Copeia, 2002(2), pp. 344–355

Analysis of the Contact Zone between the Dusky Salamanders *Desmognathus fuscus fuscus* and *Desmognathus fuscus conanti* (Caudata: Plethodontidae)

RONALD M. BONETT

Two most widely distributed "subspecies" of the *Desmognathus fuscus* complex are the northern dusky salamander (*Desmognathus fuscus fuscus*) and the spotted dusky salamander (*Desmognathus fuscus conanti*). Previous mitochondrial DNA and allozyme studies of this complex have suggested that these two forms should be granted species-level recognition. However, detailed examination of populations inhabiting the region where the two taxa come into contact have not been performed to corroborate this taxonomic change. In this study, specimens were collected from four transects that traverse the putative contact zone. Allozyme and color pattern analysis were employed to determine the nature of variation across the area and evaluate the taxonomic status of *D. f. conanti*. Phylogenetic analyses of allozyme data divide the populations sampled into at least four major groups, two of which are referable to *Desmognathus fuscus* (groups A and C) and two referable to *D. conanti* (groups B and D). This study supports the distinctiveness of *D. conanti* by revealing parapatry between populations of groups A and B in western Kentucky with only a minor amount of hybridization between them and identifies color pattern differences that are effective for characterizing most group B individuals. The data presented here suggest that a longitudinal division may exist within *D. conanti* in central Alabama. Group C occurs primarily on the Piedmont of North Carolina and Virginia and appears to hybridize with group A in central Virginia. Groups C and D are treated, conservatively, as *D. fuscus* and *D. conanti*, respectively; but further study may reveal that each is a distinct species.

THE spotted dusky salamander, *Desmognathus fuscus conanti*, was originally described from the south-central United States on the basis of its brilliant color pattern, retention of larval spots in adults, and prominently colored postocular stripe (Rossman, 1958). Karlin and Guttman (1986) examined allozyme variation across the *Desmognathus fuscus* complex and demonstrated that northern populations of *D. fuscus* are substantially different from southern populations. A recent mitochondrial DNA-based phylogeny of desmognathine salamanders elevated *D. f. conanti* to the level of species (Titus and Larson, 1996). This phylogeny suggests that *Desmognathus fuscus fuscus* from northern areas (Vermont) are not even sister taxa to *D. f. conanti* (western Kentucky and Georgia) and that *D. fuscus* from North Carolina are phylogenetically distinct from both *D. f. fuscus* and *D. f. conanti*. If Titus and Larson's (1996) phylogeny accurately places members of the *D. fuscus* complex with respect to other congeners, then this complex actually contains at least three species. However, the most recent comprehensive work on the salamanders of the United States and Canada retains the subspecific status of *D. f. conanti* (Petranka, 1998).

Molecular approaches have become widely used for assessing evolutionary relationships and species boundaries within plethodontids and have allowed delineation of cryptic taxa not distinguishable on the basis of morphology (Larson and Chippindale, 1993). During the past two decades, allozyme analysis has been used to identify cryptic species within many species complexes in the Plethodontidae, for example, *Eurycea bislineata* (Jacobs, 1987), *Plethodon glutinosus* (Highton et al., 1989), *Desmognathus ochrophaeus* (Tilley and Mahoney, 1996), and Texas *Eurycea* (Chippindale et al., 2000). I used allozyme and color pattern analysis to determine the nature of variation among the taxonomically ambiguous populations of the *D. fuscus* complex in the south-central United States to evaluate the taxonomic status of *D. f. conanti*.

MATERIALS AND METHODS

Sample collection.—Desmognathus fuscus were collected from 59 localities in the central portion of the eastern United States from March 1999 to May 2000 (see Material Examined). Initial localities were selected to create four transects that traverse the putative contact zone identified by Petranka (1998; Fig. 1). Two transects to

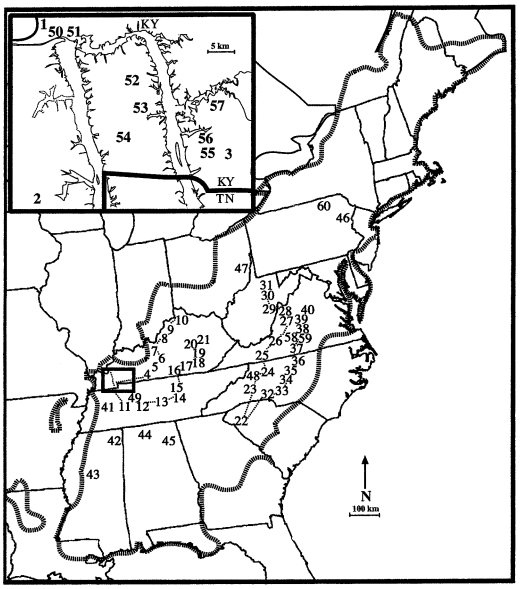


Fig. 1. Localities where *Desmognathus* (1–59 putative *Desmognathus fuscus*, 60 *Desmognathus ochrophaeus*) were collected for this study. Light dotted lines that connect some localities, represent original transects. Hatched lines follow the perimeter of the distribution of *D. fuscus* proposed by Petranka (1998). Inset map (upper left) is an enlargement of the squared region on the main map.

the west of the Blue Ridge Physiographic Province originated from the type locality of *D. f. conanti* in Livingston County, Kentucky (Rossman, 1958). Two other transects to the east of the Blue Ridge share a common origin on the western Piedmont of South Carolina. Additional localities far north (Pennsylvania and Ohio) and far south (Mississippi and Alabama) of the putative contact zone provided comparative specimens. After the initial electrophoretic survey was performed, supplemental localities were collected to examine fine scale variation between certain groups (explained below). *D. ochrophaeus* from Pennsylvania were used as the outgroup for phylogenetic analysis.

Specimen processing and deposition.—All specimens were transported alive to East Stroudsburg University, Pennsylvania, where they were housed at ~5 C for at least five days prior to being processed. Specimens were sacrificed by submerging in a 1% solution of 2-phenoxyethanol for approximately 5 min. Snout–vent lengths (SVL) and general color pattern descriptions were made for each individual (i.e., presence of larval spotting, color of postocular stripe, other features). For all specimens with a SVL of 21 mm or greater, samples of liver, stomach, and skeletal muscle were taken for allozyme analysis and vouchers were preserved with 10% formalin and stored in 70% ethanol. Specimens having a SVL less than 21 mm were frozen whole after color pattern notations were made. The entire specimen was then homogenized for electrophoresis, and no voucher specimens exist for these individuals. Specimens homogenized whole represent less than 5% of the specimens examined.

