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GENERAL NOTES

TEST OF ALTERNATIVE HYPOTHESES CONCERNING THE ORIGIN OF *REITHRODONTOMYS RAVIVENTRIS*: GENETIC ANALYSIS

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Based upon exomorphologic characteristics (Hooper, 1952; Fisler, 1965), it has been accepted that the origin of the endangered species, *Reithrodontomys raviventris*, was from the parental stock of *R. megalotis*. Shellhammer (1967) investigated standard karyotypes and found no data to refute the hypothesized evolutionary origin of this species. However, in a cladistical analysis of G-banded chromosome data, Hood et al. (1984) concluded that *R. raviventris* and *R. montanus* are more closely related to each other than either is to *R. megalotis*.

Incongruence of the results of the two methodologies is perplexing and, without additional data, investigators must rely on the relative value of the methods to produce credible phylogenies. Warner (1983:282) questioned the value of using G-banded chromosomes in depicting phylogenies. He stated: "It appears that at the current level of technical resolution, chromosomal banding patterns cannot provide sufficient unambiguous information to assess phylogenetic relationships within groups . . . in which there has been extensive repatterning of the karotypes (see Baker and Bickham, 1980). In these groups other types of data (e.g., morphological, immunological, and allozymic) are probably much more informative than are chromosomal data." If Warner is correct in his conclusions, the genic data for *Reithrodontomys*, a group in which karyotypic megaevolution has occurred, should lend support to the systematic hypothesis based on the morphological data (Hooper, 1952; Fisler, 1965) and not that proposed by Hood et al. (1984).

In the following analysis of genic variation within the genus *Reithrodontomys* we provide critical data to discriminate between alternative systematic hypotheses concerning the origin and status of the endangered species, *R. raviventris*. Finally, we document by examination of additional species and loci, the amount of genic evolution that has accompanied the variable, often extensive, amounts of chromosomal evolution in *Reithrodontomys* (Hood et al., 1984). Arnold et al. (1983) examined electrophoretic variation at 20 presumed loci for six species of *Reithrodontomys* (*megalotis, montanus, humulis, fulvescens, sumichrasti,* and *mexicanus*). We examined 30 presumed loci from eight species of *Reithrodontomys* [*raviventris* (both subspecies), *creper, zacatacae, megalotis, montanus, humulis, fulvescens,* and *sumichrasti.*] See Hood et al. (1984) for justification of specific status for *R. zacatacae*.

Methods.—Heart, kidney, and liver homogenates were prepared from each specimen. Starch gel electrophoresis and protein staining techniques followed those of Selander et al. (1971) and Harris and Hopkinson (1977). The following 30 loci were examined (nomenclature and abbreviations follow Harris and Hopkinson, 1977): aconitase (ACON-2), albumin (ALB), alcohol dehydrogenase (ADH), aldolase (ALD), creatine kinase (CK-4), diaphorase (DIA), fumarate hydratase (FH), glucose-6-phosphate dehydrogenase (Gd), glucose phosphate isomerase (GPI), L-glutamate dehydrogenase (GLUD), glutamate oxaloacetate transaminase (GOT-1, GOT-2), glutamate pyruvate transaminase (GPT), glyceraldehyde phosphate dehydrogenase (GAPDH), isocitrate dehydrogenase (ICD-1), lactate dehydrogenase (LDH-1, LDH-2), malate dehydrogenase (MDH-1, MDH-2), malic enzyme (ME-1, ME-2), mannose phosphate isomerase (MPI), peptidase (PEP-A, PEP-B), phosphoglucomutase (PGM-3), phosphogluconate dehydrogenase (PGD), purine nucleoside phosphorylase (NP), sorbital dehydrogenase (SORDH), and superoxide dismutase (SOD-1, SOD-2). Loci were designated numerically with "1" being the most anodally migrating isozyme of an enzyme with the more cathodal loci given increasingly larger numbers. Allozymes of a given locus were designated by "a" representing the most anodal allele. Allele designations and frequencies are given in Table 1. Allelic designations of this study and those of Arnold et al. (1983) do not match because, with the addition of different taxa and the presence or absence of rare alleles, the relative designations of electromorphs change.

