# BIOCHEMICAL POLYMORPHISM AND SYSTEMATICS IN THE GENUS *PEROMYSCUS*. VI. THE *BOYLII* SPECIES GROUP

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ABSTRACT.—An analysis of electrophoretic variation in proteins encoded by 21 genetic loci in 275 individuals belonging to the *Peromyscus boylii* species group yielded the following systematic conclusions. Populations of *P. boylii rowleyi* and *P. b. levipes* in four Mexican states and four states in the southwestern United States, separated by up to 3000 kilometers, share common alleles at virtually all loci, and thus show no evidence of representing more than one species. *P. (b.) attwateri* from Arkansas differs from *P. boylii* in allelic composition at several loci, and in all probability represents a distinct species, as suggested by other authors on the basis of morphology and karyotype. *P. stephani* from the Gulf of California is very similar genically to *P. boylii*, and, on the basis of this and other evidence, should be removed from the subgenus *Haplomylomys* and placed in the *boylii* species group of the subgenus *Peromyscus*. Populations referable to *P. pectoralis* from Ciudad Victoria, Mexico, Ranger, Texas, and Big Bend, Texas, show considerable allelic differences from one another but form a single cluster in a dendrogram of biochemical similarities. The nature of the allelic differences suggests that two or more species currently are classified as *P. pectoralis*.

Of the approximately 60 named species of *Peromyscus*, only *P. maniculatus* and *P. leucopus* are more widely distributed than the brush mouse, *P. boylii*. Populations conventionally assigned to about 15 subspecies of *P. boylii* are distributed from Honduras northward to California in the west and to Missouri and Arkansas in the east (Fig. 1). Because several of the presumed subspecies are strongly differentiated morphologically, Hooper (1968) believed that "it is extremely doubtful that all of the populations known by the name *P. boylii* are conspecific." Recent chromosomal (Lee *et al.*, 1972) and morphological (Schmidly, 1973) evidence has suggested that certain populations in Texas, Oklahoma, Kansas, Missouri, and Arkansas, previously named *P. b. attwateri*, are specifically distinct from more western populations designated as *P. b. rowleyi*. Several forms apparently retain their identities where they are sympatric in the central highlands of Mexico. In particular, the relationships of the central Mexican subspecies *P. b. levipes* to surrounding forms, *P. b. rowleyi*, *P. (b.) aztecus*, *P. (b.) evides*, *P. b. ambiguus*, and *P. b. spicilegus*, remain undetermined.

In this study, we have examined electrophoretic variation at 21 genetic loci in 226 specimens of *P. boylii* complex from various parts of its range. We have also included analyses of *P. stephani* and three populations of *P. pectoralis*, other presumed members of the *boylii* species group. Our primary objectives are (1) to provide genetic information relevant to the problem of determining the taxonomic status of populations within the *P. boylii* complex, and (2) to determine affinities of members of the *P. boylii* group to other species groups of *Peromyscus*. *Peromyscus boylii* and *P. pectoralis* are added to a growing

