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Ancestry of an Isolated Subspecies of Salamander, *Ambystoma tigrinum stebbinsi* Lowe: The Evolutionary Significance of Hybridization

THOMAS R. JONES,*,[1] ERIC J. ROUTMAN,† DAVID J. BEGUN,*,[2] AND JAMES P. COLLINS*

*Department of Zoology, Arizona State University, Tempe, Arizona 85287-1501; and †Department of Biology, San Francisco State University, San Francisco, California 94132

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Most phylogenetic systematists assume speciation results in dichotomously branching phylogenies. Hybridization that gives rise to a new lineage can produce character homoplasy that might obscure a species' true history. We report the results of a restriction-enzyme analysis of mitochondrial DNA (mtDNA) variation in three tiger salamander subspecies (*Ambystoma tigrinum mavortium, Ambystoma tigrinum nebulosum,* and *Ambystoma tigrinum stebbinsi*) and compare the results to studies of morphological and allozymic variation in these taxa. Allozymically, *A. t. mavortium* and *A. t. nebulosum* share most of their genomes (although each has several unique alleles), yet color pattern and mtDNA haplotypes are distinct. Color pattern and allozyme data suggest that *A. t. stebbinsi* shares a common ancestor with *A. t. mavortium,* while the *A. t. stebbinsi* mtDNA haplotype is derived from an *A. t. nebulosum* haplotype. Thus, our data suggest that *A. t. stebbinsi* originated through hybridization between *A. t. mavortium* and *A. t. nebulosum.* That hybridization can produce recognizably distinct evolutionary entities has long been recognized for plants, but the evolutionary significance of hybridization in animals should be examined more closely. Conservation agencies must recognize that hybrids and hybrid taxa are not necessarily evolutionary "mistakes," and they might have significant importance in the production of natural biodiversity.

INTRODUCTION

The basic principles of modern phylogenetic systematics suggest that speciation results in a dichotomously branching phylogeny that can be reconstructed by finding hierarchically nested sets of shared, derived characters. Often, however, incompatible character distributions frustrate the choice of a "best" tree. One might apply successive weighting (Farris, 1969, 1988) or additional data (Kluge, 1989) to resolve character ambiguity, or use inferential statistics to justify choice of one branching pattern over another (e.g., Felsenstein, 1988; Templeton, 1986). Nonetheless, incompatible character distributions could result from events such as hybridization (McDade, 1992; Smith, 1992). Although, the evolutionary significance of hybridization is generally accepted by botanists (Wagner, 1983), among zoologists it continues to exist on the fringes of mainstream phylogenetics [exceptions include hybridization events that have given rise to polyploid lineages with clonal or hemiclonal reproduction (e.g., Duellman and Trueb, 1986; Dawley and Bogart, 1989; Echelle *et al.,* 1989; Sites *et al.,* 1990; Spolsky *et al.,* 1992; and references therein)]. However, the importance of introgressive hybridization to speciation has been hypothesized for several kinds of fishes (e.g., Smith, 1973), for which there is recent empirical support in some groups (DeMarais *et al.,* 1992; Smith, 1992; Dowling and DeMarais, 1993; Echelle and Echelle, 1993). Hybridization might be a generally important component of diversification, yet because hybrids often exhibit characteristics of both parental forms hybridization is difficult to document or reconcile with cladistic methods, especially if characters have differing modes of transmission (see McDade, 1990, 1992; Smith, 1992).

We report the results of a study of mtDNA restriction site variation in a geographically subdivided and variable species, the tiger salamander (*Ambystoma tigrinum* Green). *A. tigrinum* is distributed throughout much of North America, comprising eight subspecies recognized by color patterns of metamorphosed animals (Dunn, 1940; Stebbins, 1985). We focus on *A. t. stebbinsi,* a geographically restricted race whose taxonomic status has been debated (Gehlbach, 1965; Stebbins, 1985; Jones *et al.,* 1988), plus *A. t. mavortium* and *A. t. nebulosum,* and compare our results to studies

[1] Present address: Biology Department, Grand Canyon University, 3300 W. Camelback Road, Phoenix, AZ 85017.

[2] Present address: Section of Ecology and Evolution, Center for Population Biology, University of California, Davis, CA 95616.

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of morphological and allozymic variation in these races (Pierce and Mitton, 1980; Pierce *et al.*, 1981; Collins *et al.*, 1988; Fernandez, 1988; Fernandez and Collins, 1988; Jones *et al.*, 1988; Jones (1989); Jones and Collins, 1992). Our general goal is understanding genetic subdivision within and between races of southwestern United States *A. tigrinum* (Collins *et al.*, 1980; Jones, 1989). In this paper we use mtDNA restriction site phylogenies to address the question, has hybridization and the subsequent creation of new combinations of genes been an important source of evolutionary potential in southwestern *Ambystoma*? We discuss our results in the broader context of current thought regarding the evolutionary significance of hybridization, as well as practical application to issues of conservation and biodiversity.