Phylogenetic relationships among the species of *Reithrodontomys* were determined by a locus-by-locus cladistical analysis (Patton et al., 1981; Baverstock et al., 1979). Outgroup comparisons were used to determine primitive and derived character states. *Peromyscus maniculatus* was used as the principal outgroup species. Specimens of *Neotoma micropus* and *Sigmodon hispidus* were used as additional outgroups when the nature of the allozymic characters could not be determined from the data for *Peromyscus maniculatus*. An allele shared between one or more of the outgroup taxa and one or more species of *Reithrodontomys* was considered primitive for *Reithrodontomys* (Watrous and Wheeler, 1981).

To determine genetic similarity between the species, Nei's (1972) identity and distance values were also calculated. From the matrices of identity and distance values, cluster analyses were performed using UPGMA (Sneath and Sokal, 1973).

Results and discussion.—Results from the electrophoretic analysis of 30 presumed loci are presented in Table 1. Seven loci were monomorphic in all *Reithrodontomys* examined, and 23 loci were variable in the genus. A locus-by-locus cladistic analysis, using *Peromyscus maniculatus, Neotoma micropus,* and *Sigmodon hispidus* as outgroups, is shown in Fig. 1. A phenogram constructed from clustering analyses (UPGMA) using Nei's (1972) identity and distance values is presented in Fig. 2. Several conclusions can be drawn from our results.

Our samples of *R. raviventris* contained specimens of both subspecies, *R. r. raviventris* and *R. r. halicoetes*. These subspecies generally cannot be distinguished on the basis of external morphological characteristics (Fisler, 1965:92). We found no fixed differences which distinguish the two subspecies. At two polymorphic loci, GPI and ICD-1, an allele was present in one subspecies but not the other (Table 1). However, our small sample sizes could easily result in a failure to detect these alleles in one of the subspecies. It does appear safe to conclude that little genic differentiation has accompanied the morphological differentiation that is the basis for the recognition of the two subspecies.

Four synapomorphies [CK-4(b), ICD-1(a), ICD-1(c), and MPI(d)] document that *R. raviventris* shared a common ancestry with *R. montanus* after diverging from the other six species examined in this study. Therefore, the biochemical data support the conclusions from G-band chromosomal analysis (Hood et al., 1984) rather than the conclusions drawn in studies of morphology (Fisler, 1965). Hooper (1952) pointed out that it is extremely difficult to identify all specimens of *R. montanus* and *R. megalotis*. To date no one has shown that there is sufficient morphological differentiation among the three species to substantiate whether *R. raviventris* originated from *R. megalotis* or *R. montanus*.

A sister clade to the *montanus-raviventris* group is composed of *R. megalotis, R. zacatacae,* and *R. sumichrasti*. These associations appear in both the phenetic and cladistical analyses. Two synapomorphies, PEP-A(c) and PGD(d), identify the three taxa as sharing a common ancestry after diverging from the remaining species of *Reithrodontomys* examined. This same *megalotis* group was recognized in the study of chromosomal banding data (Hood et al., 1984). Within this group extensive chromosomal rearrangements have occured. However, in the 30 loci we examined, no fixed genic differences were found to have accompanied the extensive chromosomal evolution. Although these data do not conclusively show that the chromosomal evolution that distinguishes these three taxa occurred over a short time period, we suggest that the *megalotis* group is a potential example of rapid chromosomal evolution with little or no genic differentiation accompanying the process.

The genic data suggest two other relationships that are defined by a single genic synapomorphy. First, a common ancestry for *R. humulis* and *R. fulvescens* is suggested by ICD-1(b). Hooper (1952) indicated that the most recent common ancestor of *R. humulis* is from the *megalotis-burti* group and not from the *fulvescens* group. Also, Gd(b) suggests a common ancestry for *R. humulis, R. fulvescens, R. montanus, R. megalotis, R. zacatacae,* and *R. sumichrasti* after separating from *R. creper*. It must be noted that *R. humulis* has allele "c" at the loci Gd and not allele "b." Two alternative pathways are possible. One is that allele "c" was derived from the primitive allele as was allele "b." Alternatively, allele "c" may represent a further derived character state of allele "b." We chose to represent the latter possibility because of the synapomorphy between *R. fulvescens* and *R. humulis* and because this interpretation is compatible with the split of the genus into two subgenera (*R. creper* is in the subgenus *Aprodon* and all other species examined are in the subgenus *Reithrodontomys*). However, we recognize that when clades are identified on the basis of a single synapomorphy, care should be taken not to over-extend the systematic implications.

