PHENETIC AND CLADISTIC ANALYSES OF BIOCHEMICAL EVOLUTION IN PEROMYSCINE RODENTS

John C. Patton, Robert J. Baker and John C. Avise

Abstract--An approach to applying principles of Hennigian cladistics to qualitative, uncoded electrophoretic data is suggested. This approach is contrasted with more conventional phenetic analyses, using a data base of newly collected biochemical information on nine genera and 14 species of New World rodents, Cricetidae. Phenetic dendrograms and cladistic trees based on protein electrophoretic data were evaluated against a model phylogeny for these taxa derived from nonprotein information. In two cases, the cladistic analysis of our electrophoretic data gives a somewhat better fit to the model phylogeny than does the phenetic analysis: (1) In suggesting only a distant affiliation between *Ochrotomys* and *Neotoma*, and (2) in defining *Peromyscus*-*Neotomodon* as a distinct clade. In most other respects, the phenetic and cladistic interpretations fit the model phylogeny equally well. Whether or not this cladistic method of analysis will ultimately prove more precise than standard phenetic approaches, it does offer advantages of greater testability since individual character states are defined along all branches of a tree. Our results indicate that electrophoretic data provide useful systematic information even at intergeneric taxonomic levels.

INTRODUCTION

In the past decade numerous authors have documented the power and efficiency of protein electrophoresis to examine genetic relatedness of populations (Avise, 1974). While the general utility of the electrophoretic approach is no longer in serious question, one remaining area of contention concerns how best to analyze and interpret data derived from these techniques. At least two basic philosophies are prevalent: phenetics and cladistics.

genase (SDH), creatine kinase (CK-2), glutamate oxalate transaminase (GOT-1 and -2), indophenol oxidase (IPO), and esterase (EST-1 and -6 of Selander et al., 1971).

Two methods of data analysis were employed. First, Nei's (1972) identity and distance values between species pairs were subjected to an unweighted pair-group method of cluster analysis using arithmetic means (Unweighted Pair Group Method of Analysis - UPGMA; Sneath and Sokal, 1973). This is a common phenetic procedure. Second, cladistic analyses of each discrete electrophoretic character were performed, utilizing outgroup comparisons as described by Hennig (1966) and as discussed by Bonde (1977). The development of the tree is described in detail later. We have chosen the method of Hennig (1966) due to the ease of analysis (it does not require a computer) and because the characters need not be coded or submerged in a summary distance or similarity measure.

RESULTS

Electromorph mobilities and electrophoretic conditions for 15 loci are given in Table 1. Banding patterns and tissue specificities were similar to gene products previously reported in *Peromyscus* (Selander et al., 1971; Mascarello and Shaw, 1973). Gels stained for creatine kinase (CK) showed two areas of activity in samples from heart and kidney. The most anodal band (CK-1) was indistinct and could not be scored reliably. The more cathodal zone of activity (CK-2) banded sharply but no variation was found within or between the taxa analyzed. All other loci demonstrated interspecific variation.

Phenetic Analysis

Table 2 lists genetic identities ($\bar{I}$) and distances ($\bar{D}$) between species, computed according to Nei's (1972) formulae. These data were summarized in a dendrogram which provides a two-dimensional representation of phenetic relationships among the taxa (Fig. 2).

As noted in Table 1, only 13 loci were analyzed in *N. alstoni*; hence, three different estimates of $\bar{I}$ and $\bar{D}$ were obtained. First, the 13 loci analyzed for *N. alstoni* were examined over all taxa. The second esti-

Table 1. Designations of electromorphs of loci examined using *Peromyscus polionotus* as the standard. Electromorphs taken from Avise et al. (1979a) are enclosed in parentheses. Species abbreviations are as given in Table 2 and locus abbreviations in text.

Locus	Species														Buffers*	Tissue**
	P.m.	P.p.	P.l.	P.f.	N.a.	O.l.	O.t.	B.t.	R.f.	R.m.	O.n.	N.m.	S.h.	O.p.		
CK-2	100	100	100	100	100	100	100	100	100	100	100	100	100	100	7,5	H&K
MDH-2	-100	-100	-100	-100	-100	-100	-100	-100	-100	-100	-80	-80	-80	-80	1,6,4	[illegible]
LDH-2	100	100	100	100 30	100	20	20	20	20	20 105	20	20	20	120	1,6,2,4,5	H&K,He
MDH-1	100	100	100	100	100	100	100	100	70	70	100	100	90	100	1,6,4	H&K,He
IDH-2	-100	-100	-100	-100	-100	-50	-50 20	-50	-50	-50	-50	-120	-120	-120	1,6,4	H&K
GOT-2	-100	-100	-100	-64	-64	-100	-100	-75	-64	-64	-100	-100	-64	-70	1,2,6	H&K,L
EST-6	100 102	100	100	100		100	100	100	95	65	100	102	100	94	4	He
IDH-1	100	100	100	127	127	100	100	60	100	100	110	100	135	120	1,6,4	H&K
GOT-1	100 130	100 (130)	100	170 (130)	170	100	100	170	100	100	170	170	175	180	1,2,6	H&K,L
SDH	100	100	5	100	100	100	100	350	100	100	-10	-50	140	120	1,2,6	H&K
ME	100	100	120	100	200 175	160	180	40	165	105	90	168	170	210	1,4	H&K
IPO	100	100	100 60	130	100	100	100	-20	10	10	40	105	95	45	5,7,2,6	H&K,L
6-PGD	100 (117)	100 (117)	117	139	139	132	150	95 85	90	141 135	125	160	104 82	134	6,1	H&K,He
EST-1	100 90	100	90 120	98		50 40 30 10	50 40	70	55	65 72	102	75	25	60	3	He
LDH-1	98	100	98	95	99	98.5	98.5	99	99	99	85	110	99	99	4,1,6,2,5	H&K