MATERIALS AND METHODS

We analyzed mtDNA variation in populations of *A. t. stebbinsi* from the San Rafael Valley in Arizona, *mavortium* from eastern New Mexico and presumably introduced populations in southeastern Arizona, *nebulosum* from Arizona and New Mexico, and a contact zone between *mavortium* and *nebulosum* in central New Mexico (Appendix); *mavortium* is distributed primarily in grasslands and desert east of the Rocky Mountains, *nebulosum* is widely distributed in the southern Rocky Mountains and intermontane southwestern United States (Gehlbach, 1967). Detailed distribution maps are available in Stebbins (1986), Jones *et al.* (1988), Jones (1989), and Jones and Collins (1992). Allozymic frequencies were available for all populations except sites M11-M16, MBA, and S9 (Jones *et al.*, 1988; Jones, 1989; Jones and Collins, 1992). Comparative mtDNA data were also available for *mavortium* from Colorado and Nebraska (Routman, 1993).

We removed liver or skeletal muscle samples from animals killed in a lethal solution of Tricane (MS 222), stored them at −78°C, and then ground tissues to power in liquid nitrogen. We used protocols in Hillis and Davis (1986) for preparation of nucleic acids (see Routman, 1993) and followed DeSalle *et al.* (1986) for restriction digestion, electrophoresis, and autoradiography. Eleven restriction endonucleases with 6-bp recognition sequences were used to digest mtDNA samples (Table 1). We transferred DNA from gels to nylon filters using a modified Southern blotting technique (Maniatis *et al.*, 1982), and filters were probed with ^{32}P-labeled mtDNA and exposed to X-ray film to allow visualization of mtDNA fragments. Hybridization techniques followed DeSalle *et al.* (1986), substituting random primer DNA labelling for nick translation. We probed filters initially with cloned *Xenopus laevis* mtDNA; DNA of most individuals was probed with *A. t. tigrinum* mtDNA that had been cloned into plasmid PUC 19 (Routman, 1993). We mapped restriction sites with double digestion, partial digestion, and partial probing. Each unique combination of restriction sites denotes a haplotype. We were unable to map a few haplotypes, which we deleted from the analysis. However, none of those was similar to haplotypes found in subspecies other than their own and would not have affected our conclusions regarding subspecific relationships.

We used parsimony analysis of presence/absence mtDNA restriction site data with the branch and bound algorithm in PAUP 3.1.1 (Swofford, 1993), weighting all character states equally. Trees were rooted with *A. t. tigrinum* mtDNA haplotype TC (Routman, 1993), the most common among 13 populations of *A. t. tigrinum* in Missouri and Illinois (Routman, 1993). Initially, ingroup taxa were unconstrained; in later analyses we forced the monophyly of *stebbinsi* and *mavortium*.

RESULTS

Including the outgroup, we mapped 12 mtDNA haplotypes (Table 1). We found 3 haplotypes among 9 *mavortium* populations (n = 36) and 7 haplotypes in 10 *nebulosum* populations sampled (n = 21) (Table 2). No haplotypes were shared between *mavortium* and *nebulosum*. Within the zone of contact between subspecies 1 population (n = 2) contained both *mavortium* (A1-m) and *nebulosum* (A56-n) haplotypes, and samples from 4 populations (n = 8) had only *nebulosum* mtDNA. Only 1 mtDNA haplotype (A31-s) occurred in samples of 7 *A. t. stebbinsi* populations (n = 17). This haplotype was not found in either *mavortium* or *nebulosum*. A31-s differs from the most common *nebulosum* haplotype (A56-n) and other hyplotypes in that clade (Fig. 1) by the absence of one site, *XmnI*-3.

Cladistic analysis revealed three equally most parsimonious trees each with 16 steps; consistency (CI) and retention (RI) indices were 0.750 and 0.882, respectively, informative characters only (Fig. 1). Note that the *stebbinsi* haplotype (A31-s) falls well within the *nebulosum* clade in each tree. Constraining monophyly of *stebbinsi* and *mavortium* produced four trees with 20 steps (4 steps longer than unconstrained cladograms; CI = .600, RI = .765). The differences between the trees are statistically significant [Wilcoxon signed rank test, P = 0.02 (see Templeton 1983; 1986)].

DISCUSSION

Phylogenetic History of A.t. stebbinsi

Our mtDNA results are best understood relative to color pattern and allozymic variation in *A. tigrinum* in the American Southwest.