Allozyme analysis.—Allozyme analysis can be useful for examination of contact zones in diploid organisms (e.g., plethodontid salamanders) because it facilitates the distinction between homozygous and heterozygous individuals (Weising et al., 1995). Examination of multiple loci can be beneficial because each locus represents an independent estimate of nuclear gene flow between populations (Hillis et al., 1996). In a survey of 21 protein-coding loci Karlin, and Guttman (1986) showed substantial latitudinal differences in the *D. fuscus* complex for several loci. Among them were glucose phosphate isomerase (''Gpi'' = Gpi-A), lactate dehydrogenase-1 (''Ldh-1'' = Ldh-A), 1-leucyl-1-tyrosine peptidase (''Pep-2'' = Pep-B), amino-aspartate transferase (''Aat-2'' = sAta-A), and 6-phosphogluconate dehydrogenase (''6-Pgdh'' = 6Pgdh-A). I primarily focused on the loci that Karlin and Guttman (1986) previously found to have complete or nearly complete differences between northern and southern populations of *D. fuscus* plus additional loci that I found to be variable. Horizontal starch gel electrophoresis was employed to examine 499 specimens for the seven variable loci on two buffer systems (Table 1). Electrophoretic methods used were similar to those of Tilley and Mahoney (1996) with the following modifications: Ldh-A and Pep-B were resolved on a lithium hydroxide buffer system (LIOH; Murphy et al., 1996); sIcdh-A was resolved using Tris-Borate-EDTA buffer system (TBE; Karlin and Guttman, 1986).

Phylogenetic analysis.—Genotypes of each individual were placed into BIOSYS-1 (Swofford and Selander, 1981) to generate Cavalli-Sforza and Edwards (1967) chord distances. The data matrix of distances was transferred into PHYLIP (vers. 3.57c, J. Felsenstein, Univ. of Washington, Seattle, 1995, unpubl.) for neighbor-joining analysis (Saitou and Nei, 1987). This method was chosen because it demonstrated a high level of accuracy in phylogenetic reconstruction using allozyme data (Wiens, 2000). *Desmognathus ochrophaeus* (population 60) was used as an out-

TABLE 1. ENZYME SYSTEMS EXAMINED IN ELECTROPHORESIS.

Enzymes examined	Loci scored	E.C. number	Buffer system*
Aspartate aminotransferase	sAta-A	2.6.1.1	2
Glucose-6-phosphate isomerase	Gpi-A	5.3.1.9	1
Isocitrate dehydrogenase	sIcdh-A	1.2.1.42	2
L-Lactate dehydrogenase	Ldh-A and Ldh-B	1.1.1.27	1
Peptidase, leucylglycylglycine	Pep-B	3.4.–.–	1
Phosphogluconate dehydrogenase	6Pgdh-A	1.1.1.44	2

* Buffer systems: 1. Lithium hydroxide (Selander et al., 1971); 2. Tris-EDTA-borate, pH 9.1 (Karlin and Guttman, 1986).

group in the neighbor-joining analysis, because it previously was identified as a member of a complex that is sister to the clade that includes the ''subspecies'' of *D. fuscus* (Titus and Larson, 1996). In addition, generalized frequency coding (GFC; Smith and Gutberlet, 2001), a method for coding polymorphic characters, was used to calculate noncumulative frequency codes. These codes were used in a parsimony analysis performed using the branch-and-bound search option in PAUP* (vers. 4.0b3a, D. L. Swofford, Sinauer Assoc. Inc., Sunderland, MA, 2000, unpubl.). Strict consensus was used to summarize features shared among equally parsimonious trees. Nonparametric bootstrapping using heuristic searches based on 1000 pseudoreplications were used to determine confidence at each node of the consensus phylogram.

Color pattern analysis.—In the original description, *D. f. conanti* was characterized as being proportionately most similar to *D. f. fuscus* but with prominently colored postocular stripes, and larval spots that persist into adulthood (Rossman, 1958). It appears that the brilliant postocular striping of *D. f. conanti*, pointed out by Rossman (1958) can be more precisely described as being orange in coloration (pers. obs.; J. MacGregor, pers. comm.). Because of the ontogenetic variation in coloration exhibited by most species of *Desmognathus* (Conant and Collins, 1998), specimens were categorized with respect to the SVL of each individual. Specimens used in this analysis ranged from 20 mm SVL, the maximum size at metamorphosis for *D. fuscus* (Juterbock, 1990), to 78.0 mm SVL, the largest specimen measured in this study. To account for ontogenetic color pattern variation, individuals were divided into four arbitrarily chosen size categories of equal size (size category 1: 20.0–34.5, 2: 34.6–49.0, 3: 49.1–63.5, and 4: 63.6–78.0). Populations were grouped according to the major clusters determined by phylogenetic analysis of allozyme data; within each group, samples were pooled and then subdivided into their respective size category. Specimens were scored for the presence or absence of larval spots on the dorsum and orange postocular stripes. Two-by-two contingency tables with no fixed margins (Zar, 1996) were used to compare the proportions of individuals that possess a given character among equivalent size categories of the groups. Initial comparisons of size categories between groups A and C (for explanation of phylogenetic groups see below) showed presence of an orange postocular stripe and larval spot persistence to be independent of group. Because postocular stripe coloration and larval spot persistence were used to distinguish *D. f. conanti* from other *D. fuscus* (Rossman, 1958), and to reduce the number of overall comparisons, groups A and C (*D. fuscus*) were pooled and compared to group B (*D. conanti*). Group D specimens and all larvae (< 20 mm SVL) were not included in the color pattern analysis because each were represented by only small numbers, and for many of them color pattern data are not available.

