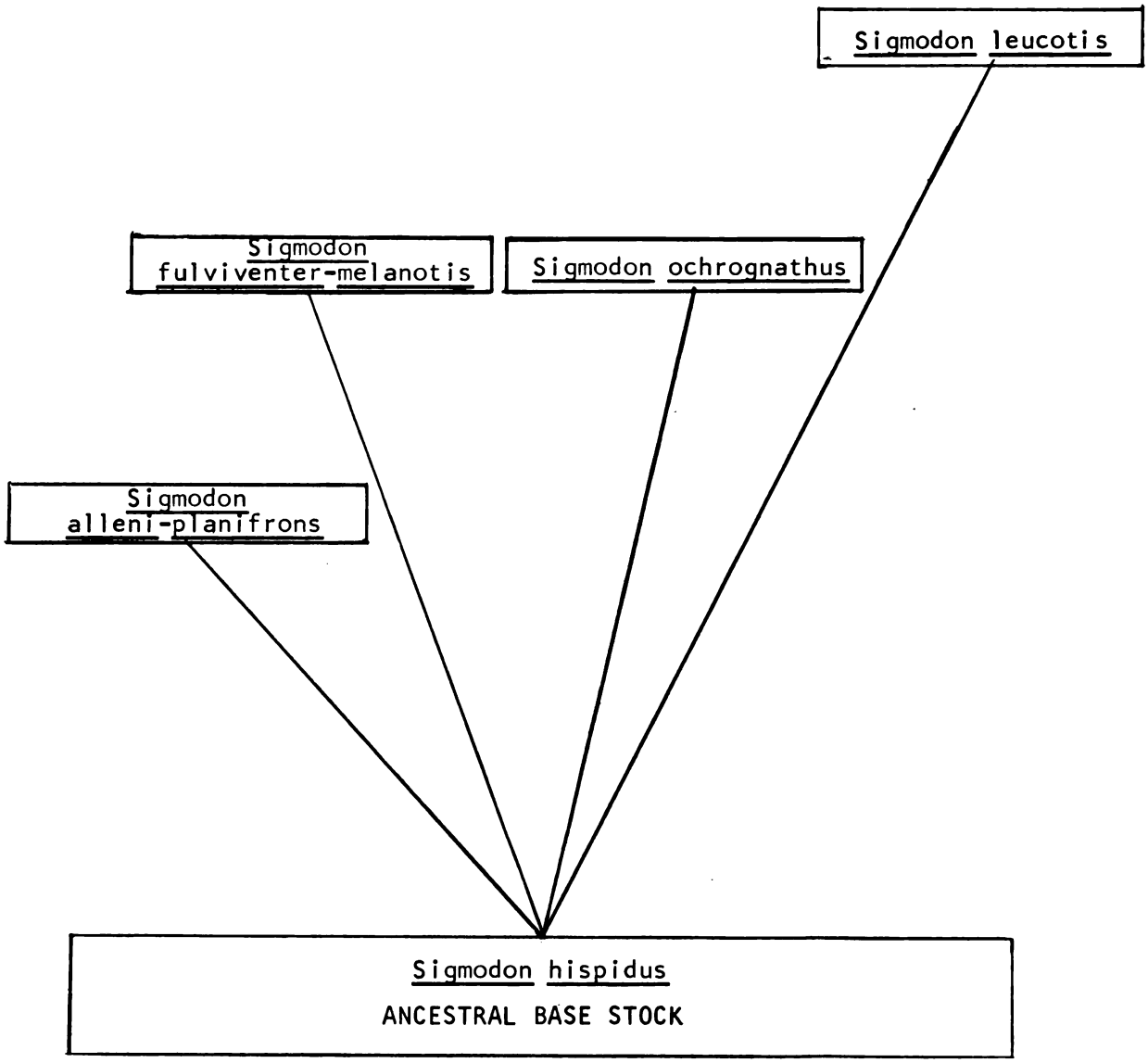


Figure 10. A diagramatic sketch indicating possible lines of evolution in Sigmodon.

