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FINAL REPORT

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THE ENDANGERED SPECIES ACT, SECTION 6

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Project No: E-1-4

ENDANGERED AND THREATENED SPECIES CONSERVATION

Job No. 36

Houston Toad Taxonomic Relationships

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FINAL REPORT

STATE:	Texas	**PROJECT NO.:**	E-1-4

PROJECT TITLE: Endangered and Threatened Species Conservation.

PERIOD COVERED: September 1, 1991 through August 31, 1992.

JOB NUMBER: 36

JOB TITLE: Houston Toad Taxonomic Relationships.

JOB OBJECTIVE: To investigate the taxonomic relationship between *Bufo houstonensis* and other closely related congeners, specifically, *Bufo americanus* and *Bufo terrestris*.

ACCOMPLISHMENTS

See attached report.

SIGNIFICANT DEVIATIONS

None.

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ABSTRACT

The products of 20 enzyme-coding loci were examined for 147 toads representing 4 of the 6 members of the *Bufo americanus* species group, including the endangered *B. houstonensis*. No diagnostic alleles were found for any of the taxa examined. The data support previously hypothesized phylogenetic relationships and reflect current zoogeographic positions of the taxa involved. A pattern of reticulate hybridization among taxa and between populations in historic times is suggested. The data do not refute the hypothesis that *Bufo houstonensis* is a distinct evolutionary lineage. They also do not suggest genetic divergence between isolated populations of *B. houstonensis*. Examination of sequence or restriction-site variation in nuclear and mt-DNA is required to reveal in detail the extent of reticulation among the lineages examined in this study.

Systematic Status of the Endangered Houston Toad, *Bufo houstonensis*, Based on Allozymic Comparisons

Section 6 Research Report

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Introduction and Background

The Houston Toad, ***Bufo houstonensis***, is a small member of the ***Bufo americanus*** species group. It is endemic to southcentral Texas and occurs as relict populations on sandy soils in Post-Oak Woodland and Gulf Coastal Prairie vegetation communities. ***Bufo houstonensis*** is believed to be closely related to ***B. americanus*** and to have recently diverged from it sometime during the Pleistocene (Blair, 1972a). Prior to it's description the Houston Toad had been confused by some (e.g. Harwood, 1932) with another member of the ***americanus*** group, ***B. terrestris***, and subsequently had been considered by some (e.g. Blair, 1957) conspecific with ***B. americanus***. ***Bufo americanus charlesmithi*** occurs in northeast Texas and along the Red River to Cooke County, whereas ***B. terrestris*** is native to the southeastern United States and most closely approaches the range of the Houston Toad in eastern Louisiana and southern Mississippi east of the Mississippi River. Concerns over the future existence of ***B. houstonensis*** on the landscape prompted the U. S. Fish and Wildlife Service to list it as an endangered species in 1970. A Recovery Plan (USFWS, 1984) was established, and one of the tasks identified in that plan was to determine the systematic relationship between ***Bufo houstonensis*** and ***B. americanus charlesmithi***. This report addresses that question by examining the genetic relationships among 4 members of the *americanus* species group which occur within close geographic proximity to each other in the southcentral United States using allozyme elctrophoresis.

Methods

Specimens from the following taxa and localities were examined: *Bufo houstonensis*; Bastrop County, Texas (25 specimens), Leon County, Texas (11 specimens): *Bufo americanus*; Jackson County, Arkansas (23 specimens), Cooke County, Texas (14 specimens), Lamar County, Texas (6 specimens): *Bufo terrestris*; Pearl County, Mississippi (24 specimens): *Bufo woodhousii velatus*; Rusk County, Texas (6 specimens), Marion County, Texas (8 specimens), Smith County, Texas (11 specimens), Nacogdoches County, Texas (4 specimens): *Bufo woodhousii woodhousii*; Austin County, Texas (1 specimen), Montgomery County, Texas (3 specimens), Guadalupe County, Texas (5 specimens), Robertson County, Texas (1 specimen), Freestone County, Texas (3 specimens), Limestone County, Texas (1 specimen), Falls County, Texas (1 specimen).