RESULTS

Allozymes.—Genotype arrays of the loci examined are provided in Appendix 1. The neighbor-joining analyses using Cavalli-Sforza and Edwards (1967) chord distance demonstrated that the populations sampled are divisible into at least five groups (Fig. 2). Four of the groups comprise the 59 putative populations of *D. fuscus* (sensu lato), and the fifth division is the single population of *D. ochrophaeus* (population 60). For the remainder of this paper, the four groups within the *D. fuscus* complex will be referred to as A, B, C, and D. The parsimony-based tree using GFC (Fig. 3) was congruent with the neighbor-joining tree, with respect to major population groups. Bootstrap analysis provides strong support for some of the major groupings (A + C = 84%, B = 72%, D = 99%). The geographic ranges of groups A–D and putative hybrid populations are shown in Figure 4. The following summary of the groups excludes putative hybrid populations (explained below),

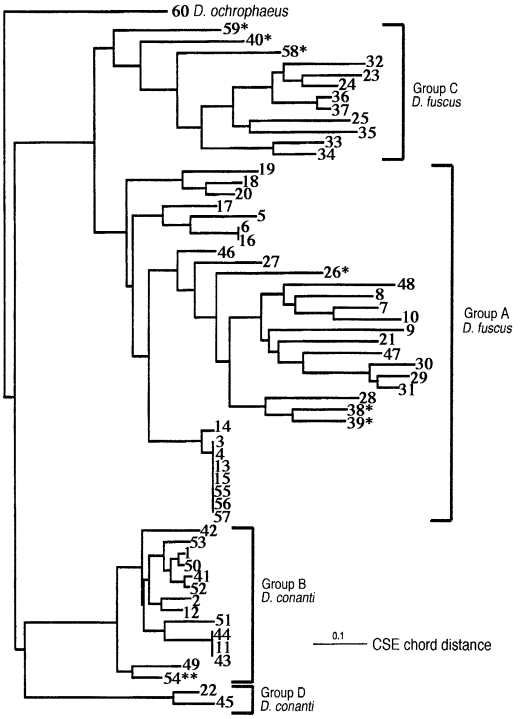


Fig. 2. Neighbor-joining tree, based on Cavalli-Sforza and Edwards (1967) chord distances. This tree demonstrates the separation of putative *Desmognathus fuscus* populations (1–59) into four disjunct groups (A and C, *D. fuscus*; B and D, *Desmognathus conanti*). Putative hybrid populations are indicated by * (A/C hybrids) or ** (A/B hybrids).

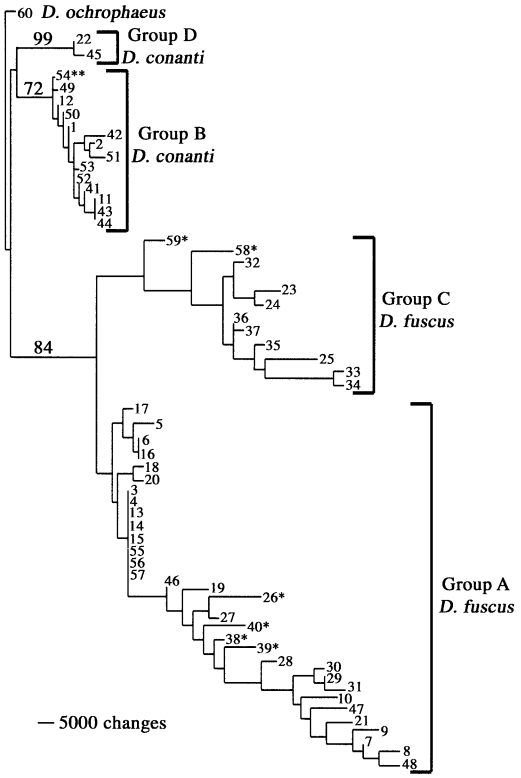


Fig. 3. Parsimony tree based on allozyme data for 59 putative *Desmognathus fuscus* populations with *Desmognathus ochrophaeus* placed as an outgroup. Bootstrap analysis based on 1000 pseudoreplications generated bootstrap values (> 50%) for the major groupings that are reported adjacent to their corresponding node, and relative branch lengths are shown. This tree demonstrates the separation of putative *D. fuscus* populations (1–59) into four disjunct groups (A and C, *D. fuscus*; B and D, *Desmognathus conanti*). Putative hybrid populations are indicated by * (A/C hybrids) or ** (A/B hybrids).

so that generalizations about the allelic composition of groups can be made.

Group A (population: 3–10, 13–21, 27–31, 38–39, 46–48, and 55–57) extends from northeastern Pennsylvania to eastern Ohio, southwest to the western portion of the Highland Rim of east-central Tennessee and the eastern tributaries of the Cumberland River in Kentucky. Of the loci surveyed only the allele 6Pgdh-A^{a} was found to be fixed across all group A populations. This is a result of the tremendous allelic diversity of this widespread group. However, there are many alleles (sAta-A^{c}, sIcdh-A^{a,e}, Ldh-A^{b,c,e}, and Ldh-B^{b,c,d,e,h}) that are shared only among group A populations. Group B (population: 1–2, 11–12, 41–44, and 49–54) occurs in Kentucky west of the Cumberland River, including populations inhabiting the Land Between the Lakes Region and the type locality of *D. f. conanti* (3.4 km south of Smithland, Livingston County, Kentucky; Rossman, 1958). In Tennessee, group B populations are distributed throughout the tributaries of the Tennessee and Cumberland Rivers west of the Nashville Basin. The remainder of the group B populations exist in the northern half of Mississippi and northwestern Alabama. Group B contains many alleles (sAta-A^{b}, Gpi-A^{c}, sIcdh-A^{b}, Ldh-A^{g}, Ldh-B^{i}, and 6Pgdh-A^{a}) that were found to be fixed for all individuals surveyed, as well as a variety of alleles (sIcdh-A^{b}, Ldh-A^{g}, Ldh-B^{i}, Pep-B^{a}, and Pep-B^{f}) that have been identified only in populations from this group. Group C (population: 23–25, 32–37, 40, and 58–59) occurs primarily on the Piedmont of North Carolina and Virginia. Group C populations showed fixed (sIcdh-A^{c}, Ldh-A^{d}, and Ldh-B^{g}) or nearly fixed (6Pgdh-A^{c}) allelic differences with respect to other groups, and also several alleles (sAta-A^{d}, Gpi-A^{b}, Gpi-A^{d}, Pep-B^{e}, and 6Pgdh-A^{b}) that are not shared with other groups. Group D includes only two populations,