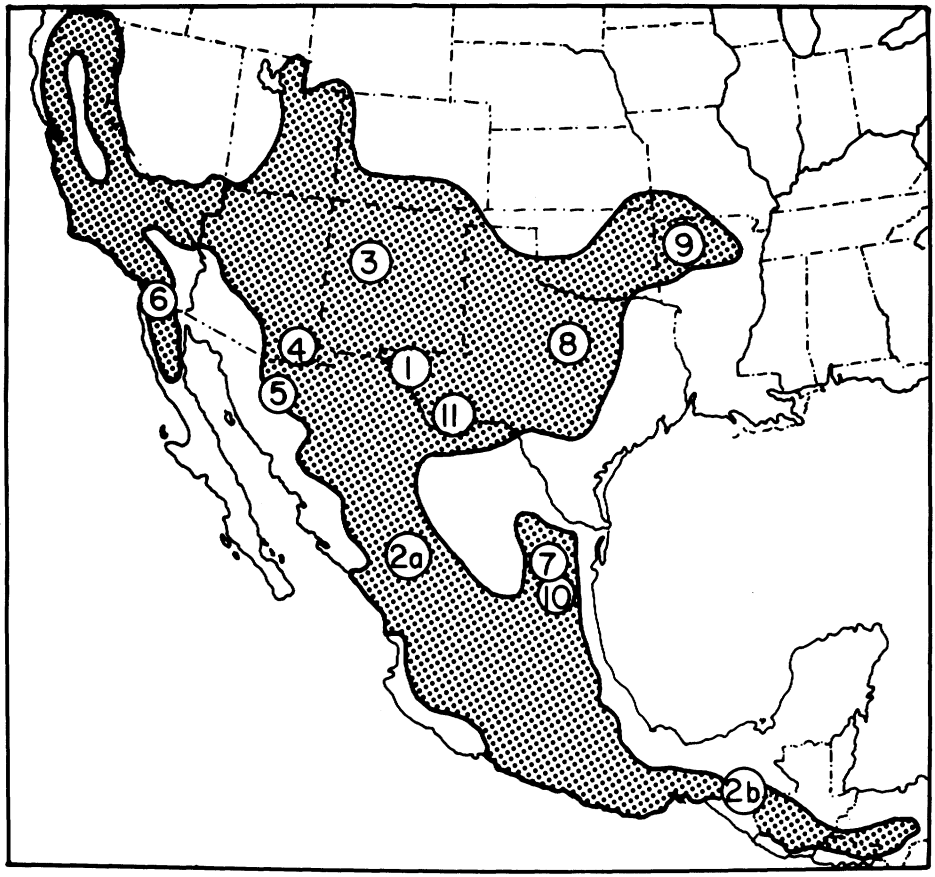


FIG. 1.—Sample localities for members of the *boylii* species group. Numbers correspond to those in Table 1.

biochemical dendrogram (Avise *et al.*, 1974), which now includes 15 named species of the genus *Peromyscus*.

## MATERIALS AND METHODS

Specimens of the *Peromyscus boylii* complex were collected at the following localities in the United States and Mexico: (1) Franklin's Mtn., El Paso, Texas (N = 15 specimens); (2) 25 mi. W Durango, Mexico, on hwy. 40 (N = 20); (3) 22.9 mi. W Durango, Mexico, on hwy. 40 (N = 9); (4) 6.2 mi. NNW Jitotol, Chiapas, Mexico (N = 2); (5) eastern city limits of Albuquerque, New Mexico (N = 3); (6) 2.0 mi. W Tajique, New Mexico (N = 10); (7) Barefoot Lookout, 11.4 mi. W Portal, Arizona (N = 13); (8) 6.9 mi. W Portal, Arizona (N = 4); (9) 7.1 mi. W Portal, Arizona (N = 19); (10) Texas Canyon, E of Benson, Arizona, on IH-10 (N = 6); (11) 4.7 mi. N Cumeral on hwy. 15, Sonora, Mexico (N = 28); (12) 4.5 mi. N Cumeral on hwy. 15, Sonora, Mexico (N = 30); (13) 3.5 mi. N Mt. Laguna on hwy. 51, San Diego Co., California (N = 20); (14) 2.5 mi. N Mt. Laguna on hwy. 51, San Diego Co., California (N = 27); (15) 7.0 mi. W Ciudad Victoria on hwy. 101, Tamaulipas, Mexico (N = 6); (16) 1.1 mi. W Fayetteville, Arkansas, on hwy. 62. In

TABLE 1.—*Samples of the* boylii *species group of the subgenus* Peromyscus. *Estimates of genic variability are based on 20 loci. Subspecies names are those given by Hall and Kelson (1959) and Schmidly (1973).*

| Sample number | Number of individuals | Species | Subspecies | Collection area | Mean percentage of loci | |
|---|---|---|---|---|---|---|
| | | | | | Heterozygous per individual | Polymorphic per population[a] |
| 1 | 15 | *P. boylii* | *rowleyi* | El Paso, Texas | 2.2 | 0.10 |
| 2[a] | 29 | *P. boylii* | *rowleyi* | Durango, Mexico (2 sites) | 2.0 | 0.05 |
| 2[b] | 2 | *P. boylii* | *levipes* | Chiapas, Mexico[b] | 2.4 | 0.05 |
| 3 | 13 | *P. boylii* | *rowleyi* | Albuquerque and Apache Canyon, New Mexico | 3.0 | 0.05 |
| 4 | 42 | *P. boylii* | *rowleyi* | Portal and Texas Canyon, Arizona | 2.6 | 0.10 |
| 5 | 58 | *P. boylii* | *rowleyi* | Northern Sonora, Mexico (2 sites) | 0.8 | 0.05 |
| 6 | 47 | *P. boylii* | *rowleyi* | San Diego Co., California (2 sites) | 0.9 | 0.05 |
| 7 | 6 | *P. boylii* | *ambiguus* | Ciudad Victoria, Mexico | 3.1 | 0.14 |
| 8 | 4 | *P. attwateri* | | Fayetteville, Arkansas | 1.2 | 0.05 |
| 9 | 25 | *P. pectoralis* | *laceianus* | Ranger, Texas (2 sites) | 3.0 | 0.05 |
| 10 | 7 | *P. pectoralis* | *collinus* | Ciudad Victoria, Mexico | 2.0 | 0.14 |
| 11 | 3 | *P. pectoralis* | *laceianus* | Big Bend, Texas | 1.6 | 0.05 |