Color pattern variation. *A. t. mavortium* and *A. t. nebulosum* are largely allopatric and have different

TABLE 1

Character State Distributions for 22 Mapped Restriction Sites for *A. tigrinum* mtDNA

	Restriction sites																					
	*Bam*HI			*Bst*EII	*Eco*RI			*Eco*RV				*Stu*I				*Xba*I		*Xmn*I				
Haplotype	−1	−2	−3	−2	−2	−3	−4	−1	−2	−3	−5	−1	−3	−4	−5	−2	−4	−2	−3	−4	−5	−6
TC-t	0	1	0	1	1	1	0	1	1	1	0	1	1	0	0	1	0	1	0	1	0	1
A1-m	0	1	1	0	1	0	0	0	1	0	0	1	1	0	0	1	0	1	0	1	0	1
A6-m	0	1	1	0	1	0	0	0	1	0	1	1	1	0	0	1	0	1	0	1	0	1
A16-m	0	1	1	0	1	0	0	0	1	0	1	1	1	0	0	1	0	0	0	1	0	1
A31-s	0	1	1	0	1	0	0	0	1	1	0	0	1	1	0	0	0	1	0	0	1	0
A56-n	0	1	1	0	1	0	0	0	1	1	0	0	1	1	0	0	0	1	1	0	1	0
A57-n	0	1	1	0	1	0	0	0	1	1	0	0	1	1	1	0	0	1	1	0	1	0
A68-n	1	0	0	0	1	1	0	0	1	1	0	1	0	0	0	0	1	1	0	1	0	0
A69-n	0	1	1	0	1	0	1	0	1	1	0	0	1	1	0	0	0	1	1	0	1	0
A71-n	0	1	1	0	1	0	0	0	1	1	0	0	1	1	0	0	0	1	1	0	1	0
A73-n	0	1	1	0	0	0	0	0	1	1	0	0	1	1	0	0	0	1	1	0	1	0
A76-n	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	1	0

Note. Haplotype designations (e.g., TC, A1) are followed by a lowercase letter indicating the subspecies to which they were confined (t = *tigrinum*, m = *mavortium*, s = *stebbinsi*, n = *nebulosum*). Character states are 1 = present, 0 = absent. Invariant mapped sites are not shown in the table, including the following enzymes: *Bcl*I, *Bgl*II, *Pst*I, and *Pvu*II.

TABLE 2

Distribution of mtDNA Haplotypes in Three Subspecies of *A. tigrinum*

	Haplotype										
Population	A1-m	A6-m	A16-m	A31-s	A56-n	A57-n	A68-n	A69-n	A71-n	A73-n	A76-n
A. t. mavortium											
M08	1	1	—	—	—	—	—	—	—	—	—
M10	—	—	2	—	—	—	—	—	—	—	—
M11	—	—	2	—	—	—	—	—	—	—	—
M12	—	—	2	—	—	—	—	—	—	—	—
M13	—	—	5	—	—	—	—	—	—	—	—
M14	1	—	5	—	—	—	—	—	—	—	—
M15	—	9	—	—	—	—	—	—	—	—	—
M16	4	2	—	—	—	—	—	—	—	—	—
MBA	—	—	2	—	—	—	—	—	—	—	—
A. t. nebulosum											
N09	—	—	—	—	—	—	—	—	—	—	2
N14	—	—	—	—	—	—	1	1	—	—	—
N17	—	—	—	—	—	—	2	—	—	—	—
N18	—	—	—	—	—	—	—	—	—	2	—
N20	—	—	—	—	2	—	—	—	—	—	—
N21	—	—	—	—	3	—	—	—	—	—	—
N22	—	—	—	—	2	—	—	—	—	—	—
N25	—	—	—	—	2	—	—	—	—	—	—
N27	—	—	—	—	1	1	—	—	—	—	—
N32	—	—	—	—	2	—	—	—	—	—	—
N35	—	—	—	—	—	—	—	—	2	—	—
A. t. stebbinsi											
S01	—	—	—	3	—	—	—	—	—	—	—
S02	—	—	—	6	—	—	—	—	—	—	—
S03	—	—	—	2	—	—	—	—	—	—	—
S05	—	—	—	2	—	—	—	—	—	—	—
S06	—	—	—	2	—	—	—	—	—	—	—
S08	—	—	—	1	—	—	—	—	—	—	—
S09	—	—	—	1	—	—	—	—	—	—	—
NM Contact zone											
X01	—	—	—	—	2	—	—	—	—	—	—
X04	1	—	—	—	1	—	—	—	—	—	—
X05	—	—	—	—	2	—	—	—	—	—	—
X09	—	—	—	—	2	—	—	—	—	—	—
X10	—	—	—	—	2	—	—	—	—	—	—

Note. Numerals indicate number of individuals in a population with a particular haplotype. See Appendix for localities.