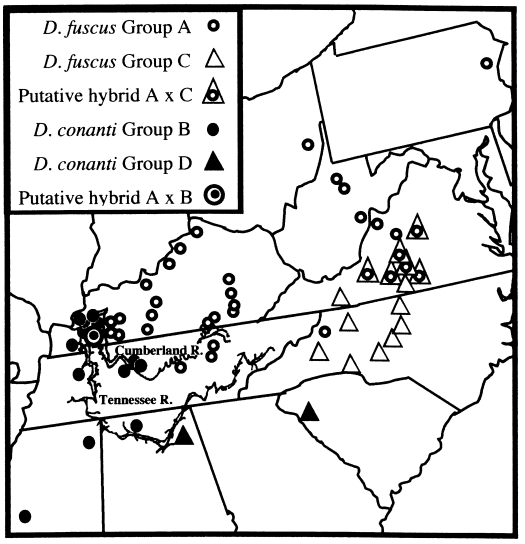
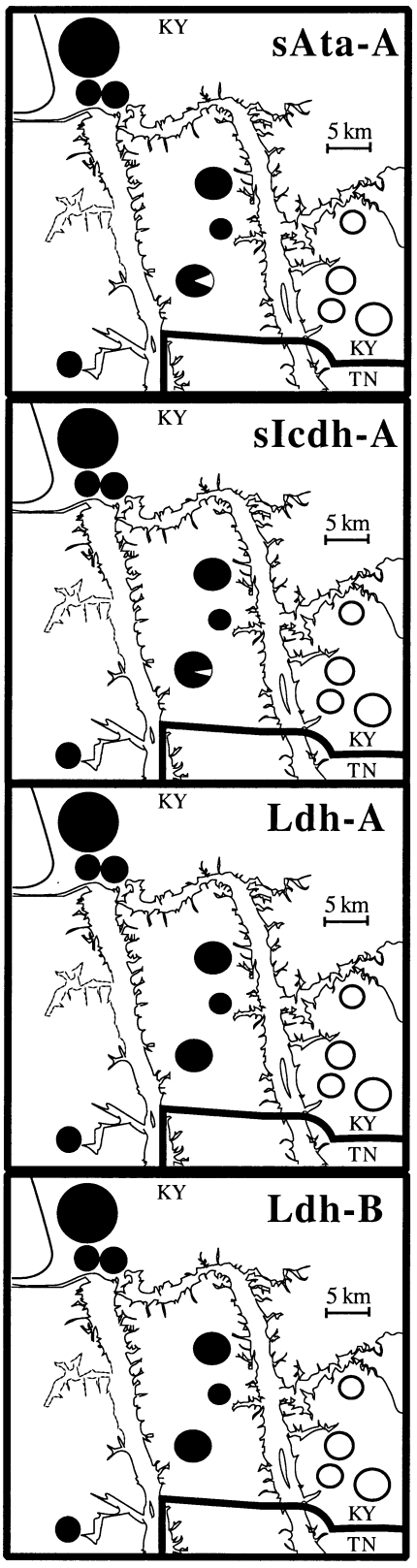


Fig. 4. Mapped representation of phylogenetic division of sampled populations into four groups (A–D) and putative hybrid populations.

one from western South Carolina (22) and the other from northeastern Alabama (45). These populations are similar to group B populations in having the alleles sAta-A^b, Gpi-A^c, and 6Pgdh-A^a but are distinguishable by the diagnostic alleles sIcdh-A^f, Ldh-A^f, and Ldh-B^f.

Contact zones.—Phylogenetic analysis shows parapatric populations in western Kentucky to be apart of two distinct groups (A and B). Fine-scale examination of allele frequencies at the contact zone between groups A and B revealed that the boundary between them is relatively discrete, with concordant changes to alternate alleles for the loci sAta-A, sIcdh-A, Ldh-A, and Ldh-B across this region (Fig. 5). Only a few individuals in a single population (54), at the interface of these groups, were found to be heterozygous for alleles that are otherwise diagnostic in adjacent populations of the two groups. This suggests that only a limited amount of very localized gene flow exists between groups A and B, and supports their distinctness.

In western Virginia where groups A and C come into contact at least six putative hybrid populations (26, 38–40, and 58–59) were identified. These populations have combinations of



→

Fig. 5. Inset map from Figure 1 demonstration the relatively discrete boundary between group A (open circles) and group B (filled circles) populations for the loci sAta-A, sIcdh-A, Ldh-A, and Ldh-B. Circles are proportionate to sample sizes (See Materials Examined).

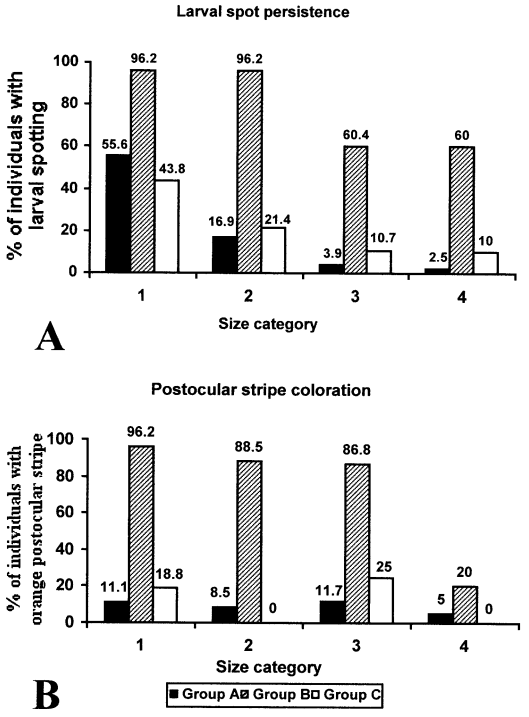


Fig. 6. Comparison of percent larval spot persistence (A) and postocular stripe coloration (B) for the size categories of groups A–C.

alleles that are monoallelic elsewhere. This indicates gene flow between groups A and C.

Color pattern.—Two by two contingency tables with no fixed margins (Zar, 1996) demonstrated that post-metamorphic (> 20 mm SVL) *conanti* (group B) have significantly higher proportion of individuals that retain larval spots than *fuscus* (groups A and C) for all size categories ($P < 0.001$). For size categories 1–3, a significantly higher proportion of *conanti* had an orange postocular stripe than did *fuscus* ($P < 0.001$). There was no significant difference ($0.9 > P > 0.5$) in postocular stripe coloration between *conanti* and *fuscus* for the largest category of individuals, size category 4. This is somewhat expected since color patterns tend to fade with increasing size in most species in the genus *Desmognathus* (Conant and Collins, 1998). Figure 6 shows the percentage of individuals with larval spotting and orange post ocular stripes among groups, with the separation of *fuscus* into its respective groups (A and C).

Discussion

The original focus of this study was the contact zone between *D. f. fuscus* and *D. f. conanti*. This study makes it clear that this question is only relevant for the western populations and also corroborates the suggestion by Titus and Larson (1996) that the spotted dusky salamander, *D. conanti* should be recognized as a distinct species. This is supported under both the phylogenetic (Cracraft, 1989) and evolutionary (Wiley, 1981; Frost and Hillis, 1990) species concepts. Under the phylogenetic species concept, *D. conanti* is distinctive by having allozyme and morphological differences that indicate that these populations represent a distinct unit. Under the evolutionary species concept, allozyme (this study) and mitochondrial (Titus and Larson, 1996) characteristics of *D. conanti* support that this taxon represents a distinct evolutionary lineage. Furthermore, a discrete species boundary has been identified between *D. conanti* and *D. fuscus* in western Kentucky, just east of the type locality of *D. conanti*. In this region, substantial allozyme divergence exists between parapatric populations of *D. fuscus* and *D. conanti* with a very limited amount of hybridization.