[a] Polymorphic if frequency of common allele $\leq 0.95$.
[b] From the laboratory colony of John King.

TABLE 2.—*Biochemical similarity coefficients between samples of* P. boylii, P. pectoralis, *and* P. stephani *calculated by Roger's (1972) formula. These values were included in a larger matrix used to form the dendrogram of 15* Peromyscus *species in Fig. 3.*

| Sample number | Species | Subspecies | Sample number | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 1 | *P. boylii* | *rowleyi* | 1.00 | .96 | .95 | .96 | .95 | .97 | .89 | .80 | .64 | .72 | .60 | .85 |
| 2 | *P. boylii* | *rowleyi* | | 1.00 | .99 | .99 | .98 | .96 | .93 | .81 | .63 | .71 | .60 | .86 |
| 3 | *P. boylii* | *levipes* | | | 1.00 | .99 | .98 | .95 | .93 | .81 | .63 | .71 | .60 | .86 |
| 4 | *P. boylii* | *rowleyi* | | | | 1.00 | .98 | .95 | .93 | .81 | .63 | .71 | .60 | .86 |
| 5 | *P. boylii* | *rowleyi* | | | | | 1.00 | .94 | .94 | .81 | .62 | .70 | .60 | .86 |
| 6 | *P. boylii* | *rowleyi* | | | | | | 1.00 | .91 | .81 | .63 | .71 | .60 | .85 |
| 7 | *P. boylii* | *levipes* | | | | | | | 1.00 | .80 | .68 | .71 | .65 | .80 |
| 8 | *P. attwateri* | | | | | | | | | 1.00 | .53 | .66 | .58 | .71 |
| 9 | *P. pectoralis* | *laceianus* | | | | | | | | | 1.00 | .75 | .84 | .67 |
| 10 | *P. pectoralis* | *collinus* | | | | | | | | | | 1.00 | .79 | .65 |
| 11 | *P. pectoralis* | *laceianus* | | | | | | | | | | | 1.00 | .64 |
| 12 | *P. stephani* | | | | | | | | | | | | | 1.00 |

addition, samples of *P. pectoralis* were collected (1) 3.0 mi. S Ranger, Texas (N = 14); (2) 7.0 mi. S Ranger, Texas (N = 11); (3) 7.0 mi. W Ciudad Victoria, hwy. 101, Tamaulipas, Mexico (N = 7); and (4) 100 yds. N Big Bend National Park, Texas, on hwy. 385 (N = 3).

Mice were live-trapped and shipped to the laboratory in Austin for processing. Techniques of preparation of tissue extracts, electrophoresis, and protein staining are described by Selander *et al.* (1971); methods of designating alleles are discussed by Smith *et al.* (1973) and Avise *et al.* (1974).

When small samples of *P. boylii* from nearby localities were not demonstrably heterogeneous at any of the polymorphic loci, they were pooled to form larger samples numbered in Table 1. The pooled collections were treated as operational taxonomic units (OTU's) for similarity comparisons. In addition, biochemical information on several previously studied species of *Peromyscus* was used in a comparative analysis of genic similarity. These species are as follows; *P. polionotus* (Selander *et al.*, 1971); *P. floridanus* (Smith *et al.*, 1973); *P. leucopus* and *P. gossypinus* (Smith *et al.*, in preparation); *P. sejugis, P. stephani, P. eremicus,* and six other species of the subgenus *Haplomylomys* (Avise *et al.*, 1974). Allele frequencies for each species were calculated from pooled samples.