SUMMARY

Blood serum proteins from seven species, totaling 156 specimens, of laboratory acclimated cotton rats, Sigmodon, from the United States, México, Honduras, and Panamá were separated by the acrylamide dis electrophoresis technique of Ornstein and Davis (1961), Davis (1964), and modified by Wright and Mallmann (1966). The resulting protein patterns in the acrylamide gel and their corresponding densitometric tracings were analyzed to determine the amount of variation between individuals within a population sample, between population samples of the same species from different geographical localities, and between species.

The electrophoretic serum patterns of some population samples within a species were distinctive. Other samples were not distinguishable from each other either because of the variability within the individual population samples or because of close similarity with other population samples. One case of transferrin polymorphism within a single population was noted, suggesting the occurence of a limited gene exchange. Between certain geographically-separated population samples of S. hispidus, transferrin polymorphism, consisting of five different phenotypes (transferrin patterns A,B,C,D, and E), was observed to follow a definite arrangement which allowed for quick regional identification.

Because of such polymorphism, no form of a "species curve" or "species pattern" was apparent in the wide ranging, ubiquitous S. hispidus, but was present in a species such as S. leucotis, a spatially and ecologically restricted cotton rat.

Differences between species were evidenced in the transferrins, some of the prealbumins, and to a lesser degree, in the postalbumin

components in the blood sera. On this basis S. hispidus, S. leucotis and S. ochrognathus could be distinguished from each other while S. fulviventer and S. melanotis appeared conspecific as did S. alleni and S. planifrons. A table and key for electrophoretically differentiating the studied species and a diagram of their possible relationships are presented.

LITERATURE CITED

Ahl, A. S.
1968. Electrophoretic examination of hemoglobin and plasma proteins from three altitude groups of Peromyscus maniculatus nebrascensis. Comp. Biochem. Physiol. 24:427-435.

Ashton, G. C.
1958. Serum protein variations in horses. Nature 182:1029-1030.

Ashton G. C. and A. W. H. Braden.
1961. Serum β-globulin in polymorphism in mice. Aust. J. Biol. Sci. 14:248-253.

Auernheimer, A. H., W. Cutter, and F. O. Atchley.
1960. Electrophoretic studies on blood serum proteins of rodents. Jour. Mamm. 41:405-407.

Bailey, V.
1902. Synopsis of the North American species of Sigmodon. Proc. Biol. Soc. Washington, 15:101-116.

Baker, R. H.
1963. Geographical distribution of terrestrial mammals in Middle America. Amer. Midl. Nat., 70: 208-249, 1 fig.

Baker, R. H. and J. K. Greer.
1962. Mammals of the Mexican state of Durango. Michigan State Univ., Publ. Mus., Biol. Ser. 2:25-154.

Blair, W. F.
1958. Distributional patterns of vertebrates in the southern United States in relation to past and present environments. In C. L. Hubbs, ed. Zoogeography. Amer. Assoc. Adv. Sci. pp.433-468.

Brown, J. H. and C. F. Welser.
1968. Serum albumin polymorphisms in natural and laboratory populations of Peromyscus. J. Mamm. (in press).

Cockrum, E. L.
1948. The distribution of the hispid cotton rat in Kansas. Trans. Kansas Acad. Sci. 51:306-312.

Davis, B. J.
1964. Disc electrophoresis-II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. 121:404-427.

Engle, R. L. Jr., and K. R. Woods.
1960. Comparative biochemistry and embryology. In F. W. Putnam, ed. The plasma proteins. Academic Press, New York. 2:183-265.

Goodman, M., A. Kulkarni, E. Poulik, and E. Reklys.
1965. Species and geographic differences in the transferrin polymorphism of macaques. Sci. 147:884-886.

Hall, E. R., K. R. Kelson.
1959. The mammals of North America. Ronald Press Co., New York, 2:viii 547-1083.

Hibbard, C. W.
1960. An interpretation of Pliocene and Pleistocene climates in North America. 62nd Annual Report, Mich. Acad. Sci., Arts, Ltrs., 1959-1960, pp.5-30.