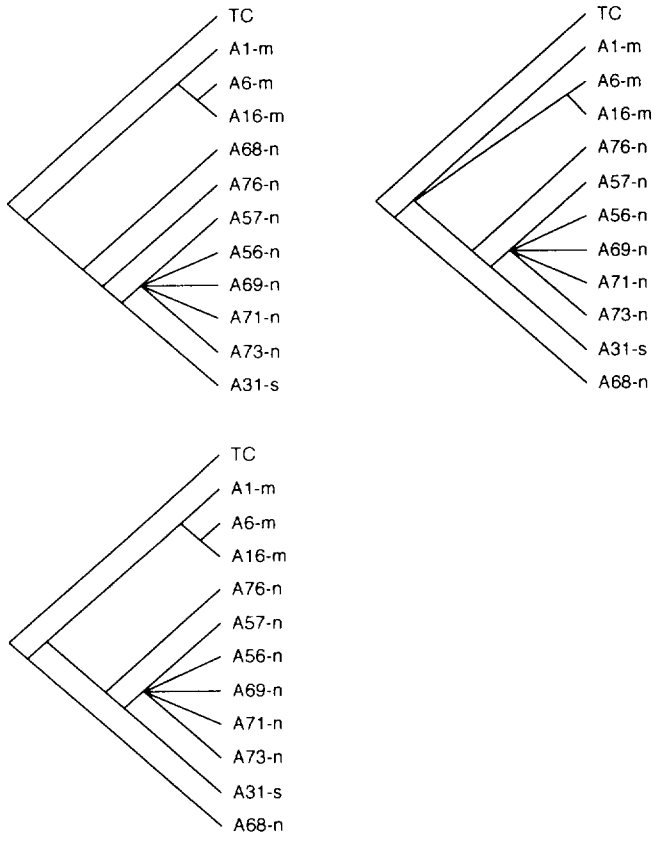

FIG. 1. Three equally most parsimonious cladograms based on analysis of mtDNA restriction sites found in selected populations of *A. t. mavortium, A. t. nebulosum,* and *A. t. stebbinsi;* outgroup is the most common haplotype detected in a survey of *A.t. tigrinum* (Routman, 1993). See Table 1 for haplotype terminology.

and distinctive color patterns. Adult, metamorphosed *mavortium* have narrow to broad vertical bars or large blotches sometimes reduced to distinct spots, yellow to olive in color, on the dorsum and sides of the body. Metamorphosed *nebulosum* can vary from bright yellow with black spots or reticulations, to nearly all black with small yellow flecks (Fernandez, 1988; Fernandez and Collins, 1988).

About 60% of mature, metamorphosed, field-collected *stebbinsi* from the San Rafael Valley, Arizona have dorsal color patterns indistinguishable from *mavortium* color patterns. Others have a distinctive, reticulate dorsal pattern in which the light coloration forms a branching network of light on dark ground color with scattered light spots, and a uniformly dark venter (Collins *et al.,* 1988; Jones *et al.,* 1988); this pattern does not conform to any reported for *mavortium* or *nebulosum* (Dunn, 1940; Gehlbach, 1967; Stebbins, 1985). Immature animals conforming to Lowe's (1954) original description and raised in the laboratory, developed either *mavortium* or reticulate patterns (Jones *et al.,* 1988). Although some *stebbinsi* have a unique reticulate color pattern, the *mavortium* pattern in a large percentage of *stebbinsi* suggests that *stebbinsi* shares a common history with *mavortium.*

Allozyme variation. Throughout their ranges, *nebulosum* and *mavortium* form genetically cohesive units, and are both characterized by several unique alleles (Pierce and Mitton, 1980; Pierce *et al.,* 1981; Jones, 1989; Jones and Collins, 1992). There are no fixed, allozymic differences between these taxa in the southern portion of their distribution (Jones and Collins, 1992), while in Colorado *mavortium* and *nebulosum* share no alleles at LDH-2 (Pierce and Mitton, 1980). Genetic variability is relatively high in both taxa: for 23 populations of *nebulosum* (22 loci) and 9 populations of *mavortium* (21 loci), respectively, mean heterozygosity ($\bar{H}$ ± standard error) = .118 ± .033 and .143 ± .046; percent locus polymorphism = 72.7 and 57.1; mean number of alleles per locus = 2.1 ± 0.2 and 2.0 ± 0.2 (Jones, 1989; Jones and Collins, 1992; Jones, unpublished).

A. t. stebbinsi was virtually monomorphic at 21 loci (Jones *et al.,* 1988); only two individuals were polymorphic: one for one allele at GPI, the other for one IDH allele. Genetic variability for 7 populations of *stebbinsi* was quite low: $\bar{H}$ = .0015 (among the lowest for salamanders), percent locus polymorphism = 9.5, mean number of alleles per locus = 1.1 ± 0.07 (Jones and Collins, unpublished). When compared to *mavortium, nebulosum,* and other *A. tigrinum* subspecies (Jones, 1989) this suggests that *stebbinsi* experienced a severe or prolonged bottleneck (see Leberg, 1992).