Coefficients of genic similarity between OTU's were based on allele frequencies at 21 loci and were calculated according to Rogers' (1972) formula. The resulting matrix of similarity coefficients (Table 2) was then subjected to an unweighted pair group method of cluster analysis, using arithmetic means (Sokal and Sneath, 1963). Heterozygosity values (based on 20 loci) for members of the *boylii* species group were obtained by direct counts of numbers of loci heterozygous in each individual.

## RESULTS

*Genic heterozygosity.*—Individuals of *P. boylii* and *P. pectoralis* have an average of 2.1 per cent of their loci in heterozygous condition (Table 1). This estimate of genic heterozygosity is considerably lower than the mean of about 6 per cent reported for many vertebrates, including other species of *Peromyscus* (Selander and Johnson, 1973). However, the sample of loci on which our estimate is based includes only a single esterase, as opposed to as many as seven esterase loci scored in *P. polionotus* and other species. Since esterases are an unusually variable class of enzymes (Selander and Johnson, 1973), our esti-

TABLE 3.—*Alleles at 22 loci in samples of* Peromyscus boylii *and* P. pectoralis. *Alleles are designated according to proportional electrophoretic mobility relative to the common* P. polionotus *allele. Protein abbreviations are those of Selander* et al., *(1971). Frequencies of alleles at polymorphic loci are given in parentheses; all other loci are monomorphic. All populations are monomorphic for the following:* Ldh-$1^{100}$, Mdh-$2^{-100}$, Sdh-$1^{100}$, Ipo-$1^{100}$, Got-$2^{-64}$, Ept-$A^{100}$, Ept-$B^{100}$, *and* Ept-$C^{-100}$. *Hemoglobins are scored according to phenotype.*

| Species and sample number | Es-6 | Ldh-2 | Mdh-1 | Idh-1 | G-6-Pd | Pgd-1 | Gpd-1 | Pgm-1 | Pgi-1 | Got-1 | Trf-1 | Alb-1 | HB |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *P. boylii* | | | | | | | | | | | | | |
| 1 | 100 | 100 | 100 | 110 | 93 | 139 | 125(.73)<br>142(.27) | 100 | —100 | 130 | 113 | 97(.87)<br>100(.13) | 2 |
| 2 | 100 | 100 | 100 | 110 | 93 | 139 | 125 | 100(.98)<br>132(.02) | —100 | 130(.98)<br>136(.02) | 113 | 97(.34)<br>100(.66) | 2 |
| 3 | 100 | 100 | 100(.96)<br>72(.04) | 110 | 93 | 139 | 125 | 100(.96)<br>68(.04) | —100 | 130(.96)<br>136(.04) | 113 | 97(.23)<br>100(.77) | 2 |
| 4 | 100 | 100 | 100 | 110(.99)<br>100(.01) | 93 | 139 | 125(.95)<br>142(.04)<br>100(.01) | 100(.99)<br>132(.01) | —100 | 130 | 113 | 97(.27)<br>100(.72)<br>95(.01) | 2 |
| 5 | 100 | 100 | 100 | 110(.95)<br>88(.05) | 93 | 139 | 125 | 100 | —100 | 130 | 113 | 100(.96)<br>97(.04) | 2 |
| 6 | 100 | 100(.98)<br>162(.02) | 100 | 110 | 93 | 139 | 125 | 100 | —100 | 130 | 113(.79)<br>105(.21) | 97 | 2 |
| 7 | 100 | 100 | 100 | 110 | 93 | 139(.92)<br>126(.08) | 125 | 100 | —100 | 130(.92)<br>100(.08) | 105 | 100(.92)<br>95(.08) | 2<br>2 |
| *P. attwateri* | | | | | | | | | | | | | |
| 8 | 101 | 100 | 100 | 106 | 93 | 139 | 125 | 100 | —100 | 130 | 88 | 103 | 2 |
| *P. pectoralis* | | | | | | | | | | | | | |
| 9 | 100 | 100 | 100 | 110 | 100 | 124 | 142 | 100(.98)<br>132(.02) | 5 | 100 | 105 | 103.5(.50)<br>101(.50) | 8 |
| 10 | 100 | 100(.93)<br>162(.07) | 100(.93)<br>72(.07) | 110(.86)<br>100(.14) | 93 | 126 | 142 | 100 | 5 | 100 | 104 | 103 | 5 |
| 11 | 100 | 100 | 100 | 110(.50)<br>100(.50) | 100 | 126 | —[a] | 100 | 5 | 100 | 105 | 103 | 8 |

[a] No activity.

mates of heterozygosity in the *P. boylii* species group may be biased downward, relative to published values for other *Peromyscus* species.

