

IMMUNOELECTROPHORETIC RELATIONSHIPS AMONG FOUR SPECIES OF WOODRATS (CRICETIDAE: *NEOTOMA*)

MICHAEL M. SHIPLEY, FREDERICK B. STANGL, JR.,
RODNEY L. GATE, AND CRAIG S. HOOD

*Department of Biology, Midwestern State University,
Wichita Falls, TX 76308 (MMS, FBS, RLC)*

Department of Biology, Loyola University, New Orleans, LA 70118 (CSH)

ABSTRACT—Immunological relationships among four species of woodrats (*Neotoma floridana*, *Neotoma micropus*, *Neotoma albigula*, and *Neotoma mexicana*) are cladistically analyzed, using a *Peromyscus* outgroup. Within this group, *N. micropus* and *N. floridana* comprise an antigenically derived sister group, while *N. mexicana* retains the most primitive antigenic composition. *Neotoma albigula* is intermediate but appears more closely related to the *N. micropus*-*N. floridana* clade. These results are concordant with a consensus series of relationships proposed on the basis of morphology, karyology, and other biochemical data sets. This agreement supports the potential utility of immunoelectrophoresis as a systematic tool.

Evolutionary relationships within the genus *Neotoma* have been investigated using a variety of morphological (Goldman, 1910; Burt and Barkalow, 1942; Hooper, 1960; Carleton, 1980), biochemical (Birney, 1973; Zimmerman and Nejtek, 1975, 1977), and cytogenetic (Birney, 1973; Mascarello and Hsu, 1976; Koop et al., 1985) techniques. Of particular interest have been four closely related and widely available species of woodrats from the southcentral and southeastern United States. *Neotoma micropus* is a species of the southern Great Plains, whose range interfaces to the east with *Neotoma floridana* of the southeastern deciduous woodlands. The western range of *N. micropus* overlaps broadly with that of *Neotoma albigula*, and to a lesser extent, with the range of *Neotoma mexicana*.

Immunoelectrophoresis has not been widely employed as a systematic tool in mammalogy. Earlier applications (Birney, 1973; Baranov et al., 1976, 1978) have demonstrated the utility of the technique in characterizing levels of genetic variation for specific proteins (e.g., lipoproteins, esterases) but afforded no systematic resolution. More recent studies, using tissue homogenates (e.g., blood, liver) to generate antisera, have proven a useful tool in reconstructing evolutionary relationships of an array of Old World fruit bat

genera (Haiduk, 1983) and between closely related species of prairie dogs (McCullough, 1987).

The present study assesses immunoelectrophoretic relationships of *N. micropus*, *N. floridana*, *N. albigula*, and *N. mexicana*, whose interrelationships have been relatively well ascertained by a variety of independent studies. Therefore, this study also provides an ideal test of the further utility of immunoelectrophoresis as a systematic tool in mammalogy.

MATERIALS AND METHODS—Specimens of four species of woodrats (*N. floridana*, *N. micropus*, *N. albigula*, and *N. mexicana*) and the outgroup taxon, *Peromyscus attwateri*, were collected from natural populations (Appendix 1). Livers from three individuals of each species were pooled and macerated. Deionized water was added until a 15% homogenate was obtained. Samples were subsequently freeze-thawed in liquid nitrogen three times before adding a 10% Triton X-100 solution (10 ul/ml homogenate) to free cell membrane proteins.

Antibody production and immunoelectrophoresis followed Grabar and Williams (1955), as modified by Williams (1971) and Haiduk (1983). Homologous tests were conducted to characterize the number of antigenic determinants between each species recognized by each antiserum. Heterologous tests were performed to establish the maximum number of antigens shared be-

TABLE 1—Comparisons of immunoelectrophoretic precipitin bands among four species of *Neotoma* and the *Peromyscus* outgroup. Values presented represent the total number of precipitin bands detected for each set of homologous and heterologous reactions.

Antigen source	Antisera source				
	<i>N. floridana</i>	<i>N. micropus</i>	<i>N. albigula</i>	<i>N. mexicana</i>	<i>Peromyscus</i>
<i>N. floridana</i>	11	10	14	9	12
<i>N. micropus</i>	11	11	14	10	11
<i>N. albigula</i>	11	11	11	10	10
<i>N. mexicana</i>	11	10	10	9	10
<i>Peromyscus</i>	8	8	7	8	11

tween two species, and absorption tests were used to confirm the homology of precipitin lines resulting from the heterologous tests.