Hooper, E. T.
1949. Faunal relationships of recent North American rodents. Univ. Mich., Misc. Publ. Mus. Zool., 72:1-28.

Johnson, M. L.
1968. Applications of blood protein electrophoretic studies to problems in mammalian taxonomy. Syst. Zool. 17:23-30.

Johnson, M. L. and M. J. Wicks.
1959. Serum protein electrophoresis in mammals—taxonomic implications. Syst. Zool. 8:88-95.

Johnson, M. L. and M. J. Wicks.
1964. Serum-protein electrophoresis in mammals: significance in the higher taxonomic categories. In C. A. Leone, ed. Taxonomic biochemistry and serology. Ronald Press Co. New York. x 728 pp. illus.

Jones, J. K.
1964. Distribution and taxonomy of mammals of Nebraska. Univ. Kansas Publ., Mus. Nat. Hist. 16:1-356.

Kristjansson, F. K.
1963. Genetic control of two pre-albumins in pigs. Genetics 48: 1059-1063.

Longworth, L. G.
1959. Moving boundry electrophoresis-practice. In M. Bier, ed. Electrophoresis, theory, methods and applications, Academic Press, Inc., New York. pp.91-136.

Moore, D. H.
1945. Species differences in protein patterns. Jour. Biol. Chem. 161:21-32.

Moore, D. H.
1959. Clinical and physiological applications of electrophoresis. In M. Bier, Electrophoresis, theory, methods, and applications, Academic Press, Inc. New York. pp.369-425.

Nadler, C. F. and C. H. Hughes.
1966. Serum protein electrophoresis in the taxonomy of some species of the ground squirrel subgenus Spermophilus. Comp Biochem. Physiol. 18:639-651.

Nelson, E. W. and E. A. Goldman.
1933. Three new rodents from southern México. Proc. Biol. Soc. Washington, 46:195-198.

Ornstein, L. and B. J. Davis.
1962. Disc electrophoresis. Parts I and II. Distillation Products Industries. Rochester, New York. 22 pp.

Petermann, M. L.
1960. Alterations in plasma protein patterns in disease. In F. W. Putnam, ed. The plasma proteins. Academic Press Inc., New York. 2:309-343.

Petersen, M. K.
1966. Electrophoretic blood serum patterns in selected species of Peromyscus. Thesis for M.S. degree, Dept. Zool., Michigan State University. 43 pp.

Petersen, M. K.
1968. Electrophoretic blood-serum patterns in selected species of Peromyscus. Amer. Midl. Natur. 79:130-148.

Popp, R. A. and D. M. Popp.
1962. Inheritance of serum esterases having different electrophoretic patterns among inbred strains of mice. J. Heredity 53:111-114.

Rasmussen D. I.
1964. Blood group polymorphism and inbreeding in natural populations of the deer mouse Peromyscus maniculatus. Evol. 18:219-229.

Riley, V.
1960. Adaption of orbital bleeding technique to rapid serial blood studies. Proc. Soc. Expt. Biol. & Med. 104:751-754.

Russell, M. A. and R. Semeonoff.
1967. A serum esterase variation in Microtus agrestis (L.). Genet. Res. 10:135-142.

Sargent, J. R.
1965. Methods in zone electrophoresis. British Drug Houses Ltd. Poole, Dorset, England. 107 pp.

Shaw, C. R.
1965. Electrophoretic variation in enzymes. Sci. 149:936-943.

Slaughter, B. H.
1966. The Moore Pit local fauna; Pleistocene of Texas. Jour. Paleontology, 40:78-91.

Welser, C. F., H. J. Winkelmann, E. B. Cutler, and E. Barto.
1965. Albumin variations in the white-footed mouse, Peromyscus. Genetics. 52:483.

Wright, G. L. Jr., and W. L. Mallmann.
1966. Differential disc electrophoresis of serum proteins. Proc. Soc. Expt. Biol. & Med. 123:22-27.

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