Large proportions of the total genic variability in samples of *P. boylii* and *P. pectoralis* are contributed by two loci, albumin and isocitrate dehydrogenase-1. Several other loci are highly variable but could not be consistently scored; these include alcohol dehydrogenase, isocitrate dehydrogenase-2, malic enzyme, and at least one other esterase. Rasmussen and Jensen (1971) have previously reported polymorphism in serum esterases in *P. boylii.*

*Allozymic variation.*—Allele frequencies at 21 loci in samples of *P. boylii* and *P. pectoralis* are presented in Table 3. Common alleles for other species of *Peromyscus* are given by Avise *et al.* (1974). Because banding patterns and tissue specificities of proteins for *P. boylii* and *P. pectoralis* are similar to those previously described in other *Peromyscus* species (Selander *et al.*, 1971; Smith *et al.*, 1973; Avise *et al.*, 1974), we need not discuss them in detail here.

Although subunits of mammalian hemoglobin are known to be encoded by at least two genetic loci, we have scored hemoglobin zymograms according to phenotype and have conservatively considered the types as products of a single locus in calculating similarities. We did not observe intrasample variation in hemoglobin, but hemoglobin polymorphism has been reported in samples of *P. boylii rowleyi* from Arizona (Rasmussen, 1968). The hemoglobin pattern exhibited by *P. b. rowleyi, P. b. levipes,* and *P. attwateri* is the same as that in *P. stephani* and corresponds to that described by Foreman (1968). It differs from patterns represented in *P. pectoralis* (Fig. 2).

Almost without exception, populations of *P. boylii levipes* and *P. b. rowleyi* share common alleles at all loci (Table 3). Similarity values between these populations invariably are greater than 0.89 ($\bar{S} = 0.95$) on a scale from 0 to 1.0, where 1.0 indicates genetic identity. Populations of *P. attwateri* from Arkansas and of *P. pectoralis* from three widely separated collecting sites (Ciudad Victoria, Mexico, Ranger, Texas, and Big Bend, Texas) all show considerable genic differences from one another and from the *P. b. levipes-rowleyi* assemblage. In no case are similarity values between these taxa greater than 0.84, and the average value is 0.69 (Table 2). Thus, at least five distinct genic groups appear to be represented by our samples of *P. boylii* and *P. pectoralis* (sample numbers 1 to 7, 8, 9, 10, and 11 in Table 1).

Various members of the above groups are essentially monomorphic for different alleles at several loci (Fig. 2). For example, *P. attwateri* differs from the other groups in allelic composition at the esterase-6 locus, although in overall genic character it is most similar to *P. b. rowleyi-P. b. levipes* and is markedly different from all samples of *P. pectoralis* (Table 2). Also, the sample of *P. pectoralis* from Mexico shares the glucose-6-phosphate dehydrogenase allele of *P. boylii,* which is different from the common allele in other *P. pectoralis* samples. Because information on single loci may greatly distort overall estimates of relationships, taxonomic decisions should be based on patterns of variation in a large number of protein systems.