*1 = T.C. 6.7/T.C. 6.3[1] 2 = T.C. 8.0[1] 3 = T HCl[1] 4 = T MAL[1] 5 = Poulik[1] 6 = JRP[2] 7 = RSL[3] with recipes given in references below.

**H&K = heart and kidney, L = liver, and He = hemolysate

[1]Selander et al., 1971. [2]Ayala et al., 1972, (modified as described in text). [3]Ridgeway et al., 1970.

Table 2. Genic similarities reported for taxa examined. Figures in upper right diagonal are Nei's $\bar{I}$ and those in lower left are Nei's $\bar{D}$.

Species*	P.m.	P.p.	P.l.	P.f.	N.a.	O.l.	O.t.	B.t.	R.f.	R.m.	O.n.	N.m.	S.h.	O.p.
P.m.		.892	.739	.516	.498	.591	.591	.248	.332	.347	.245	.280	.107	.140
P.p.	.114		.670	.526	.475	.615	.614	.270	.333	.348	.267	.267	.136	.133
P.l.	.303	.401		.403	.402	.545	.545	.278	.275	.287	.275	.275	.140	.137
P.f.	.661	.643	.909		.672	.345	.345	.341	.270	.282	.270	.202	.206	.135
N.a.	.691	.745	.918	.398		.347	.347	.343	.339	.354	.203	.203	.207	.203
O.l.	.526	.487	.606	1.064	1.058		.847	.415	.478	.481	.410	.341	.208	.137
O.t.	.526	.487	.606	1.064	1.058	.166		.396	.461	.464	.393	.341	.208	.137
B.t.	1.395	1.309	1.279	1.075	1.069	.880	.923		.338	.335	.405	.270	.275	.203
R.f.	1.102	1.099	1.292	1.311	1.082	.738	.775	1.086		.749	.267	.200	.271	.133
R.m.	1.058	1.055	1.248	1.268	1.038	.731	.769	1.094	.290		.192	.192	.266	.139
O.n.	1.407	1.322	1.292	1.311	1.593	.892	.935	.904	1.322	1.653		.467	.271	.200
N.m.	1.274	1.322	1.292	1.599	1.593	1.075	1.075	1.309	1.609	1.653	.762		.271	.267
S.h.	2.238	1.998	1.968	1.582	1.576	1.569	1.569	1.292	1.305	1.326	1.305	1.305		.271
O.p.	1.967	2.015	1.985	2.004	1.593	1.991	1.991	1.597	2.015	1.971	1.609	1.322	1.305	

*P.m. = *Peromyscus maniculatus*; P.p. = *Peromyscus polionotus*; P.l. = *Peromyscus leucopus*; P.f. = *Peromyscus floridanus*; N.a. = *Neotomodon alstoni*; O.l. = *Onychomys leucogaster*; O.t. = *Onychomys torridus*; B.t. = *Baiomys taylori*; R.f. = *Reithrodontomys fulvescens*; R.m. = *Reithrodontomys megalotis*; O.n. = *Ochrotomys nuttalli*; N.m. = *Neotoma micropus*; S.h. = *Sigmodon hispidus*; O.p. = *Oryzomys palustris*.