The products of 20 enzyme-encoding loci were examined using standard horizontal starch-gel electrophoresis (Murphy et al., 1990). Genetic distances were calculated among the species using the program BIOSYS-1 (Swofford and Selander, 1981), and these distances were used to construct UPGMA phenograms and Distance Wagner trees (Swofford and Olsen, 1990).

The loci examined were aspartate amino transferase, both mitochondrial (AAT-M) and supernatant (AAT-S) forms; two aconitase hydratase loci (ACO-1 and ACO-2); adenylate kinase (AK); creatine kinase (CK); fructose-bisphosphatase (FBP); glyceraldehyde-3-phosphate dehydrogenase (G3PDH); β-glucuronidase (β-GLUR); glutathione reductase (GR); two isocitrate dehydrogenase loci (IDH-1 and IDH-2); malate dehydrogenase (MDHP); mannose-6-phosphate isomerase (MPI); three peptidase loci (PEP-A, PEP-B, and PEP-D); phosphoglucomutase (PGM); pyruvate kinase (PK); and superoxide dismutase (SOD). Staining for all enzymes followed Murphy et al. (1990).

Results

Twelve loci were polymorphic within or among the species examined (Table 1). None of the species or subspecies showed fixed differences at any of these loci, although the allelic frequencies were often quite different among the species. The Rogers' genetic distances (Table 2) among the taxa ranged from a low of 0.126 (*Bufo woodhousii woodhousii* to *Bufo woodhousii velatus*) to a high of 0.274 (*Bufo terrestris* to *Bufo w. woodhousii*; Table 2). *Bufo houstonensis* was most similar to *B. americanus* (Rogers' D = 0.142) and most divergent from*Bufo terrestris* (D = 0.212). Among the taxa examined, only *B. w. woodhousii* and *B. w. velatus* were more similar in their allozymes (D = 0.126) than *B. houstonensis* and *B. americanus*.

In the UPGMA phenogram based on genetic distances (Fig. 1), *B. americanus* and *B. houstonensis* cluster together, as do *B. w. woodhousii* and *B. w. velatus*. *Bufo terrestris* falls outside of the other species. The Distance Wagner tree is very similar, except that the mid-point rooting of this tree falls between *B. w. woodhousii* and *B. w. velatus* on one hand and *B. terrestris, B. houstonensis,* and *B. americanus* on the other (Fig. 2).

Table 1. Allelic frequencies for 20 enzyme-encoding loci in seven groups of southeastern *Bufo*. Key to groups: 1: *Bufo americanus*, Arkansas; 2: *Bufo americanus*, Texas; 3: *Bufo houstonensis*, Bastrop County; 4: *Bufo houstonensis*, Leon County; 5: *Bufo terrestris*, Mississippi; 6: *Bufo woodhousii velatus*, Texas; 7: *Bufo woodhousii woodhousii*, Texas.

Locus	Group 1 (n=23)	2 (n=20)	3 (n=25)	4 (n=11)	5 (n=24)	6 (n=29)	7 (n=15)
AAT-M							
A	.000	.000	.000	.000	.000	.017	.000
B	1.000	1.000	1.000	1.000	.979	.983	1.000
C	.000	.000	.000	.000	.021	.000	.000
AAT-S							
A	.935	.425	.280	.227	.292	.190	.033
B	.000	.000	.380	.545	.292	.328	.000
C	.000	.350	.000	.000	.125	.207	.767
D	.065	.250	.340	.227	.292	.276	.200
ACO-1							
A	.043	.000	.580	.455	.750	.586	.567
B	.957	.600	.420	.545	.250	.414	.433
C	.000	.400	.000	.000	.000	.000	.000
ACO-2							
A	.543	.025	.640	.182	.083	.586	.867
B	.457	.475	.040	.045	.396	.414	.133
C	.000	.500	.320	.773	.521	.000	.000
AK							
A	.783	1.000	1.000	1.000	.458	1.000	1.000
B	.217	.000	.000	.000	.542	.000	.000
CK							
A	.217	.325	.300	.455	.000	.638	.433
B	.196	.675	.160	.545	.229	.362	.567
C	.587	.000	.540	.000	.771	.000	.000
FBP							
A	.391	.000	.000	.000	.688	.000	.000
B	.239	.825	.520	.955	.313	1.000	1.000
C	.370	.175	.480	.045	.000	.000	.000
G3PDH							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 1 (cont).