Based on allozymic variation Jones *et al.* (1988) suggested that *stebbinsi* was more closely related to *mavortium* than to *nebulosum.* Although cladistic analysis of allozymes suggests a sister group relationship between *stebbinsi* and *mavortium* (Fig. 2; Jones, 1989), a close look at the data suggests a more complex interpretation. Three allozymic loci are of particular interest for determining the ancestry of *stebbinsi.* At MPI *stebbinsi* is fixed for a "*mavortium* allele" (MPI^{110}). MPI^{110} is absent in all New Mexico and Arizona *nebulosum* thus far examined, except for 5 populations located near a *mavortium–nebulosum* contact zone in west-central New Mexico (Jones and Collins, 1992). Those populations also exhibit other, more typical *mavortium* alleles. *A. t. stebbinsi* is also fixed for $ACON\text{-}1^{95}$, an allele 20 times more frequent in *mavortium* (0.178, n = 42) than in *nebulosum* (0.009, n = 164) populations sampled outside of the influence of the contact zone (Jones, 1989; Jones and Collins, 1992). At MDH-1 *stebbinsi* is fixed for a common *nebulosum* allele ($MDH\text{-}1^{92}$; detected in 18 of 23 populations) that is absent in *mavortium* (Jones, 1989; Jones and Collins, 1992). These data are difficult to reconcile with the hypothesis that *stebbinsi* is derived exclusively from either *mavortium* or *nebulosum.*

Our mtDNA restriction site analysis suggests a dif-

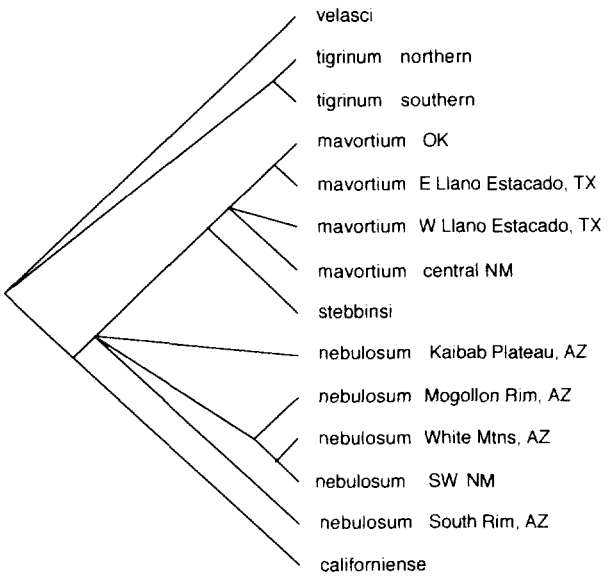

FIG. 2. Cladogram based on analysis of allozymes in selected *A. tigrinum*. All data and choice of outgroups (*A. t. tigrinum* and *A.t. velasci*) were based on analyses in Jones (1989). Allozymes were coded as present or absent. The cladogram is a strict consensus tree of 12 equally parsimonious trees [length 113 steps, CI = .473 (informative sites only), RI = .556]. Trees were constructed with the heuristics option in PAUP 3.1.1 (Swofford, 1993). The OTU's are geographic population composites of *mavortium* from Oklahoma, Texas, and New Mexico, and of Arizona and New Mexico *nebulosum, stebbinsi* from three San Rafael Valley sites, a Michigan sample of *tigrinum,* two southern (North Carolina and South Carolina) *tigrinum* populations, nine *californiense* populations, and one population of *velasci* from Durango, Mexico (site 9, Shaffer, 1983).

ferent relationship among *mavortium, nebulosum,* and *stebbinsi* than that determined using allozymes and morphology. While mtDNA data indicate the maternal genome of *stebbinsi* is derived directly from *nebulosum,* the nuclear markers show a contribution from both *mavortium* and *nebulosum.* When combined with the general difficulty of clearly resolving the relationships among these taxa using data from all allozyme loci (Fig. 2; Jones *et al.,* 1988; Jones, 1989; Jones and Collins, 1992), our results support a hypothesis of mixed ancestry in *stebbinsi* (Smith, 1992). Such a mixture could have resulted from either historical accident, i.e., lineage sorting in isolated populations, or hybridization between the presumed parental subspecies.