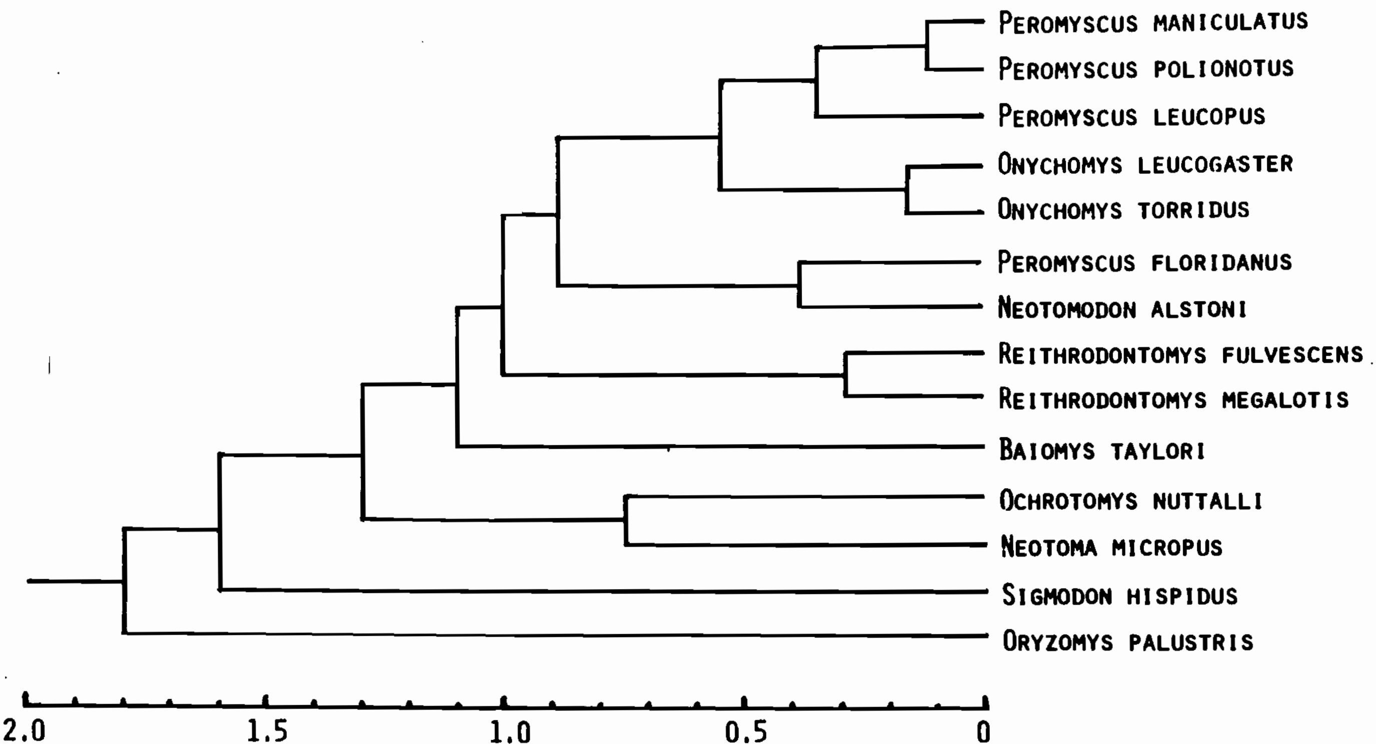

Fig. 2. Unweighted Pair Group Method of Analysis (UPGMA) dendrogram depicting possible relationships within the cricetine rodents based on genetic distances at 13-15 protein loci.

mate employed 15 loci in all comparisons except those involving Neotomodon for which 13 loci were compared. Finally, both loci not scored for N. alstoni were considered unique, thereby maximizing the distance between N. alstoni and its closest relative. In all cases N. alstoni was phenetically allied most closely to P. floridanus. Similarity and distance values were 0.778 (0.251), 0.778 (0.251), and 0.672 (0.398), respectively. Identity values between N. alstoni and P. floridanus were considerably greater than the identity values between Onychomys and the P. maniculatus-polionotus-leucopus assemblage. The implication is that N. alstoni and P. floridanus are phenetically more closely related to one another than the other Peromyscus forms are to Onychomys. Only minor changes in the phenograms resulted from clustering the three different distance matrices.

Cladistic Analysis

Because results of cladistic analyses employing qualitative characters may be sensitive to mistakes in character identification (see DISCUSSION), we will discuss in detail the scoring and cladistic interpretation of individual electromorphs.

LDH-1: Seven alleles were identified for LDH-1 (Table 1). Outgroup comparison indicated that Ldh-1^{99} was the plesiomorph (ancestral form) for the cricetines examined. Ldh-1^{99} was identified in S. hispidus, Oryzomys palustris, R. fulvescens, R. megalotis, B. taylori, and N. alstoni. Synapomorphic (shared derived) electromorphs were found in P. maniculatus and P. leucopus (Ldh-1^{98}) and Onychomys leucogaster and Onychomys torridus (Ldh-$1^{98.5}$). All other taxa possessed autapomorphs (derived characters unique to a taxon).

LDH-2: Five electromorphs were identified. Onychomys leucogaster, Onychomys torridus, B. taylori, R. fulvescens, R. megalotis, O. nuttalli, and N. micropus possessed Ldh-2^{20}. The electromorph for S. hispidus has also been tentatively scored as Ldh-2^{20}, although its product in some samples occasionally appeared to migrate slightly slower. At present, Ldh-2^{20} should be considered the plesiomorph. The only synapomorph, Ldh-2^{100}, was found in Neotomodon and all Peromyscus. Other alleles were autapomorphs. It should be noted that this scoring does not agree with that presented by

Smith et al. (1973) where Ldh-2^{95} was described in *P. floridanus*. The causes of the discrepancy are unknown.