In Kentucky, populations of *D. conanti* are restricted to the areas immediately north and west of the Cumberland River. In central Tennessee, populations of "*D. fuscus*" have previously been illustrated as contiguous across the state (Petranka, 1998), although they are actually geographically separated by the well-drained Nashville Basin (see "*D. fuscus*" spot map in Redmond and Scott, 1996). It appears that populations of *D. conanti* and *D. fuscus* tend to localize along the headwaters of springs in this plateau (pers. obs.), this may create a mosaic of isolated populations that occupy habitat islands between the two species, rather than exhibiting close parapatry. In general, this region seems to have some biogeographic significance. The Cumberland and Tennessee Rivers originated approximately 5 million years ago (Potter, 1955) and have a variety of endemic mussels (M. E. Gordon and J. B. Layzer, unpubl.) and fishes (Page and Burr, 1998) that inhabit the headwaters of each. Additional species boundaries in this region include other stream-associated plethodontid salamanders (*Eurycea longicauda* and *Eurycea guttolineata*; Carlin, 1997), terrestrial plethodontids (*Plethodon glutinosus* and *Plethodon mississippi*; Highton et al., 1989), and shrews (*Blarina brevicauda* and *Blarina carolinensis*; George et al., 1982).

Even considering the data for all of the populations examined in this study, the distribution of *D. conanti* still needs more attention. It is clear that populations in the vicinity of Livingston County, Kentucky, constitute at least a portion of the northern limit for *D. conanti*, but it is unclear whether populations from southern

Illinois, which appear to have some distinct color pattern characteristics (D. B. Means, pers. comm.), should also be included. The suggested distribution of *D. f. conanti* (here considered *D. conanti*, Petranka, 1998) extends from western Kentucky, south to the Gulf of Mexico east of the Mississippi River, with disjunct populations in northern Louisiana and Arkansas (explained below). Because of sampling limitations, my study only provides evidence that *D. conanti* occurs as far south as central Mississippi. Karlin and Guttman (1986) showed two electrophoretically different subgroups, one northern and the other southern, within the distribution of *D. conanti*. There are several aquatic and semiaquatic endemic vertebrate taxa that are distributed parapatrically with respect to adjacent watersheds that traverse the Gulf Coastal Plain. Among them are fishes (*Fundulus*; Page and Burr, 1998), frogs (*Rana*; Young and Crother, 2001; Conant and Collins, 1998), and turtles (*Graptemys*; Ernst and Barbour, 1989). Means (2000) hypothesized that steephead ravines that occur in the Gulf Coastal Plain served as ''evolutionary engines'' in the speciation of many of the endemics that exist there. Further studies are needed to determine the southern limit of the distribution of *D. conanti*; examination of variation among Gulf Coastal Plain drainage populations may prove to be enlightening.

The allozyme data presented here suggest a longitudinal separation within *D. conanti* in north-central Alabama by elucidating two phylogenetically distinct clades (groups B and D). I am currently considering group D populations to be *D. conanti*, because sampling of this group is far too limited to speculate on its distribution or interaction with adjacent groups.

Two disjunct fragments of *D. conanti* are reported from west of the Mississippi River (outlined in Fig. 1). The populations that occur in northeastern Arkansas are distributed along a highland region known as Crowley's Ridge. *Desmognathus* were collected there approximately three decades ago and were presumed to be *D.* cf. *fuscus* (Means, 1974), but recent excursions in search of *Desmognathus* on Crowley's Ridge were unsuccessful (pers. obs., 2001; D. B. Means, pers. comm., 2000). Populations from southern Arkansas were also included in the study of Means (1974), but there is no information available on their current status. I have initiated studies of these disjunct populations from west of the Mississippi River to clarify their status.

The remainder of the *D. fuscus* complex, including groups A and C, is incredibly expansive, and may be quite specious. Titus and Larson (1996) found *D. fuscus* from Vermont to be only distantly related to *D. fuscus* from the piedmont of North Carolina. If their samples of *D. fuscus* correspond to my groups A and C, respectively, then the putative hybrid populations identified here are likely the result of secondary contact between groups A and C, and additional species should be recognized.

This study has identified a discrete species boundary between *D. fuscus* and *D. conanti* and reveals groupings of populations within both species that may lead to further taxonomic subdivision. More studies are necessary to clarify the true species diversity within this complex. Currently, I am addressing some of these issues in more detail, and incorporating nuclear and mitochondrial DNA sequence data to answer some of the many questions that remain in this widespread complex.

Materials Examined

Specimens examined listed below are organized as follows: Species.—STATE: county: (population) locality, catalog number(s). Population numbers correspond to those in Figure 1. Vouchers were deposited in the Collection of Vertebrates, the University of Texas at Arlington, Arlington, Texas (UTA) or the Museum of Natural History, Appalachian State University, North Carolina (ASU).

Desmognathus conanti.—ALABAMA: Jackson County: (45) Buck's Pocket S.P., UTA 54359–67; Limestone County: (44) Elk River S.P., UTA 54349–58. KENTUCKY: Calloway County: (2) Goose Creek, UTA 54044–50; Livingston County: (1) 3.4 km South of Smithland, UTA 54019–43; (50) Lee Creek, 0.6 km East of Rt. 60, UTA 54404–10; (51) Cooper-James Creek, UTA 54411–15; Lyon County: (52) Race Track Hollow, 0.6 km North of the Trace, UTA 54416–28; Trigg County: (53) Ferguson Spring, no vouchers available; (54) Stream adjacent to Rt. 68, 1 km West of the Trace, UTA 54428–40. MISSISSIPPI: Carrol County: Near junction of Rt. 55 with Rt. 82, no voucher available; Tishamingo County: (42) Tishamingo South P., UTA 54337–47. SOUTH CAROLINA: Anderson County: (22) Pendelton Swamp, ASU 23228–29. TENNESSEE: Benton County: (41) near Camden, UTA 54329–36; Cheatham County: (11) Half Pone Creek, near Rt. 12, UTA 54109–15; (12) ravine near exit 188 of I40, UTA 54116–33; Montgomery County: (49) Fredonia, UTA 54401–3.