A lineage sorting hypothesis assumes that an ancestral *mavortium–nebulosum–stebbinsi* population gave rise to contemporary taxa. The ancestral lineage would have included the range of morphological and genetic variation we now see, and became split into three geographically separate areas. Populations in those three areas each would have contained a random sample of the ancestral allozyme and mtDNA polymorphisms. Genetic drift in the three descendant populations could have sorted polymorphic features of nuclear and mitochondrial DNA inherited from the ancestral population. Such a result would be consistent with the pattern of variation seen in *stebbinsi;* i.e., our data support a hypothesized bottleneck in which drift would likely operate efficiently, and any combination of "pre-*nebulosum*" and "pre-*mavortium*" markers could be expected. Nonetheless, there is no such evidence of a bottleneck in the other two subspecies (both are widespread and quite variable). Without a bottleneck (and in the absence of strong selection) we would expect some combination of the "ancestral" mtDNA haplotypes to be found in *mavortium* and *nebulosum* (e.g., Moran and Kornfield, 1993), which we have not found. The retention of ancestral characters in some populations is especially likely in species with low levels of gene flow among breeding populations, such as in *A. tigrinum* (Routman, 1993). In addition, a survey of 40 *mavortium* populations in Colorado and Nebraska (n = 388) revealed no *nebulosum* haplotypes (Routman, 1993). Thus, although we cannot rule out the possibility that *stebbinsi* characteristics are the result of sorting of ancestral polymorphisms, we do not believe it is probable.

Alternatively, we hypothesize that *stebbinsi* is more likely a result of past hybridization between *mavortium* and *nebulosum.* We suggest ancestral *nebulosum* and *mavortium* were geographically isolated and had unique mtDNA attributes. Contact between the two subspecies occurred at some time, resulting in a hybrid zone in the San Rafael Valley, perhaps analogous to the present situation in hybrid fishes (*Notropis;* Dowling and Hoeh, 1991) or mice (*Mus;* Ferris *et al.,* 1983). Subsequently, the geographic ranges of *nebulosum* and *mavortium* shifted to their present positions, leaving relict hybrid populations in San Rafael Valley. The hybrid populations would have been intermediate in various genetic characters, somewhat like that seen in the present New Mexico contact zone (Jones and Collins, 1992). Isolation and reduction in population size resulted in fixation of a *nebulosum*-like mtDNA haplotype, and alleles coding for the *mavortium*-like and *nebulosum*-like electromorphs. The *stebbinsi* haplotype (A31-s) either evolved its difference from *nebulosum* haplotypes in situ, or was present in the ancestral *nebulosum* population and is now extinct or was not discovered in our sampling.

Comparative data provide a historical framework consistent with our hypothesized origin of *stebbinsi.* Paleontological evidence for Pleistocene north–south and east–west faunal exchange in southwestern New Mexico and southeastern Arizona is abundant (e.g., Van Devender and Worthington, 1977; Mead *et al.,* 1984; Harris, 1987, 1989, 1990; Czaplewski *et al.,* 1989). Biogeographic evidence links the San Rafael Valley area (including the Huachuca and Patagonia Mountains) with both southern Rocky Mountain and

Great Plains habitats. Plio-Pleistocene elevational shifts in southwestern vegetation zones (Van Devender and Spaulding, 1979; Hall, 1985) provide a mechanism by which both high elevation and low elevation amphibian communities (including *nebulosum* and *mavortium,* respectively) may have expanded and contracted geographically. Brown (1982) reviewed floristic similarities between the upland habitats of northern and southeastern Arizona, and between grassland communities of southeastern Arizona, northern Sonora, and the southern Great Plains. Distributions of several animals support former north–south continuity, while distributions of others demonstrate biogeographical affinities with Great Plains habitats to the east (e.g., Stebbins, 1985; DeMarais, 1986; Hoffmeister, 1986; Jones *et al.,* 1988; Rosen and Schwalbe, 1988). The narrowly restricted distribution of some taxa, including a hydrobiid snail (*Pyrgulopsis thompsoni*; Hershler and Landye, 1990) and an orchid (*Spiranthes delitescens;* Sheviak, 1990), in and immediately surrounding the San Rafael Valley, further suggest a unique evolutionary history for that area.

Such biogeographic hypotheses can sometimes be supported with data for similar patterns in other taxa from the same geographical area. The origin of *S. delitescens,* known only from the San Rafael Valley and adjacent headwater tributaries of the San Pedro River, suggests an interesting parallel to our hypothesized history of *stebbinsi.* Sheviak (1990) provided evidence that *S. delitescens* arose through hybridization and amphiploidy, and although its origin is obscure, he suggested the parental species likely included *S. vernalis,* a species common in the southern Great Plains, and an unidentified northern species. Sheviak (1990; personal communication) identified two taxa that may have been the northern ancestor of *S. delitescens,* a California–Northern Cordilleran form, *S. porrifolia,* or *S. romanzoffiana* which occurs at high elevations in northern Arizona and in the southern Rocky Mountains. Populations of the latter also occur in disjunct "Pleistocene relict" habitats in the Pinaleño Mountains (Kearney and Peebles, 1951) in southeastern Arizona, corroborating its former southern distribution.