MDH-1: Mdh-1^{100} is proposed as the plesiomorph because it was found both in the neotomine-peromyscine line and in *Oryzomys palustris*. A synapomorph (Mdh-1^{70}) appeared in the *Reithrodontomys* species. *Sigmodon hispidus* possessed an autapomorph (Mdh-1^{90}).

MDH-2: Since Mdh-2^{-80} was common to *S. hispidus*, *Oryzomys palustris*, *N. micropus*, and *O. nuttalli*, it has been designated as the plesiomorph. The synapomorph is Mdh-2^{-100}, common to all *Reithrodontomys*, *Baiomys*, *Onychomys*, *Neotomodon*, and *Peromyscus* taxa examined.

ME: Due to the rapid rate of evolution in this protein, it was impossible to designate a plesiomorph for all taxa examined. Thirteen electromorphs were identified and each was considered unique (autapomorphic) for its respective taxon. The exception involves the *Peromyscus-Neotomodon* complex (cladistically defined by LDH-2), within which three taxa shared Me-1^{100} (*P. maniculatus*, *P. polionotus*, and *P. floridanus*).

IDH-1: Six electromorphs have been identified for IDH-1. No electromorphs appeared to be shared between the South American cricetines and the neotomine-peromyscine taxa. Within the neotomine-peromyscine line 8 of the 12 taxa shared Idh-1^{100}: *N. micropus*, *R. fulvescens*, *R. megalotis*, *Onychomys leucogaster*, *Onychomys torridus*, *P. leucopus*, *P. polionotus*, and *P. maniculatus*. Whether Idh-1^{100} represented an apomorph or plesiomorph for the South American, neotomine-peromyscine forms cannot be resolved at present. However, Idh-1^{100} must be considered plesiomorphic for the neotomine-peromyscine complex. Idh-1^{127} appeared to be a synapomorph for *P. floridanus* and *N. alstoni*. All other derived electromorphs appeared to be restricted to a single assayed taxon.

IDH-2: Four IDH-2 electromorphs were observed. Idh-2^{-120} was shared by *N. micropus*, *S. hispidus*, and *Oryzomys palustris* and was therefore plesiomorphic. Idh-2^{-50} was shared by the *Ochrotomys*, *Reithrodontomys*, *Baiomys*, and *Onychomys* taxa, thus defining Idh-2^{50} as a synapomorph which was also the apparent plesiomorph for the peromyscine forms. Idh-2^{-100} was synapomorphic for *Neotomodon* and *Peromyscus* in the peromyscine lineage. Idh-2^{20} was unique to *Onychomys torridus*.

6-PGD: Fifteen electromorphs were identified for the taxa examined. No allele could be proposed as

plesiomorphic except within the Neotomodon-Peromyscus complex. Data presented here and in Avise et al. (1974a) indicated that 6-Pgd^{117} appears synapomorphic for P. maniculatus, P. polionotus, and P. leucopus. Peromyscus maniculatus and P. polionotus shared 6-Pgd^{100}, while P. floridanus and N. alstoni shared 6-Pgd^{139}. All other alleles have been considered autapomorphic.

SDH: No electromorph was shared between the South American cricetines and the neotomine-peromyscines. Reithrodontomys megalotis, R. fulvescens, Onychomys torridus, Onychomys leucogaster, N. alstoni, P. floridanus, P. polionotus, and P. maniculatus possessed Sdh^{100}. Six other electromorphs were presumed autapomorphic.

GOT-1: Five electromorphs have been identified at this locus. Since none were shared by the neotomine-peromyscine forms and the South American cricetines, it is impossible at this time to propose a plesiomorph for the entire assemblage. Got-1^{170} was found in N. micropus, O. nuttalli, B. taylori, N. alstoni and P. floridanus, and is apparently plesiomorphic for the neotomine-peromyscine forms. Got-1^{100} was found in R. megalotis, R. fulvescens, Onychomys torridus, Onychomys leucogaster, P. leucopus, P. polionotus, and P. maniculatus and is apomorphic. Got-1^{130} was found only in P. maniculatus in the specimens examined here, but Smith et al. (1973) reported the electromorph in both P. polionotus and P. floridanus. Got-1^{130} must also be considered apomorphic. Sigmodon hispidus and Oryzomys palustris possessed autapomorphs.

GOT-2: Got-2^{-64} was present in both the neotomine-peromyscine forms and S. hispidus and is therefore considered plesiomorphic. Got-2^{-100} was found in N. micropus, O. nuttali, Onychomys torridus, Onychomys leucogaster, P. leucopus, P. polionotus, and P. maniculatus. Autapomorphic alleles were found in B. taylori and Oryzomys palustris.