Locus	Population 1 (n=23)	2 (n=20)	3 (n=25)	4 (n=11)	5 (n=24)	6 (n=29)	7 (n=15)
β-GLUR							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
GR							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IDH-1							
A	.000	.000	.000	.000	.000	.000	.633
B	.109	.625	.240	.091	.417	.966	.367
C	.891	.375	.760	.909	.583	.034	.000
IDH-2							
A	.435	.000	.300	.045	.000	.000	.000
B	.565	1.000	.700	.955	1.000	1.000	1.000
MDHP							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
MPI							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEP-A							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PEP-B							
A	.435	.200	.640	.545	.104	.086	.067
B	.565	.800	.340	.455	.500	.914	.933
C	.000	.000	.000	.000	.396	.000	.000
PEP-D							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
PGM							
A	.000	.000	.000	.000	.625	.069	.000
B	1.000	.900	.880	.864	.292	.690	.467
C	.000	.100	.120	.136	.063	.241	.533
D	.000	.000	.000	.000	.021	.000	.000
PK							
A	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Table 1 (cont).

Locus	1 (n=23)	2 (n=20)	3 (n=25)	4 (n=11)	5 (n=24)	6 (n=29)	7 (n=15)
SOD							
A	.848	.475	.060	.455	.313	.414	.267
B	.130	.000	.000	.000	.292	.000	.000
C	.022	.525	.000	.000	.083	.552	.000
D	.000	.000	.160	.273	.000	.000	.667
E	.000	.000	.000	.000	.000	.017	.067
F	.000	.000	.620	.273	.000	.000	.000
G	.000	.000	.160	.000	.292	.017	.000

Table 2. Genetic distances among five taxa of southeastern *Bufo*. Below diagonal: Modified Rogers' distance (Wright, 1978); above diagonal: Rogers' (1972) genetic distance.

Population	1	2	3	4	5
B. americanus	*****	.142	.220	.186	.232
B. houstonensis	.232	*****	.212	.179	.208
B. terrestris	.317	.307	*****	.232	.274
B. w. velatus	.270	.290	.352	*****	.126
B. w. woodhousii	.336	.323	.399	.243	*****