Finally, Plio-Pleistocene fossil records verify the historical presence of *Ambystoma* in southeastern Arizona. Brattstrom (1955) reported *A. tigrinum* in the San Pedro Valley, east of the Huachuca Mtns. in the late Pliocene. Fossil *Ambystoma* cf. *A. tigrinum* are also known from Pleistocene deposits in Papago Springs Cave in the Canelo Hills bordering the northern end of San Rafael Valley (J. I. Mead, personal communication). Although Czaplewski *et al.* (1989) bracketed the age of the entire fossil assemblage at about 31 to 107.6 ky B.P., the Papago Springs *Ambystoma* are from the younger deposits, i.e., Wisconsin Glacial (J. I. Mead, personal communication).

Generality of the Phenomenon

A. t. stebbinsi is probably the result of hybridization between *mavortium* and *nebulosum* at some time in the past. Color pattern, allozyme makeup, and mtDNA gene phylogeny combine to suggest its mixed genealogy, while a distinctive reticulate color pattern, high percentage of branchiate adults, absence of cannibalistic morphology found in *mavortium* and *nebulosum* (Collins *et al.,* 1993), depauparate allozyme profile, and characteristic mtDNA genome indicate its individual evolutionary heritage (see Collins *et al.,* 1988; Jones *et al.,* 1988). In general, conditions leading to the situation we have described can occur whenever distinct taxa meet in a hybrid zone and there is differential introgression of nuclear and mitochondrial genes (e.g., see Arnold, 1993). In fact, it is likely that the initial hybridization event that ultimately led to the taxon we refer to as *stebbinsi* produced a geographic pattern of introgression of nuclear and mtDNA characters similar to those reported for other organisms that currently hybridize (see discussion in Wilson *et al.,* 1985, and references therein). However, the *stebbinsi* example is especially significant with regard to our understanding of speciation and the use of cladistic analyses to trace the history of lineage formation (see also McDade, 1990; Smith, 1992). Unlike extant hybrid zones, through which introgression presumably continues, the *stebbinsi* populations are now geographically isolated and are not subject to continued reticulation or swamping of features through backcrossing. Many species exhibit patterns of geographically structured variation and have isolated populations. A comparison of characters in isolated populations to those of potential ancestral lineages might also yield evidence for hybridization.

Clearly, hybridization has been an important component of evolutionary diversification (Wagner, 1983; Dowling and DeMarais, 1993), and as Harrison (1990: 116) pointed out, hybrid zones might not be simply evolutionary "proving grounds or way-stations but are potential sites of species origination, sources not just sinks." Grant and Grant (1992) have provided empirical evidence suggesting hybridization results in conditions that might be favorable for rapid or major evolutionary change, although the mechanisms leading to successful introgression following hybridization are unknown. Although extant hybrid zones are interesting in this regard (reviews by Barton and Hewitt, 1985; Hewitt, 1988; Harrison, 1990), we think isolated populations are particularly relevant. If allopatric speciation is common (Mayr, 1963), then these separate populations might be one step farther into the process, and techniques to differentiate between homoplasy and hybridization should receive more attention, while patterns of reticulation should be sought in a wider variety of organisms.

Finally, part of our early work with *stebbinsi* was stimulated by requests from Arizona Game and Fish Department and the U.S. Fish and Wildlife Service (USFWS) to examine the status of this geographically restricted salamander (Collins *et al.*, 1988; Jones *et al.*, 1988). *A. t. stebbinsi* is currently listed by USFWS as a Category 2 species, i.e., although listing under the Endangered Species Act of 1973 might be appropriate, more data are necessary before such a ruling can be supported (United States Fish and Wildlife Service, 1989). Thus, its evolutionary history underscores further the implications of hybridization for endangered species management. O'Brien and Mayr (1991) raised several important issues with respect to the legal treatment of hybrids by state and federal resource management agencies. These issues have received considerable attention and suffer from misinterpretation of data, e.g., management of red wolves (see Wayne and Jenks, 1991). In that light we reiterate important points raised by Dowling *et al.* (1992). First, as we argue, hybridization might be an important evolutionary mechanism in a variety of organisms, therefore all cases in which rare or endangered taxa or populations are thought to have experienced hybridization (either in the recent or evolutionary past) must be considered carefully and individually. Also, "to deny protection due to a lack of [perceived] 'purity' defeats the purpose of our efforts" (Dowling *et al.*, 1992:8). Taxa that are recognized on the basis of unique sets of characters assume no less importance evolutionarily or legally if their origin is determined to be the result of past hybridization.