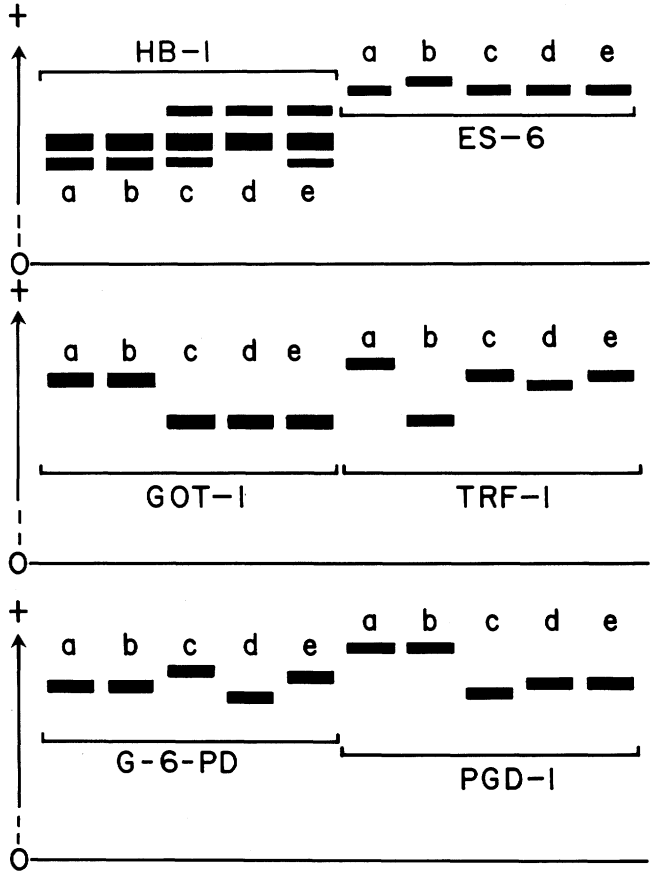


FIG. 2.—Zymogram patterns for six proteins in populations of the *boylii* species group. All individuals in given groups showed identical patterns. a, *P. boylii;* b, *P. attwateri;* c, *P. pectoralis*, Ranger, Texas; d, *P. pectoralis*, Ciudad Victoria, Mexico; e, *P. pectoralis*, Big Bend, Texas.

A dendrogram resulting from cluster analysis of biochemical similarity coefficients for 15 named *Peromyscus* species is shown in Fig. 3. *P. stephani* (an insular endemic) clusters closest to the *P. boylii rowleyi-P. b. levipes* assemblage. The *P. attwateri* sample joins this cluster at a much lower level of similarity, $S = 0.79$. The three samples of *P. pectoralis* cluster together, although the branching points of this cluster indicate that these populations are quite distinct; they are no more similar to one another than is *P. stephani* to *P. b. rowleyi-P. b. levipes.*

With the exception of *P. stephani,* the relative positions of other *Peromyscus* species have not changed from our previous dendrogram (Avise *et al.*, 1974), with the addition of *P. boylii* and *P. pectoralis.* Absolute branching points

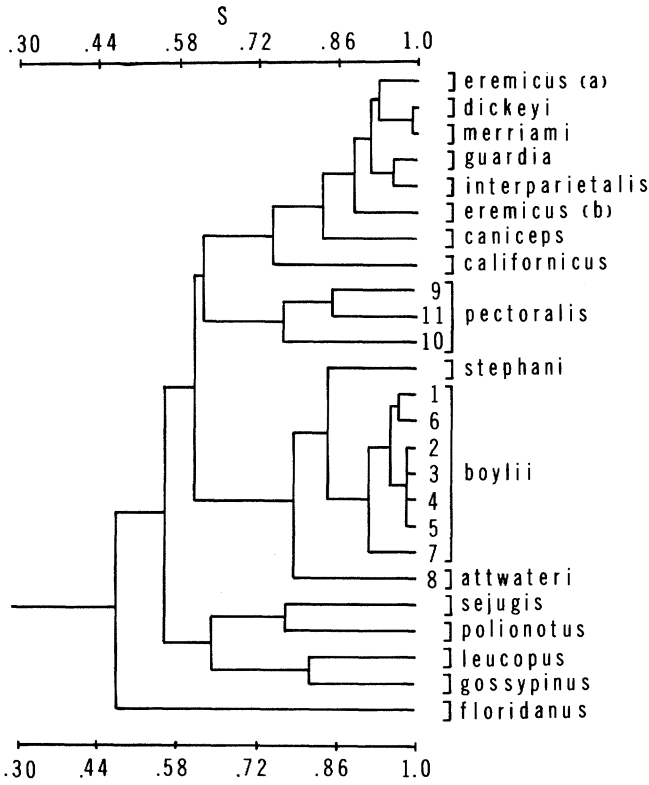


FIG. 3.—Biochemical similarity dendrogram of *Peromyscus*.

within the dendrogram have changed slightly because different numbers of loci were used in the calculations of similarities. All members of the subgenus *Haplomylomys* form a single large cluster. *Peromyscus sejugis* is most similar to *P. polionotus;* both are members of the *maniculatus* species group in the subgenus *Peromyscus*. The sibling species *P. leucopus* and *P. gossypinus* are very similar and join other members of the subgenus *Peromyscus* in a larger cluster. *Peromyscus floridanus,* subgenus *Podomys,* exhibits the lowest degree of similarity to other species of the genus. More detailed discussions of the genetic relationships of these previously studied *Peromyscus* species are given by Avise *et al.* (1974). Almost without exception, the biochemical information supports systematic conclusions based on morphological, chromosomal, and ecological evidence.