IPO: No electromorph was shared between any neotomine-peromyscine form and any South American cricetine form. Onychomys torridus, Onychomys leucogaster, N. alstoni, P. leucopus, P. polionotus, and P. maniculatus all shared Ipo^{100}. Reithrodontomys fulvescens and R. megalotis shared Ipo^{70}. Seven other electromorphs were considered autapomorphs.

EST-1: This highly variable esterase was represented by 16 electromorphs in the specimens examined.

Phenetic and Cladistic Analyses

No electromorph could be designated as a plesiomorph for all taxa examined. Synapomorphs were found only for members of Peromyscus and Onychomys. All other forms possessed autapomorphic alleles.

EST-6: This erythrocytic esterase was represented by four electromorphs. $Est\text{-}6^{100}$ was present in both major lineages examined and is therefore ancestral. All other observed alleles were autapomorphic.

DISCUSSION

Phenetic Analysis

The UPGMA dendrogram revealed a fair concordance with current ideas regarding systematic relationships of peromyscine rodents. However, at least four questionable results were apparent. First, the phenetic linking of P. maniculatus, P. polionotus, and P. leucopus to Onychomys is surprising since their supposed congener, P. floridanus, is phenetically much more distinct. Morphological and chromosomal data do not support these phenetic results (Hooper, 1968; Baker et al., 1979). A second possible problem is the apparent phenetic similarity between N. alstoni and P. floridanus. In this case, recent morphological and chromosomal data support such a relationship. Morphological similarities were noted between the external genitalia of N. alstoni and P. floridanus by Hooper (1959). Also, studies of G- and C-banded chromosomes of Peromyscus, Neotomodon, and Baiomys recently led to the conclusion that N. alstoni is more closely related to the P. floridanus, P. maniculatus, and P. leucopus groups, respectively, than to the eremicus group (Yates et al., 1979).

A third area of apparent disagreement is the placement of B. taylori outside the 2n = 48 group of rodents. If the assumptions of Yates et al. (1979) are correct that the 2n = 48 peromyscines are a monophyletic assemblage, Baiomys is the more distantly related (basal) form of the clade. This basal relationship is also supported by Hooper's (1959) work with external reproductive structures. If Reithrodontomys is the taxon most closely related to the 2n = 48 forms, a slight difference in the rates of biochemical evolution between Baiomys and Reithrodontomys (as reflected by sampling

biases in loci examined) could cause the supposed reversal seen in the phenetic dendrogram.

The final concern about accuracy of the phenetic groupings is the alignment of O. nuttalli with N. micropus. Although the systematic status of Ochrotomys is poorly understood, evidence from other sources does not suggest placing Ochrotomys with neotomines.

Cladistic Analysis

Five stages in the development of the cladistic tree for peromyscine rodents are presented in Figs. 3 and 4. For reasons discussed later, data for loci were added to the analysis in the order listed in Table 1. Figure 3A shows the result of joint consideration of CK-2 and MDH-2; Fig. 3B shows the effect of the further addition of LDH-2 and MDH-1; Fig. 3C shows the effect of the addition of IDH-2, GOT-2, and EST-6 to Fig. 3B; and, Fig. 3D shows the effects of the addition of IDH-1, GOT-1, and SDH to Fig. 3C. Figure 4 shows the final cladistic tree for the 103 character states identified for the 15 loci examined.

The composite cladistic tree is composed of a stem and 24 branches (Fig. 4). The distributions of electromorphs along all stems and branches are presented in Table 3. For loci 1-7 the plesiomorph for all taxa could be designated. These loci were used to form the skeleton of the cladistic tree. Plesiomorphs at loci 8-14 could not be designated for all taxa and, therefore, characters were initially assessed as apomorphic. Data for locus 15 (LDH-1) were added after those for EST-1 because only Est-1^{100} defines the P. maniculatus-polionotus complex. Adding the data for LDH-1 prior to adding those for EST-1 and 6-PGD would result in a phylogenetic tree linking P. maniculatus to P. leucopus rather than to P. polionotus. Such a relationship is refuted by evidence from morphology (Hooper, 1968), hybridization potential (Dice, 1968), chromosome banding (Greenbaum et al., 1978), protein electrophoresis (Avise et al., 1979a; phenetic analysis in this paper), and mitochondrial DNA sequence relatedness (Avise et al., 1979b).

The distributions of electromorphs along all stems and branches are presented in Table 3. A major advantage of this cladistic analysis is that discrete character states, such as those listed in Table 3, define the

Table 3. Discrete character states identified in rodent taxa examined. Numbers correspond to stem and branches shown in Fig. 4. Item 1 lists characters considered plesiomorphic (ancestral) for all taxa examined. Items 2 through 25 list apomorphic (derived) characters which appear to define the respective clades (Fig. 4A). Characters placed within parentheses are characters considered polymorphic in stem taxa but subsequently lost in the respective clades. Characters marked with asterisks are apomorphic electromorphs found or determined to be polymorphic. Locus abbreviations are given in text.