APPENDIX

Taxon and locality data (state, county, site name, **population code,** locality, sample sizes) for *A. tigrinum* used in mtDNA analysis.

stebbinsi: AZ, Santa Cruz Co., Meadow Valley Flat Tank No. 1 **(S1)** 5.7 km E Harshaw; 31°27′49″N, 110°38′47″W (3). Parker Canyon Tank No. 1 **(S2)** 7.4 km E Lochiel; 31°20′16″N, 110°32′42″W (6). Huachuca Tank **(S3)** 11.4 km ENE Lochiel; 31°21′12″N, 110°30′15″W (2). Upper 13 Reservoir **(S5)** 3.6 km WNW Lochiel; 31°21′18″N, 110°39′16″W (2). Grennan Tank **(S6)** 5.1 km SE Harshaw; 31°25′29″N, 110°40′47″W (2). Bodie Canyon Tank **(S8)** 13.8 km ENE Lochiel; 31°22′23″N, 110°28′59″W (1). Judy Tank **(S9)** 14.7 km ENE Lochiel; 31°23′04″N, 110°29′19″W (1).

nebulosum: AZ, Apache Co., Apache RR Tank **(N20)** 1.73 km S, 32.8 km W Alpine; 33°49′37″ 109°28′43″ (2). Udall Draw Tank **(N21)** 2.5 km S, 29 km W Springerville; 34°06′39″ 109°36′07″ (3). Paradise Creek Lake **(N32)** 15.8 km N, 33.1 km W Alpine; 33°58′35″ 109°39′40″ (2). AZ, Coconino Co., Warm Springs Lake **(N9)** 2.6 km S, 5.8 km W Jacob Lake; 36°41′25″ 112°16′52″ (2). Hearst Tank **(N18)** 4 km S, 13.6 km E Grand Canyon Village; 35°58′30″ 111°59′10″ (2). Bismarck Lake Tank **(N35)** 18.4 km N, 6.1 km W Flagstaff; 35°21′52″ 111°43′13″ (2). AZ, Navajo Co., Lonesome Lake **(N14)** 16 km S, 23 km W Heber; 34°17′62″ 100°51′05″ (2). Twin Lakes **(N17)** 16.8 km S, 11.5 km W Heber; 34°16′44″ 110°43′30″ (2). NM, Catron Co., Lake Erin **(N22)** 15.6 km N, 10 km E Alpine; 33°59′02″, 109°02′22″ (2). Indian Creek Tank **(N25)** 34.5 km S, 15 km E Reserve; 33°24′06″ 108°35′40″ (2). Bull Basin Tank **(N27)** 21.5 km S, 6.75 km E Reserve; 33°31′05″ 108°41′08″ (2).

mavortium: AZ, Cochise Co., Adobe Tank **(M14)** 19.3 km NW Willcox; 32°20′30″ 110°00′39″ (6). Cochise Stronghold Tank No. 1 **(M15)** 11.3 km NW Pearce; 31°57′06″ 109°55′03″ (9). Tombstone Tank No.1 **(M16)** 7.8 km NE Tombstone; 31°45′21″ 109°59′57″ (6). AZ, Graham Co., Deer Creek Tank **(M11)** 30.2 km WNW Fort Grant; 32°40′01″ 110°16′13″ (2). Register Tank **(M12)** 18.2 km E Klondyke; 32°49′41″ 110°08′02″ (2). Sulphur Springs Valley Tank No. 1 **(M13)** 25.2 km SW Fort Grant; 32°32′43″ 110°12′13″ (5). AZ, Pima Co., Little Mormon Tank **(MBA)** 7.1 km NNE Sasabe; 31°33′02″ 111°31′11″ (2). NM, Roosevelt Co., unnamed stock tank **(M8)** ≈ 16.3 km E Elida; 33°56′26″ 103°29′06″ (2). NM, Socorro Co., unnamed stock tank **(M10)** ≈ 4.8 km S, 0.64 km E Bingham; 33°52′08″ 106°20′34″ (2).

Contact Zone: NM, Catron Co., Rene Spring Tank **(X10)** 2.56 km N, 9.36 km W Datil; 34°10′03″ 107°56′40″ (2). NM, Socorro Co., Boulder Tank **(X1)** 20.8 km N, 1.2 km E Magdalena; 34°18′12″ 107°13′51″ (2). Lane Tank **(X4)** 25.4 km S, 11.6 km W Magdalena; 33°49′35″ 107°25′16″ (2). John Henry Tank **(X5)** 19.3 km N, 9.76 km E Datil; 34°19′26″ 107°42′59″ (2). Scrapes Tank **(X9)** 38.4 km S, 35.7 km W Magdalena; 33°47′26″ 107°32′48″ (2). Madera Canyon Tank **(X12)** 0.8 km S, 13.4 km W San Antonio; 33°54′36″ 107°00′43″ (2).

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