There are several noticeable differences between our results and those of Arnold et al. (1983). The first and most extensive represents differences observed for *R. sumichrasti*. The specimen examined by Arnold et al. (1983) was obtained from the northern end of the range in Jalisco, Mexico, whereas, our specimen was obtained from the southern end of the range in Costa Rica. Hall (1981) suggested that these represent morphologically distinct and geographically isolated populations which the biochemical data suggest may be distinct species. Our small sample size and the geographic origin of the samples for *R. fulvescens* and

TABLE 1.—*Allozyme designations and frequencies for the specimens of* Reithrodontomys *and the three outgroup taxa (*Peromyscus maniculatis, Sigmodon hipidus, *and* Neotoma micropus*). For species in which more than one allele is present, the number in parentheses immediately following the allele designation is the frequency of that allele. Loci and allele designations are identified in the text. Two loci were monomorphic for all specimens; ALD and GPT.*

Taxa	Loci													
	ACON-2	ALB	ADH	CK-4	DIA	FH	Gd	GPI	GLUD	GOT-1	GOT-2	GAPDH	ICD-1	LDH-1
R. montanus	b	a	d	b (0.125) d (0.875)	b	d	b	c	b	d	a (0.25) b (0.75)	b	a (0.25) c (0.75)	c
R. raviventris raviventris	b	a	c	b (0.25) d (0.75)	b (0.25) c (0.75)	d	b	c	b	d	b	b	a (0.25) c (0.75)	c
R. raviventris halicoetes	b	a	c	b (0.25) d (0.75)	b (0.50) c (0.50)	d	b	b (0.25) c (0.75)	b	d	b	b	c	c
R. megalotis	a (0.063) b (0.938)	a	b	d	b (0.50) c (0.50)	d	b	c	a (0.125) b (0.875)	d	b	b	e	c
R. zacatacae	b	a	b	d	b (0.50) c (0.50)	d	b	c	b	d	b	b	e	c
R. sumichrasti	b	a	b	a (0.50) d (0.50)	b	d	b	c	b	d	b	b	e	c
R. fulvescens	b	a	b	d (0.50) f (0.50)	d	d	b	c	b	d	b	b	b (0.50) e (0.50)	c
R. humulis	b	b	b	d	c	d	c	c	b	c	b	a (0.50) b (0.50)	b	c
R. creper	b	c	e	d	b (0.75) c (0.25)	d	a	c	b	b	b	b	d	b
P. maniculatus	b	a	a	c (0.50) e (0.50)	b	c	a	a (0.50) c (0.50)	b	d	a	b	e	c
S. hispidus	b	a	b	d	a	b	a	c	b	a	c	b	e	c
N. micropus	b	—	b	d	b (0.50) c (0.50)	a	—	c	b	a	a	b	e	a

R. humulis may explain some of the other differences found between Arnold et al. (1983) and this study. However, for ALB and PGD, we were unable to obtain the separation of electromorphs for some taxa as reported by Arnold et al. (1983).

Relative to the proposed systematic hypotheses, our data support the conclusions from the G-band chromosomal data of Hood et al. (1984) that *R. raviventris* and *R. montanus* shared a common ancestry after diverging from *R. megalotis* and not the hypothesis based on morphology (Fisler, 1965). We find no support for Warner's contention that G-band data in groups that have undergone extensive chromosomal evolution have little systematic value. Finally, we note that with a more extensive analysis, we were able to add more resolution to the cladogram, which is a hypothesis of the phylogeny of the genus *Reithrodontomys*.

Specimens Examined

Reithrodontomys creper, Costa Rica: San Jose Prov.: 2.2 km E La Trinidad de dota, 2,600 m (2).
Reithrodontomys fulvescens, Texas: Jeff Davis Co.: 10 mi N Fort Davis (1).
Reithrodontomys humulis, North Carolina: Brunswick Co.: 5.3 mi N Wilmington (1).
Reithrodontomys megalotis, California: San Mateo Co.: 10 mi SE Pescadero (1); San Bernardino Co.: 2 mi N, 7 mi E Mentone (2); Los Angeles Co.: Whittier (2). Kansas: Ellis Co.: 2 mi S, 1 mi W Hays (2). Texas: Jeff Davis Co.: 10 mi N Fort Davis (1).
Reithrodontomys zacatacae, Mexico: Durango: 12.4 mi W El Salto (1).
Reithrodontomys montanus, Texas: Garza Co.: 6.6 km NE Southland (3); Culberson Co.: 6 mi N Kent (1).
Reithrodontomys raviventris halicoetes, California: Sonoma Co.: Mouth of Tolay Creek (2).
Reithrodontomys raviventris raviventris, California: Alameda Co.: 2.5 mi W of Newark, Newark Slough (2).
Reithrodontomys sumichrasti, Costa Rica: San Jose Prov.: 2.2 km E La Trinidad de dota, 2,600 m (1).
Peromyscus maniculatis, Pennsylvania: Westmoreland Co.: Powdermill Nature Reserve (2).
Sigmodon hispidus, Texas: Lubbock Co.: Lubbock, 4th and Quaker (1).
Neotoma micropus, Texas: Garza Co.: Post (1).