1) Ck-2^{100}, Mdh-^{100}, Mdh-2^{-80}, Ldh-1^{99}, Ldh-2^{20}, Idh-2^{-120}, Got-2^{-64}, Est-1^{100}

2) Ldh-2^{120}, Me^{210}, Idh-1^{120}, 6-Pgd^{134}, Sdh^{120}, Got-1^{180}, Got-2^{-70}, Ipo^{45}, Est-6^{94}

3) Mdh-1^{90}, Me^{170}, Idh-1^{135}, 6-Pgd^{104}, 6-Pgd^{82}, Sdh^{140}, Got-1^{175}, Ipo^{95}, Est-1^{25}

4) Idh-1^{100}, Got-1^{170}, Got-2^{-100*}

5) Ldh-1^{110}, Me^{168}, 6-Pgd^{160}, Sdh^{-50}, Ipo^{105}, Est-1^{75}, Est-6^{102}, (Got-2^{-64})

6) Idh-2^{-50}

7) Ldh-1^{85}, Me^{90}, Idh-1^{110}, 6-Pgd^{125}, Sdh^{-10}, Ipo^{40}, Est-1^{102}, (Got-2^{-64})

8) Mdh-2^{-100}

9) Me^{40}, Idh-1^{60}, 6-Pgd^{95}, 6-Pgd^{85}, Sdh^{350}, Got-2^{-75}, Ipo^{-20}, Est-1^{70}

10) Sdh^{100}, Got-1^{100*}, Ipo^{100}

11) Mdh-1^{70}, Ipo^{10}, (Got-1^{170}, Got-2^{-100})

12) Ldh-2^{105*}, Me^{105}, 6-Pgd^{141}, 6-Pgd^{135}, Est-1^{65}, Est-1^{72}, Est-6^{65}

13) Me^{165}, 6-Pgd^{90}, Est-1^{55}, Est-6^{95}

14) Ldh-1$^{98.5}$, Est-1^{50}, Est-1^{40} (Got-1^{170}, Got-2^{-64})

15) Me^{180}, Idh-2^{20*}, 6-Pgd^{150}

16) Me^{160}, 6-Pgd^{132}, Est-1^{30*}, Est-1^{10*}

17) Ldh-2^{100}, Me^{100}, Idh-2^{-100}, Got-1^{130*}

18) Idh-1^{127}, 6-Pgd^{139}, (Got-1^{100}, Got-2^{-100})

19) Me^{200}, Me^{175}, (Got-1^{130})

20) Ldh-1^{95}, Ldh-2^{30*}, Ipo^{130}, Est-1^{98}

21) Ldh-1^{98}, Est-1^{90*}, (Got-1^{170}, Got-2^{-64})

22) Me^{120}, Sdh^{5}, Ipo^{60*}, Est-1^{120*}, (Got-1^{130})

23) Est-1^{100*}, 6-Pgd^{100}

24) Ldh-1^{100}, (Est-1^{90})