Immunoelectrophoretic differences were assessed and scored as to the presence, absence, or relative mobility of specific precipitin bands. PAUP (Swofford, 1986) phylogenetic analyses were employed to systematically order the four taxa.

RESULTS—Heterologous comparisons produced two to three more detectable precipitin bands than the corresponding homologous reactions with the exception of *anti-floridana* and *anti-micropus* sera (Table 1). The presence of weakly antigenic components cross-reactive with heterologous antisera may account for these observations. Therefore, for comparisons of precipitin bands, a numbering system was employed based on the observed relative electrophoretic mobilities of all detectable cross-reactive antigens. Heterologous comparisons of *anti-albigula* serum with *Neotoma floridana* antigen produced a maximum of 14 observable precipitin bands. These bands were sequentially numbered from most anodal to most cathodal. This numbering system was applied to all other heterologous, homologous, and absorption tests. Like-numbered bands were presumed to be homologous, and those exhibiting mobility differences from the standard were so designated (Table 2). A unique band, generated only by immunoelectrophoresis of antigens of *N. micropus* and *N. floridana* with *anti-Peromyscus*, was labeled "A."

PAUP analysis produced a phylogenetic tree (Fig. 1; tree length = 26.000, consistency index = 0.846) indicating a sister-group relationship

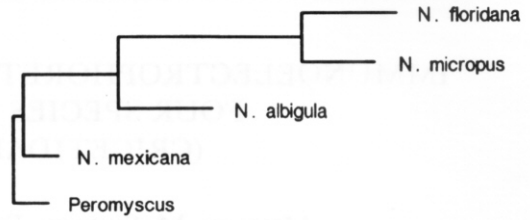


FIG. 1—Phylogenetic arrangement of four species of *Neotoma* and the *Peromyscus* outgroup taxon produced by phylogenetic analysis (PAUP; Swofford, 1986) of merged immunoelectrophoretic data sets.

between *N. micropus* and *N. floridana* and the primitive antigenic nature of *N. mexicana* relative to *N. albigula*.

DISCUSSION—Within the woodrat genus *Neotoma*, the subgenus *Neotoma* is comprised of an assemblage of 17 nominal species (Hall, 1981), whose interrelationships have not been entirely resolved. However, a considerable accumulation of data has ascertained, with some degree of confidence, the interrelationships of the four species examined in this study. *Neotoma micropus* and *N. floridana* comprise a sister group to the exclusion of *N. albigula*, and *N. mexicana* is most primitive.

The glans penis of *N. micropus*, *N. floridana*, and *N. albigula* was found by Hooper (1960) to be so similar as to suggest to him conspecific or sibling-specific status. Interfertility of the three taxa under laboratory and natural conditions (Birney, 1976) further supports the close evolutionary relationships of these taxa, leading Birney (1973, 1976) to favor inclusion of the three under a single species group.

The close morphological relationship between *N. micropus* and *N. floridana* was recognized by Goldman (1910), who placed the two species within a single group, exclusive of *N. albigula*. This arrangement, which does not conflict with Birney's (1973, 1976) views, was subsequently accepted by Carleton's (1980) comprehensive morphological assessment.

Alternatively, Burt and Barkalow (1942) found the bacula of *N. micropus* to be intermediate between those of *N. floridana* and *N. albigula* and placed each of the three species within their own species group. This alignment was later followed by Hall (1981).

In each of these studies where *N. mexicana* was included, that species was divergent from the other three and placed within its own group. Re-

TABLE 2—Immunoelectrophoretic differences among four species of *Neotoma* and the *Peromyscus* outgroup. Numbered precipitin bands are based on the standard numbering system. Specific bands are listed by absence (0), presence (1), or mobility differences (!).

Antigen source	Antisera source																			
	N. floridana		N. micropus			N. albigula				N. mexicana				Peromyscus						
	10	13	1	5	10	2	3	4	9	10	4	5	9	3	4	5	6	I	9	A
<i>N. floridana</i>	0	1	1'	0	1	0	1	0	1	1	1'	0	0	1	0	1	0	0	1	1
<i>N. micropus</i>	0	1	1'	1'	1'	0	1	1	1'	1	1'	1	0	1	0	1	1	0	0	1
<i>N. albigula</i>	1	0	1'	1'	1	1	0	1	0	0	1'	0	1	0	0	0	0	1	1	0
<i>N. mexicana</i>	0	1	1	1	1	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0
<i>Peromyscus</i>	0	0	1	1	1	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0

tention of dentine tracts on the lower first molars of *N. mexicana*, which are absent or nearly so in the other three species (Harris, 1984), further supports this separation.