TABLE 1.—*Continued.*

Loci													
LDH-2	MDH-1	MDH-2	ME-1	ME-2	MPI	PEP-A	PEP-B	PGM-3	PGD	NP	SORDH	SOD-1	SOD-2
b	c	a	b	a	d	a	a	a	e	d	b	a	b
b	c	a	b	a	d	a	a	a	f	d	b	a	b
b	c	a	b	a	d	a	a	a	f	d	b	a	b
b	c	a	b	a	b	c	a	a	d	a	b	a	b
b	c	a	b	a	b	c	a	a	d	a	b	a	b
b	c	a	b	a	b	c	a	a	d	a	b	a	b
b	c	b	a	null	b	a	a	a	b (0.50) c (0.50)	c	b	a	b
b	c	a	b	a	b	a	a	b	e	c	a	a	a
b	c	a	b	a	c	b	a	a	a	a (0.50) b (0.50)	b	a	b
a	c	a	a	b	b	a	a	a	e	e	b	a	b
a	b	—	a	a	a	—	—	a	—	g	—	—	—
b	a	—	a	null	—	—	—	a (0.50) c (0.50)	—	f	—	—	—

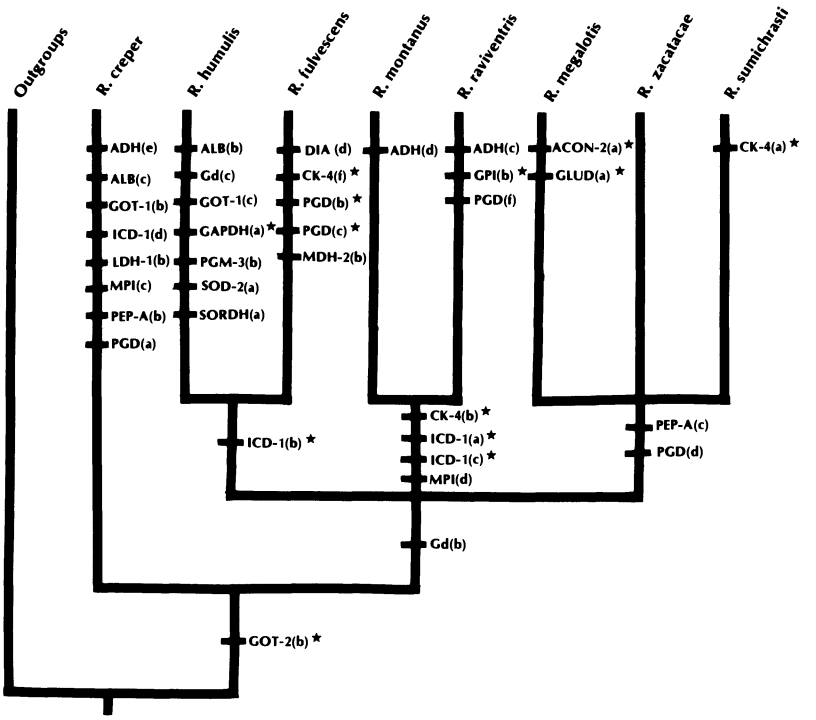

FIG. 1.—Cladogram depicting the phylogenetic relationships among eight species of *Reithrodontomys* from electrophoretic data. Unique, derived characters are indicated along the branches leading to each taxon. A star indicates that the character is polymorphic and not fixed.