25) Est-6^{102*}

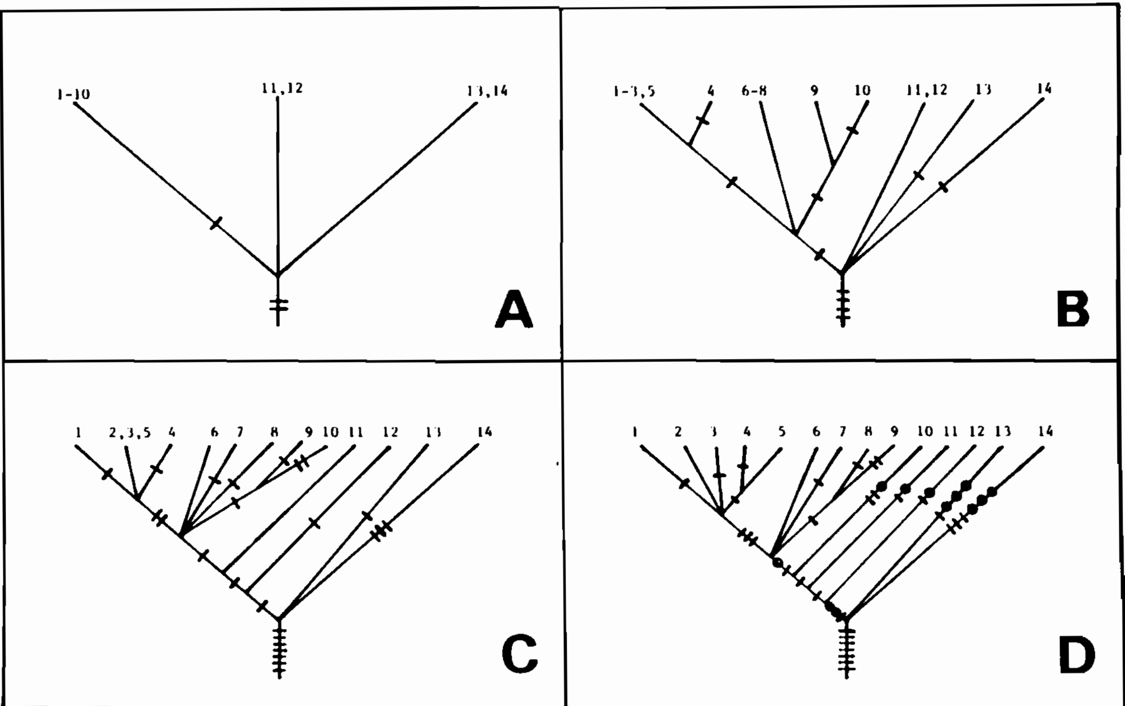

Fig. 3. Various stages in the development of the phylogenetic tree as discussed in text.

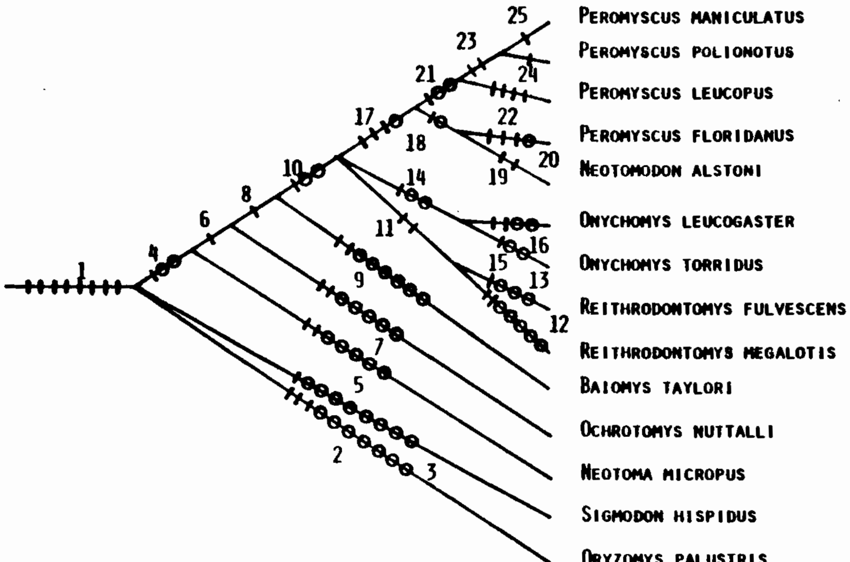

Fig. 4. Phylogenetic tree resulting from cladistic analysis of 13-15 enzyme loci in 14 cricetine taxa. A total of 103 character states were observed. Slashes represent character states for which the plesiomorph could be hypothesized, and circles represent characters entered as assumed apomorphs.

the stem and all branches of the tree. Thus, whether or not this cladistic method of analysis ultimately prove to be more precise than standard phenetic approaches, it does offer advantages of greater testability. As already illustrated, points of ambiguity in cladograms can often be recognized and the source of the dilemma specifically identified for further study.

Electrophoretic information can be cladistically analyzed because the data are expressed as discrete characters. One potential drawback is the possibility of obtaining several alternative cladistic trees from a common data base. This may result from any process leading to inconsistencies in information content of different distributions of electromorphs within a tree. For example, distinct allelic products could be scored incorrectly as synapormorphs or symplesiomorphs.

It is unclear whether sampling errors due to small sample size will be more severe for phenetic or cladistic analyses. The effect of small sample size in phenetics has recently been addressed from both theoretical and empirical standpoints (Nei, 1978; Gorman and Renzi, 1979). The bias introduced through sampling error appears not to be a serious problem, even for sample sizes as small as one or a few individuals, as long as the genetic distance ($\bar{D}$) between the OTU's (operational taxonomic units) and branch points under study is ≥ 0.15 and heterozygosity ($\bar{H}$) is ≤ 0.05. Both of these conditions held for the great majority of taxa examined in this study.

Because the present cladistic analysis utilizes a qualitative data base, it might appear to be more prone to sampling error due to small sample size. Several kinds of information could be introduced into a cladistic analysis with the discovery of additional electromorphs at the loci assayed. First, additional autapomorphic alleles might be identified. These would have no effect on the branching sequence of the cladistic tree. Second, symplesiomorphs not observed in the original sample might be found. An example from the present study would be the discovery of Got-1^{170} in <u>Onychomys</u>. This again would have no effect on the branching sequence of the cladistic tree. Third, an electromorph could be identified in clade B which was formerly considered synapomorphic or autapomorphic to clade A. This latter case could indeed cause a possible reassessment of phylogenetic relatedness of species. An

example of this situation has been discussed for the Got-1 locus of Baiomys. Fourth, entirely new sets of synapomorphs could be detected, which could also cause a reassessment of the branching framework. Since the current study is exploratory in design, we suggest that future work empirically address the issue of sampling error in cladistic analyses.