Desmognathus fuscus.—KENTUCKY: Boone County: (10) Woopler Creek, UTA 54101–08; Clark County: (21) Four Mile Creek, UTA 54205–13;

Clinton County: (16) springs near Rt. 639, UTA 54170–72; (17) Spring Creek, Bug, UTA 54173–82; Edmonson County: (6) Bylew Creek, Straw, UTA 54069–71; Laurel County: (19) Hawks Creek, UTA 54187–96; Meade County: (7) Doe Run Inn, ~5 km East of Brandenburg, UTA 54072–77; Oldham County: (8) Harrods Creek, West of Brownsboro, UTA 54078–87; Rockcastle County: (20) Renfro Creek, 0.5 km Southwest of Hummel, UTA 54197–204; Simpson County: (4) Alexander's Cave, off of Saddler Ford Rd., UTA 54060–61; Trigg County: (3) Dry Creek, 1 km West of Rt. 139, UTA 54051–59; (55) Creek adjacent to McCoy Hollow Rd., UTA 54441–44; (56) Creek near Old Dover Rd, UTA 54445–52; (57) Caney Creek, UTA 54453–58; Trimble County: (9) Milton, UTA 54088–100; Warren County: (5) Long Br., Riverside, UTA 54062–68; Whitley County: seeps near Cumberland Falls, UTA 54183–86; NORTH CAROLINA: Burke County: (23) Laurel Creek, near Morgantown, UTA 54214–21; Gaston County: (32) Gastonia, 54266–67; Guliford County: (35) Bur Mill Park, UTA 54282–86; Randolph County: (34) Ashboro, UTA 54277–81; Rockingham County: (36) Stream off of Rt. 29, 0.5 km South of 14, UTA 54287–97; Stanley County: stream along Marrow Mt. Rd, UTA 54268–76; Watauga County: (48) Hidden Valley Circle, Boone, UTA 54389–400); Wilkes County: (24) Stream near Balls Mill Rd., UTA 54222–26; OHIO: Belmont County: (47) Barkcamp S.P., UTA 54378–88; PENNSYLVANIA: Monroe County: (46) E. Brown St. Spring, East Stroudsburg, UTA 54370–77; TENNESSEE: Cannon County: (13) Springs near Woodbury, UTA 54134–49; Overton County: (15) West Obey Creek, UTA 54163–69; Putnam County: Ravens Croft, UTA 54150–62; VIRGINIA: Albemarle County: (40) Charlottesville, UTA 54323–28; Amherst County: (39) Amherst, UTA 54311–22; Campbell County: (38) stream along Rt. 626, near Leesleyville Rd., UTA 54304–10; Carrol County: (25) stream along Rt. 860, East of Blue Ridge Pkwy, UTA 54227–36; Highland County: (28) Spring off of Rt. 250, UTA 54246–47; Nelson County: (27) Montabello, UTA 54241–45; Pittsylvania County: (37) Danville, UTA 54298–303; (58) 3.2 km east of Penhook, UTA 54459–68; (59) Chatham, UTA 54469; Roanoke County: (26) Back Creek, UTA 54237–40; WEST VIRGINIA: Barbour County: (30) ravine near Pleasant Creek, UTA 54253–62; Pocahontas County: (29) Rt. 250, ~5 km West of VA border, UTA 54248–52; Taylor County: Springs near Tagert Lake, UTA 54263–65.

Desmognathus ochrophaeus.—PENNSYLVANIA: Bradford County: (60) The Bonett Farm, LeRoy Twp, UTA 54470–54479.

Acknowledgments

I give special thanks to T. LaDuke for his tremendous influence on my development and understanding of various topics in herpetology, and to S. Tilley for providing me with an introduction to electrophoretic techniques and "desmogs." Also, thanks to R. Secord, L. Baatz, J. Boundy, and L. Teixeira for their assistance in the field. I benefited greatly from helpful comments and criticisms from D. Andrews, A. Baldwin, P. Chippindale, T. Doan, T. LaDuke, J. MacGregor, M. Meadows, R. Montanucci, M. Ramirez, J. Robinson, W. Schargel, C. Spencer, R. W. VanDevender, T. Whitford, J. Wiens, and N. Woodman. P. Chippindale, B. McAllister, and E. Smith provided me with critical guidance on phylogenetic analysis. R. W. Van Devender, R. Montanucci, and S. Dula provided me with invaluable specimens for my research. R. J. Bonett, J. Smith, J. Jewett-Smith, and J. Huffman were of great assistance in acquiring laboratory supplies used in this study. I would like to thank the following institutions for providing permission to collect specimens that were examined in this study: The State of Alabama Department of Conservation and Natural Resources (99136), Kentucky Department of Fish and Wildlife Resources (9903), Louisiana Department of Wildlife and Fisheries (LNHP-99–069), State of Mississippi Department of Wildlife Fisheries and Parks, North Carolina Wildlife Resources Commission (0127), Ohio Division Natural Resources and Parks, Pennsylvania Fish and Boat Commission (209 Type I), South Carolina Department of Natural Resources (0031–99), Tennessee Wildlife Resource Agency (1404), Virginia Department of Wildlife and Inland Fisheries (013596), West Virginia Department of Natural Resources (79–1999), U.S. Department of Agriculture; Daniel Boone, Francis Marion, George Washington, Monongahela, and Sumpter National Forests; and Land Between the Lakes National Recreation Area. Funding for this study was provided by the Department of Biological Sciences, East Stroudsburg University, and a grant from the Pennsylvania Academy of Sciences. Appreciated laboratory hardware was provided by Salk Laboratories, Swiftwater, Pennsylvania.

Literature Cited

Carlin, J. L. 1997. Genetic and morphological differentiation between *Eurycea longicauda longicauda* and *E. guttolineata* (Caudata: Plethodontidae). Herpetologica 53:206–217.

Cavalli-Sforza, L. L., and A. W. F. Edwards. 1967. Phylogenetic analysis: models of estimation procedures. Evolution 21:550–570.

Chippindale, P. T., A. H. Price, J. J. Wiens, and D. M.

HILLIS. 2000. Phylogenetic relationships and systematic revision of central. Monogr. 14:1–80.

CONANT, R., AND J. T. COLLINS. 1998. A field guide to the reptiles and amphibians of eastern and central North America. Houghton Mifflin Co., Boston, MA.

CRACRAFT, J. 1989. Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation, p. 157–173. *In:* Speciation and its consequences. D. Otte and J. A. Endler (eds.). Sinauer Associates, Inc., Sunderland, MA.