Electrophoresis of the hemoglobins and serum albumins of *N. floridana*, *N. albigula*, and *N. mexicana* offered no resolution of interrelationships of these taxa (Zimmerman and Nejtek, 1975), although the species were separable by their hemoglobins. A later study by Zimmerman and Nejtek (1977), utilizing starch gel electrophoresis of whole tissue homogenates, established a sister-group relationship between *N. micropus* and *N. floridana*, to the exclusion of *N. albigula*.

Koop et al. (1985) summarized and cladistically analyzed the available chromosomal banding data on *Neotoma*. Although evolutionary affinities of the four species studied herein were not resolvable, the overall similarities of the karyotypes would appear to support the generally accepted series of relationships. Karyotypes of *N. micropus* and *N. floridana* are nearly identical, except for a single chromosomal polymorphism which prevents a reliable and consistent separation of the two species. While the karyotypes of *N. albigula* and *N. mexicana* are distinctive, that of *N. albigula* appears less divergent from the *N. micropus*-*N. floridana* clade.

Birney's (1973) phenetic analysis of immunoelectrophoretic variability of esterases was unable to separate populations of *N. micropus* from those of *N. floridana*. However, analysis of merged immunoelectrophoretic data (Fig. 1) indicate relationships concordant with the most parsimonious interpretation of relatedness among the four species of woodrats. Seven synapomorphies, in-

cluding the unique precipitin band "A," substantiate the sister-group relationship of the highly derived *N. micropus* and *N. floridana*. Sharing four synapomorphies with this clade is *N. albigula*. *Neotoma mexicana* appears antigenically primitive, sharing with the other three woodrat species only the 10 generic synapomorphies.

Recent applications of immunoelectrophoresis in mammalogy have demonstrated the potential of this technique in systematic studies. Haiduk's (1983) study of 10 genera of Old World fruit bats (Pteropodidae) incorporated a cladistical assessment of precipitin bands generated by immunoelectrophoresis of whole-tissue antigens and their antisera. His appears to be the first such study to result in any systematic resolution of examined taxa. Employing numerical phylogenetic analyses, McCullough et al. (1987) similarly were able to resolve immunoelectrophoretic relationships among five species of prairie dogs (*Cynomys*). In each of these studies, no instance of intraspecific variation for immunoelectrophoretic characters was detected, and the effects of detected convergences were minimized by the merging of data sets generated by the individual antisera.

The potential utility of a technique or method of analysis in resolving the evolutionary affinities of taxa can be evaluated by comparing results of a particular study with relationships that have been relatively well ascertained by congruence of other data sets. The four species of *Neotoma* chosen for this study appear to represent such an assemblage to us for the cladistical evaluation of precipitin bands produced by immunoelectrophoresis of whole tissue samples. Agreement of our results with a consensus series of relationships

of these closely related taxa indicate that similar studies will be useful not only in constructing hypothetical relationships of a more comprehensive assemblage of woodrats but also in providing additional independent data sets for other, less well-understood mammalian assemblages.

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APPENDIX 1

Voucher specimens are deposited at The Museum of Texas Tech University and the Collection of Recent Mammals at Midwestern State University as follows: *Neotoma floridana*—Texas: Montague Co., 6.2 mi. N. Bowie (1). Oklahoma: Cleveland Co., 2 mi. SE Norman (1); Seminole Co., 9.3 mi. E Seminole (1). *Neotoma micropus*—Texas: Knox Co., 3 mi. S Gilliland (1); Wichita Co., 7 mi. N Iowa Park (1); Garza Co., 10 mi. S Post (1). *Neotoma albigula*—Texas: Dickens Co., 1 mi. E Dickens (1); Culberson Co., 4.8 mi. SW Junction FM 2185 and 2424 (1). Oklahoma: Cimarron Co., 2.4 mi. E, 4.3 mi. S Kenton (1). *Neotoma mexicana*—New Mexico: Otero Co., 14 mi. E, 4 mi. S Cloudercroft (1); Union Co., NE side Capulin Mtn. (2). *Peromyscus attwateri*—Texas: Knox Co., 4 mi. E Benjamin (3).