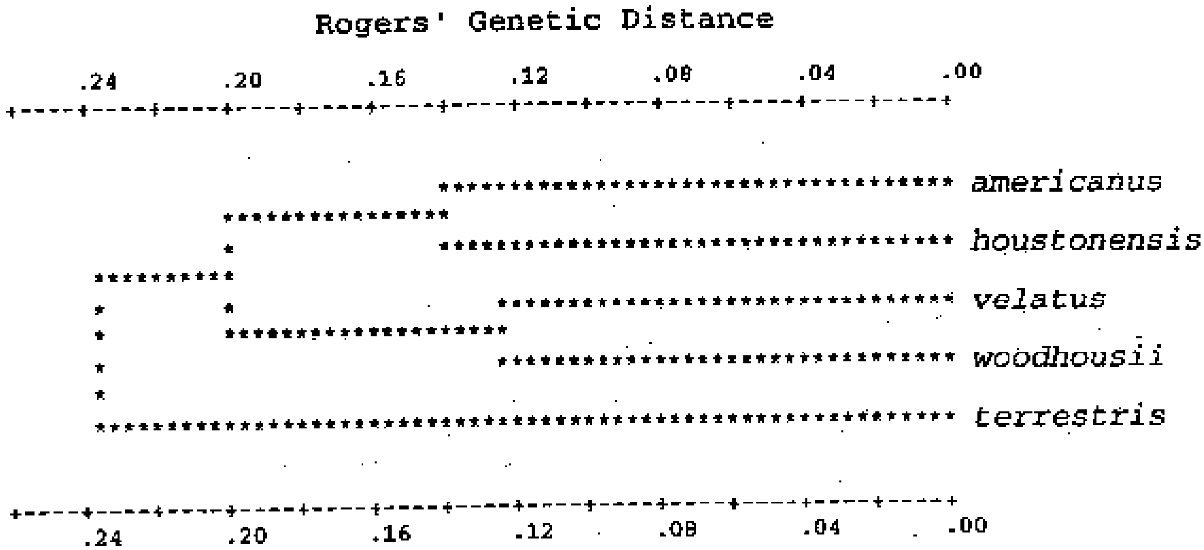

Figure 1. UPGMA phenogram of five taxa of *Bufo* from the southeastern United States.

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     Distance from root (Rogers' genetic distance)

.00       .02       .04       .06       .08       .10
+----+----+----+----+----+----+----+----+----+----+----


      ****************************** americanus
  *****
***   ************************ houstonensis
* ************************************************* terrestris
*
*                  ***************** velatus
********************
                   ******************************* woodhousii

.00       .02       .04       .06       .08       .10
+----+----+----+----+----+----+----+----+----+----+----
```

Figure 2. Distance Wagner tree of five taxa of *Bufo* from the southeastern United States.

Discussion

As has been suggested (A. P. Blair, 1957; W. F. Blair, 1963), *B. houstonensis* is closely related to *B. americanus*, and the two species have no known fixed differences in allozymes (Table 1). However, the same appears to be true of all the species examined in this study, including several species pairs that occur sympatrically. At any given sympatric locality, or given any two specific populatons, it is often possible to identify fixed allozymic differences between species (e.g. Hillis et al., 1984), but when populations from wide geographic areas are pooled (as in the present study), these differences become blurred. It is likely that the reason for this lack of fixed differences is interbreeding and hybridization among the species, which introduce alleles from one species into the gene pool of another. Species of the *Bufo americanus* group are extensively interfertile, and hybrids are known wherever two or more species occur sympatrically (Brown, 1967, 1971; Cory and Manion, 1955; Henrich, 1968; Hillis et al., 1984; Sanders, 1961; Volpe, 1959; Zweifel, 1969). Typically this occurs when premating isolating mechanisms have been breached due to natural or anthropogenic modifications of the landscape. This can lead to reinforcement of species boundaries in certain situations (Jones, 1973) or the disappearance of one or more species in areas of sympatric contact (Sullivan, 1986). This study indicates that past hybridization has been extensive enough to prevent the fixation of unique (diagnostic) alleles in any of the species examined in this report.

Should *Bufo houstonensis* be recognized as a distinct species? This species appears to be about as divergent as the other species in this group (Fig. 2), and has known (albeit minor) morphological (Sanders, 1953) and auditory (Brown, 1973) differences compared to its closest relative, *Bufo americanus*. These differences, combined with its geographical isolation and genetic divergence, indicate that *Bufo houstonensis* is a distinct evolutionary lineage (Frost and Hillis, 1990; Frost et al., 1992) and should continue to be recognized as a distinct species.

Future Work

To look in detail at the extent of reticulation among the *Bufo americanus* group lineages in the southeastern United States, it will be necessary to examine sequence or restriction-site variation in nuclear and mitochondrial DNA genes. Such studies would be useful for examining relationships among populations within the currently recognized species, and would present a clearer picture of the distinctiveness of the species within the *Bufo americanus* group. The allozyme data presented here suggest a complex and reticulating history among the species of the *B. americanus* group, but are not sufficient in themselves to illuminate the details of this history.

In addition, thorough morphometric and audiospectrographic analyses of the *Bufo americanus* group in the southeastern United States (especially eastern Texas) are needed to delimit the species in this group, and to determine the effects of hybridization on the ability of these toads to maintain their individual identities. A coordinated effort is required to integrate molecular data on hybridization with the morphological and sound analyses.

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