## DISCUSSION

*Peromyscus boylii.*—Samples of *P. boylii* from California, New Mexico, western Texas, and Arizona are very similar genically, as are those from Sonora, Durango, Tamaulipas, and Chiapas, Mexico. These populations are referable

to three described subspecies, *P. b. rowleyi, P. b. ambiguus,* and *P. b. levipes.* Some of these populations are separated by more than 3000 kilometers, and yet in almost all cases they share the same common alleles at all loci; overall biochemical similarity between populations remains high ($\bar{S} = 0.95$). This pattern of close biochemical similarity between widely separated geographic populations probably is typical of the situation for "good" species, even when populations occupy a considerable range of habitats. For example, samples of *P. leucopus* taken anywhere in North America are easily recognized biochemically, and similarity coefficients (based on more than 20 loci) between populations are always greater than 0.90 (Smith *et al.,* in preparation). A similar situation holds for populations of *P. polionotus* (Selander *et al.,* 1971), *P. gossypinus* (Smith *et al.,* in preparation), and *P. eremicus* (Avise *et al.,* 1974), as well as for many other vertebrates (Johnson and Selander, 1971; Avise and Smith, 1974) and invertebrates (Prakash *et al.,* 1969; Ayala *et al.,* 1972). Similarity coefficients between conspecific populations are almost always greater than 0.80 and are usually in the 0.90's (review in Selander and Johnson, 1973).

Whether such information provides evidence of "a tight cohesion of coadapted gene pools" (Selander *et al.,* 1969) is debatable, but, at any rate, it is extremely pertinent to systematic studies. Because similarity values between populations of *P. b. rowleyi, P. b. ambiguus,* and *P. b. levipes* are well within the range typical for conspecific populations, our evidence is compatible with the view that populations referable to these subspecies are conspecific.

Lee *et al.* (1972) described karyotypes of four specimens tentatively referred to *P. b. levipes* from Jiquilpan, Michoacan. The number of autosomal arms in the chromosome complement was 54, as opposed to 52 in samples of *P. b. rowleyi* across its range. This difference between the forms is less than that between *P. b. rowleyi* and *P. attwateri* (see beyond), which are probably specifically distinct (Lee *et al.,* 1972).

Schmidly (1973) found mean differences in several morphological characters between *P. b. levipes* from northeastern Mexico and *P. b. rowleyi.* The lack of chromosomal evidence for populations of *P. b. levipes* where they meet *P. b. rowleyi* forced him to regard both *rowleyi* and *levipes* as subspecies of *P. boylii.* Our sample of *P. b. levipes* from Ciudad Victoria, Mexico, is monomorphic for a transferrin allele ($Trf\text{-}1^{105}$) that was not observed in the other samples of *P. b. levipes.* However, this allele is common in samples of *P. b. rowleyi* from southern California.

The problem of determining the status of *P. b. levipes* is complicated by the fact that there is considerable intralocality variation in several morphological traits (Alvarez, 1961). Osgood (1909) suspected that two species were present. Patterns of genic variation within and between our populations of *P. b. rowleyi* and *P. b. levipes* provide no strong evidence that we have examined two species. However, caution is indicated, for we have recently found cases in which recognized species of *Peromyscus* are very similar genically. For example, the sibling species *P. merriami* and *P. eremicus* show $\bar{S} = 0.89$ where they occur

sympatrically, although they exhibit nearly complete differences in allelic composition at two loci (Avise *et al.*, 1974).

*Peromyscus attwateri.*—Although formerly considered a subspecies of *P. boylii, attwateri* was recently raised to species rank (Schmidly, 1973). The range of *P. attwateri* includes most of Oklahoma and parts of Kansas, Missouri, Arkansas, and central Texas. Its chromosome complement includes three pairs of large biarmed and 18 pairs of acrocentric chromosomes, whereas the *P. b. rowleyi-boylii-utahensis* assemblage has one pair of large biarmed and 20 pairs of acrocentric chromosomes (Lee *et al.*, 1972). Differences are apparent in the sex chromosomes as well. Morphological evidence has confirmed that the differences between *P. attwateri* and *P. b. rowleyi* are considerable.

The single sample of *attwateri* available to us is monomorphic at each of three loci for an allele which was not represented in our samples of *P. b. rowleyi* and *P. b. levipes.* Mean similarity between *attwateri* and the *P. b. rowleyi-levipes* samples was 0.79, indicating differences greater than those usually observed between conspecific populations. Nonetheless, *attwateri* clusters closer to subspecies of *P. boylii* (and to *P. stephani*) than to any other *Peromyscus* species, and its affinities to *P. boylii* are apparent. Our biochemical evidence supports the conclusion that considerable differences exist between *attwateri* and *P. b. rowleyi-levipes,* and that *attwateri* is specifically distinct.