ERNST, C. A., AND R. W. BARBOUR. 1989. Turtles of the world. Smithsonian Institution Press, Washington, DC.

FROST, D., AND D. M. HILLIS. 1990. Species in concept and practice herpetological applications. Herpetologica 46:87–104.

GEORGE, S. B., H. H. GENOWAYS, J. R. CHOATE, AND R. J. BAKER. 1982. Karyotypic relationships with in the short-tailed shrews, genus *Blarina*. J. Mammal. 63: 639–645.

HIGHTON, R., G. C. MAHA, AND L. R. MAXSON. 1989. Biochemical evolution in the slimy salamanders of the *Plethodon glutinosus* complex in the eastern United States. Univ. Ill. Biol. Monogr. 57:1–153.

HILLIS, D. M., B. K. MABLE, AND C. MORITZ. 1996. Applications of molecular systematics: the state of the field and look to the future, p. 515–543. *In:* Molecular systematics. D. M. Hillis, C. Moritz, and B. K. Mable (eds.). Sinauer Associates, Inc., Sunderland, MA.

JACOBS, J. F. 1987. A preliminary investigation of geographic variation and systematics of the two lined salamander, *Eurycea bislineata* (Green). Herpetologica 43:423–446.

JUTERBOCK, J. E. 1990. Variation in larval growth and metamorphosis in the salamander *Desmognathus fuscus*. *Ibid.* 46:291–303.

KARLIN, A. A., AND S. I. GUTTMAN. 1986. Systematics and geographic isozyme variation in the plethodontid salamander *Desmognathus fuscus*. *Ibid.* 42:283–301.

LARSON, A., AND P. T. CHIPPINDALE. 1993. Molecular approaches to the evolutionary biology of plethodontid salamanders. *Ibid.* 49:204–215.

MEANS, D. B. 1974. The status of *Desmognathus brimleyorum* Stejneger and an analysis of the genus *Desmognathus* (Amphibia: Urodela) in Florida. Bull. Fla. St. Mus. 18:1–100.

———. 2000. Southeastern U.S. Coastal Plain habits of the Plethodontidae, p. 287–301. *In:* The biology of plethodontid salamanders. R. C. Bruce, R. G. Jaeger, and L. D. Houck (eds.). Kluwer Academic, Plenum Publishers, New York.

MURPHY, R. W., J. W. SITES JR., D. G. BUTH, AND C. H. HAUFLER. 1996. Protiens: isozyme electrophoresis, p. 51–120. *In:* Molecular systematics. D. M. Hillis, C. Moritz, and B. K. Mable (eds.). Sinauer Associates, Inc., Sunderland, MA.

PAGE, L. M., AND B. M. BURR. 1998. A field guide to freshwater fishes. Houghton Mifflin Co., Boston, MA.

PETRANKA, J. W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC.

POTTER, P. E. 1955. The petrology and origin of the Lafayette gravel. Part 2. Geomorphic history. J. Geol. V. 63:115–132.

REDMOND, W. H. AND A. F. SCOTT. 1996. Atlas of amphibians in Tennessee. Austin Peay State Univ. Misc. Pub. 12:1–45.

ROSSMAN, D. A. 1958. A new race of *Desmognathus fuscus* from the south-central United States. Herpetologica 11:158–160.

SAITOU, N., AND M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 6:514–525.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. P. JOHNSON, AND G. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old field mouse (*Peromyscus polionotus*). Stud. Genet. VI. Univ. Tex. Publ. 7103:49–90.

SMITH, E. N., AND R. L. GUTBERLET JR. 2001. Generalized frequency coding: a method of preparing polymorphic multistate characters for phylogenetic analysis. Syst. Biol. 50:156–169.

SWOFFORD, D. L., AND R. B. SELANDER. 1981. BIOSYS-I: a Fortran program for the comprehensive analysis of electrophretic data in population genetics and systematics. J. Hered. 72:281–283.

TILLEY, S. G., AND M. J. MAHONEY. 1996. Patterns of genetic differentiation in salamanders of the *Desmognathus ochrophaeus* complex (Amphibia: Plethodontidae). Herpetol. Monogr. 10:1–42.

TITUS, T. A., AND A. LARSON. 1996. Molecular phylogenetics of desmognathine salamanders (Caudata: Plethodontidae): a reevaluation of evolution in ecology, life history, and morphology. Syst. Biol. 45: 451–472.

WEISING K., H. NYBOM, K. WOLFF, AND M. WIELAND. 1995. DNA fingerprinting of plants and fungi. CRC Press, Boca Raton, FL.

WIENS, J. J. 2000. Reconstructing phylogenies from allozyme data: comparing method performance with congruence. Biol. J. Linn. Soc. 70:613–632.

WILEY, E. O. 1981. Phylogenetics: the theory and practice of phylogenetic systematics. John Wiley and Sons, New York.

YOUNG, J. E., AND B. I. CROTHER. 2001. Allozyme evidence for the separation of *Rana areolata, Rana capito*, and the resurrection of *Rana servosa*. Copeia 2001:382–388.

ZAR, J. H. 1996. Biological analysis. 3d ed. Prentice Hall, Upper Saddle River, NJ.

DEPARTMENT OF BIOLOGY, EAST STROUDSBURG UNIVERSITY, EAST STROUDSBURG, PENNSYLVANIA 18301. PRESENT ADDRESS: DEPARTMENT OF BIOLOGY, UNIVERSITY OF TEXAS, ARLINGTON, TEXAS 76019. E-mail: desmog@exchange.uta.edu.
Submitted: 19 March 2001. Accepted: 28 Nov. 2001. Section editor: J. D. McEachran.

APPENDIX 1.