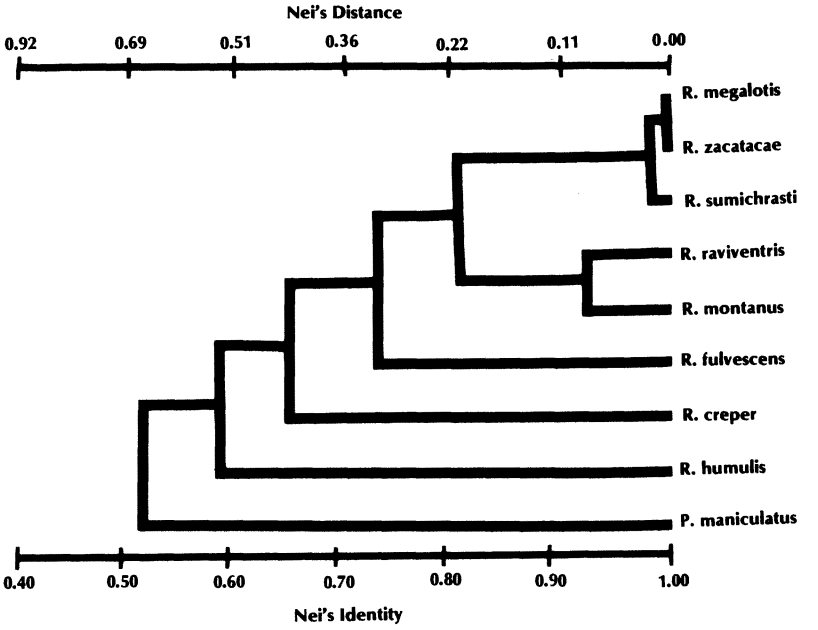

FIG. 2.—Phenogram depicting genetic identity and genetic distance among eight species of *Reithrodontomys* as derived using 30 presumed loci. The cophenetic correlation of the phenogram to the original matrix was 0.96.

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BIOCHEMICAL VARIABILITY IN A POPULATION OF BEAVER

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Beaver, once abundant in North America, were extirpated in many parts of their range during this century (Salyer, 1946). The Carolina beaver (*Castor canadensis carolinensis*) was particularly hard-hit and only recently has begun to recolonize some of its former range. Beaver were restocked in certain refuges in South Carolina and Georgia (Salyer, 1946). By the early 1960s more than a dozen colonies were established on the Savannah River Plant (SRP) in South Carolina (Jenkins and Provost, 1964). After this bottleneck in population numbers, the colonies expanded rapidly and beaver became numerous on the SRP during the late 1970s and early 1980s.

Any population that is subject to drastically reduced numbers for long periods of time should be subjected to drift and characterized by low levels of genetic variability (Spiess, 1977:337–361). Beaver on the SRP presented an ideal opportunity to examine this prediction in a natural population. Our objective was to estimate the levels of genetic variability in the beaver on this site.

Beaver ($n = 48$) were trapped on the Savannah River Plant from December 1982 through March 1983. Liver and muscle samples were taken and stored at −60° C. Thawed tissues were prepared in a spotplate with several drops of grinding buffer and minced with a scalpel (Place and Powers, 1978). A paper wick was placed on the tissues to absorb fluids. Other procedures for starch gel electrophoresis were performed as given in Selander et al. (1971). Electrostarch (Otto Hiller, Madison, Wisconsin; 9.5 g) was mixed with Connaught starch (32 g), and 400 ml of the appropriate buffer was added (Table 1).

Of the 34 proteins analyzed for the two tissue types, only three showed variability. One variant was seen for isocitrate dehydrogenase, and this individual showed the typical three-banded pattern for a dimeric molecule. The most variable loci were esterase-4, which had three alleles and showed two bands in the heterozygotes, and sorbitol dehydrogenase, which had two alleles and heterozygotes showed the banding pattern expected for a tetramer. The proportion of polymorphic loci using a 1% criterion was 8.8%, whereas at the 5% level it was 5.9%. The multi-locus heterozygosity value was 0.010. This population of beaver showed a level of variation which is slightly lower than the general value for mammals (Nevo, 1978; H = 0.033).

The SRP beaver had low levels of genetic variability as predicted from the drift hypothesis. It is possible that this population lost genetic variability as a result of a bottleneck; however, other populations that have not been subjected to drastic reductions in numbers should be examined for levels of genetic variability but such a population may not exist. If the drift hypothesis is correct, those populations should have higher levels of genetic variability than those observed in this population. On the other hand, beaver could be a species with low levels of genetic variability throughout its range even without reduction in population