*Peromyscus stephani.*—*P. stephani* is restricted to Isla San Esteban in the Gulf of California. In a previous study of the biochemical genetics of this species (Avise *et al.*, 1974), we found it to be well differentiated from its presumed relatives (*P. guardia* and other members of the subgenus *Haplomylomys*). *P. stephani* had been placed in the subgenus *Haplomylomys* by Hall and Kelson (1959) and Hooper (1968), although Hooper considered the placement tentative.

With the addition of *P. boylii* and *P. pectoralis* to our biochemical dendrogram for *Peromyscus,* the affinity of *P. stephani* becomes more apparent. It is similar to *P. boylii rowleyi* and *P. b. levipes,* with $\overline{S} = 0.85$. It appears to be even more closely allied to *P. b. rowleyi-levipes* than does *P. attwateri.*

On the basis of morphology of the phallus, Hooper and Musser (1964) suggested that *P. stephani* may be more closely related to species of the subgenus *Peromyscus.* Lawlor (1971) has recently found close similarities between *P. stephani* and *P. boylii* in several characters, including shape and position of certain skull bones, pelage, male accessory reproductive glands, and karyotypes. He concluded that *P. stephani* is more closely related to *P. boylii* than to any species of *Haplomylomys.* Our data support this conclusion. *P. stephani* should be removed from *Haplomylomys* and placed in the *boylii* species group of the subgenus *Peromyscus.*

*Peromyscus pectoralis.*—Comparatively little is known of systematic relationships among populations referred to *P. pectoralis,* a species distributed in highlands throughout much of central and northern Mexico and well into Texas. Four subspecies previously were recognized, two of which (*collinus* and

*laceianus*) are very similar in external appearance to sympatric *P. boylii* (Hooper, 1952, 1968). Specimens of *P. p. laceianus* from the Davis Mountains in Texas have been distinguished from *P. boylii* by the baculum (Clark, 1953), and those of *P. p. collinus* from Tamaulipas, Mexico, have been recognized by karyotype (Hsu and Arrighi, 1968). Recently, Schmidly (1972) has described three subspecies of *P. pectoralis* on the basis of sharp geographic discontinuities in morphological features.

We examined two populations referable to *P. p. laceianus* (Big Bend and Ranger, Texas, 520 kilometers apart) and one population of *P. p. collinus* from Tamaulipas, Mexico. These populations cluster together in the similarity dendrogram (Fig. 3), but they show considerable differences from one another. These differences are of the magnitude frequently seen between closely related species ($0.75 < S < 0.84$, and $\overline{S} = 0.79$ between the three samples) and are below the normal range of values for conspecific populations. Our samples are completely distinct from one another in allelic composition at several loci (Table 3).

If only a single species is represented by our three samples, *P. pectoralis* must be an unusually polytypic species. It is noteworthy that the genic differences between samples are not apparent within samples; that is, we have seen no intrasample variability in most of the proteins that show intersample differences. We conclude that there is a good possibility that two or more species are presently confused under the name *P. pectoralis.*

Populations of *P. pectoralis* were placed within the *boylii* species group in the classifications of Osgood (1909), Hall and Kelson (1959), and Hooper (1968). However, assignment of *P. pectoralis* forms on the basis of genic evidence is equivocal. *Peromyscus pectoralis* joins the *Haplomylomys* cluster at $S = 0.62$, and other populations in the *boylii* species group join this cluster at $S = 0.57$. This would indicate that its position is intermediate between *Haplomylomys* and members of the *boylii* group. Although most authors agree that *P. pectoralis* belongs in the *boylii* species group, it is more similar in phallic features to members of the subgenus *Peromyscus,* although its position in that subgenus is unclear (Hooper, 1958). In external appearance and habits, at least two races of *P. pectoralis* (*eremicoides* and *pectoralis*) are similar to *P. eremicus.* Our biochemical evidence suggests that *P. pectoralis* is not as closely related to *P. boylii* as is generally supposed. Whether it is genically more similar to *P. boylii* than to any other species will be determined as more *Peromyscus* are added to the dendrogram in future papers.

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