Cladistic analysis of our electrophoretic data gives a somewhat better fit to the model phylogeny for peromyscine rodents than does the phenetic analysis. First, there are no cladistic characters suggesting the close affiliation between Ochrotomys and Neotoma implied by the phenetic analysis. The synapomorph Idh-2^{-50} demonstrates Ochrotomys to have cladistic affinities with the other peromyscine forms. Second, Ldh-2^{100} and Idh-2^{-100} define Peromyscus-Neotomodon as a distinct clade, an association not apparent in the phenetic groupings. In both instances, the cladistic analysis may resolve apparent discrepancies between results of the phenetic analysis and the model phylogeny. In most other respects, the phenetic and cladistic interpretations fit the model phylogeny for peromyscine rodents equally well.

Systematic Implications

The cladistic analyses of the biochemical data yielded inferences relevant to the systematics of cricetine rodents. The classically recognized split between peromyscine and neotomine rodents is supported. Ochrotomys seems to represent a basal divergence from peromyscine stock. Beyond this level, at least four distinctive clades can be recognized from the biochemical data: Baiomys, Reithrodontomys, Onychomys, and Peromyscus (including Neotomodon). The retention of Neotomodon as a genus would require the recognition of Neotomodon, Podomys (P. floridanus), and a redefined Peromyscus (including P. maniculatus, P. polionotus, and P. leucopus) as genera, or the inclusion of P. floridanus within a redefined Neotomodon. The splitting of Peromyscus would not, however, seem warranted from these data, since none of the proposed genera would be as distinct genetically as are Onychomys, Reithrodontomys, and Baiomys from one another or from Peromyscus. Thus we agree with the suggestion of Yates et al. (1979) which includes Neotomodon within Peromyscus.

Acknowledgments--We wish to express our gratitude to a number of people who aided in the acquisition of various specimens: C. F. Aquadro, J. W. Bickham, J. R. Choate, J. Laerm, J. Layne, B. S. McDonald, G. McDonald, F. J. Patton, S. J. Patton, and V. L. Patton. We especially would like to thank H. H. Genoways, M. J. Smolen, and S. L. Williams for acquiring specimens of Neotomodon through field work supported by the M. Graham Netting Research Fund through a grant from the Cordelia Scaife May Charitable Trust. We thank I. F. Greenbaum for both his review of the manuscript and technical assistance. We also thank C. W. Kilpatrick, J. Porter, D. J. Schmidly and three anonymous reviewers for their constructive criticisms of the manuscript. This work was supported by a USPHS training grant to J. C. P., by a grant from the American Philosophical Society, and by NSF grant DEB7814195.

SPECIMENS EXAMINED

Peromyscus maniculatus - Texas (Brewster Co.): 10.5 km N Alpine, N = 2. North Carolina (Highlands Co.): 3.2 km W Norton, Coweeta Hydrologic Laboratory, N = 2.

Peromyscus polionotus - Georgia (Barrow Co.): 8.1 km NE Winder, N = 2.

Peromyscus leucopus - Georgia (Clarke Co.): 4.8 km S Athens, N = 3.

Peromyscus floridanus - Florida (Highlands Co.): 8.1 km S, 3.2 km W Lake Placid, N = 3.

Neotomodon alstoni - Mexico (Districto Federal): 1 km N Morelos, N = 5.

Onychomys leucogaster - Texas (Winkler Co): 3.2 km W Wink, N = 1; 10.5 km E Wink, N = 1.

Onychomys torridus - Arizona (Cochise Co.): 5.6 km SE Portal, N = 1; 22.5 km SE Portal, N = 1.

Baiomys taylori - Texas (Galveston Co.): 6.4 km N Texas City, N = 2; (Brazos Co.): Bryan, N = 1.

Reithrodontomys fulvescens - Texas (Galveston Co.): 6.4 km N Texas City, N = 3.

Reithrodontomys megalotis - Kansas (Ellis Co.): 3.2 km W Hays (Relict Area), N = 2.

Ochrotomys nuttalli - Georgia (Clarke Co.): 4.8 km S Athens, N = 2. North Carolina (Highlands Co.): 3.2 km W Norton, Coweeta Hydrologic Laboratory, N = 2.

Neotoma micropus - Texas (Brewster Co.): Alpine, City Dump, N = 1.

Sigmodon hispidus - Georgia (Barrow Co.): 8.1 km NE Winder, N = 4.

Oryzomys palustris - Texas (Galveston Co.): 6.4 km N Texas City, N = 3.