Locus	Population 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
sAta-A	bb:25	bb:6	cc:10	cc:2	ac:2 cc:6	cc:6	aa:6	aa:9 ac:2	aa:13	aa:8	bb:11	bb:18	cc:18	cc:14	cc:7
Gpi-A	cc:23	cc:5	cc:10	cc:1	cc:2	cc:1	cc:2	cc:2	aa:1 ac:2 cc:1	aa:2	cc:5	cc:18	cc:18	cc:14	cc:7
sIcdh-A	bb:25	bb:4	aa:10	aa:2	aa:7	aa:3	ae:1 ee:5	ae:2 ee:9	ee:5	ee:8	bb:10	bb:18	aa:18	aa:14	aa:7
Ldh-A	gg:25	gg:6	bb:10	bb:2	bb:1 be:5 ee:2	bb:3	ee:6	be:4 ee:7	bb:10 be:2	be:1 ee:7	gg:11	gg:17	bb:17	bb:14	bb:7
Ldh-B	ii:25	ii:4	ee:10	ee:2	dd:8	dd:3	ee:6	de:4 ee:7	bb:1 bh:2 hh:1	ee:8	ii:11	ii:17	ee:17	ee:14	ee:7
Pep-B	cc:7 cd:14 dd:4	cd:2 ff:1	cc:10	cc:2	cc:8	cc:3	cc:6	cc:11	cc:13	cc:8	dd:11	cc:10 cd:4 dd:1 ce:2 ee:1	cc:18	cc:13 cd:1	cc:7
6Pgdh-A	aa:25	aa:7	aa:10	aa:2	aa:8	aa:3	aa:6	aa:11	aa:13	aa:8	aa:11	aa:18	aa:18	aa:14	aa:7

Locus	Population 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
sAta-A	cc:5	cc:10	cc:4	cc:10	cc:9	aa:9	bb:2	aa:7 ac:1	aa:1 ac:2 cc:2	cc:7 cd:4	aa:3 ac:1	cc:5	ac:2	aa:4	aa:9
Gpi-A	cc:5	cc:10	cc:4	aa:7 ac:2	ac:2 cc:6	aa:2 ac:7	cc:2	cc:8	cc:5	aa:11	aa:4	aa:5	aa:2	aa:5	aa:11
sIcdh-A	aa:5	aa:10	aa:4	aa:10	aa:9	aa:3 ae:4	ff:2	cc:8	cc:5	cc:11	aa:3 ac:1	aa:5	aa:1 ae:1	aa:5	aa:6 ab:4 bb:1
Ldh-A	bb:5	bb:3 bc:6 cc:1	bb:4	bb:10	bb:9	be:2 ee:7	ff:2	dd:8	dd:5	dd:11	bb:4	bb:5	ee:2	ee:5	ee:11

Appendix 1. Continued.

Locus	Population 16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Ldh-B	dd:5	dd:5 de:4 ee:1	cc:4	cc:10	cc:9	cc:6 cd:2 dd:1	ff:2	gg:8	gg:5	gg:11	ee:4	ee:5	ef:2	hh:5	hh:11
Pep-B	cc:5	bc:1 cc:8 dc:1	bc:1 cc:3	cc:10	cc:9	cc:9	cd:1 dd:1	cd:2 dd:6	cc:1 cd:3 dd:1	cc:7 ce:3 ee:1	cc:2 ce:2	cc:5	cc:2	cc:5	cc:11
6Pgdh-A	aa:5	aa:10	aa:4	aa:10	aa:9	aa:9	aa:2	cc:8	cc:5	bc:4 cc:7	aa:4	aa:5	aa:2	aa:5	aa:11

Locus	Population 31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
sAta-A	aa:3	cc:1	cc:10	cc:5	cc:4	cc:11	cc:6	cc:5 ce:1	ac:2 cc:10	cc:6	bb:10	bb:13	bb:1	bb:10	bb:11
Gpi-A	aa:2 ac:1	cc:2	bb:10	ab:2 bb:2 bc:1	dd:5	cc:11	cc:6	aa:7	aa:12	aa:6	cc:2	cc:4	cc:1	cc:10	cc:11
sIcdh-A	aa:3	cc:2	cc:8	cc:4	cc:5	cc:11	cc:6	aa:2 ac:4 cc:1	aa:8 ac:3 cc:1	aa:3 ac:2 cc:1	bb:10	bb:13	bb:1	bb:10	ff:10
Ldh-A	ee:3	dd:2	dd:10	dd:5	dd:5	dd:11	dd:6	ee:7	ee:12	dd:4 de:2	gg:10	gg:13	gg:1	gg:10	ff:11
Ldh-B	hh:3	gg:2	gg:10	gg:5	gg:5	gg:11	gg:6	ee:7	ee:12	ee:4 eg:2	ii:10	ii:13	ii:1	ii:10	ff:11
Pep-B	cc:3	dd:2	cc:7 cd:2 dd:1	cc:1 cd:2 dd:2	cc:5	cc:7 cd:3 dd:1	cc:5 cd:1	cc:7	cc:7 cd:3 dd:2	cc:6	cc:1 cd:4 dd:5	aa:5 ac:2 af:1 cf:1 cc:1 dd:1 ff:1	dd:1	dd:10	dd:11
6Pgdh-A	aa:3	cc:2	aa:10	aa:5	cc:5	cc:11	cc:6	aa:7	aa:10 ab:2	aa:1 ac:5	aa:10	aa:13	aa:1	aa:10	aa:11

Appendix 1. Continued.

Locus	Population														
	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
sAta-A	cc:10	aa:15	aa:11 ac:1	bb:6	bb:7	bb:6	bb:13	bb:4	bb:9 bc:4	cc:4	cc:9	cc:6	cc:10	cc:1	bb:10
Gpi-A	aa:4 ac:4 cc:2	aa:11 ac:3 cc:1	aa:2 ac:4 cc:6	cc:6	cc:7	cc:6	cc:13	cc:4	cc:13	cc:4	cc:9	cc:6	cc:3	cc:1	cc:10
sIcdh-A	aa:6 ac:4	aa:3 ae:6 ee:2	ee:12	bb:5	bb:4	bb:5	bb:12	bb:2	ab:2 bb:11	aa:4	aa:7	aa:5	ac:2 cc:7	aa:1	ee:10
Ldh-A	bb:10	bb:1 be:1 ee:13	cc:12	gg:6	gg:7	gg:6	gg:13	gg:4	gg:13	bb:4	bb:9	bb:6	dd:1 de:2 ee:7	dd:1	aa:10
Ldh-B	ee:10	ee:2 eh:10 hh:3	ee:12	ii:6	ii:7	ii:6	ii:13	ii:4	ii:13	ee:4	ee:9	ee:6	ee:1 eg:2 gg:6	ee:1	aa:10
Pep-B	cc:10	bb:6 bc:4 cc:5	cc:12	cc:6	cc:3 cd:3 dd:1	dd:1 df:4 ff:1	cc:1 cd:8 dd:4	ac:1 cd:2 dd:1	cc:10 cd:3	cc:4	cc:9	cc:6	bd:1 cc:2 cd:2 cf:2 df:2 ff:1	bc:1	bc:1 cc:9
6Pgdh-A	aa:10	aa:15	aa:12	aa:6	aa:7	aa:6	aa:13	aa:4	aa:13	aa:4	aa:9	aa:6	aa:2 bb:1 ac:4 cc:2	aa:10	aa:11