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A Serological Investigation of Three Western Populations of Dusky Salamanders, Genus *Desmognathus*, with Comments on the Establishment of the Population on Crowley's Ridge

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Abstract: The blood sera of three western populations of the genus *Desmognathus* were compared by immunodiffusion and immunoelectrophoretic techniques. All populations had antigenic differences. Populations from Crowley's Ridge, Arkansas, and western Kentucky had slight differences in the albumin patterns. They were distinguishable from the Ouachita Mountain population by differing pre-albumins and albumins. These results agree with the recognition of the Ouachita Mountain populations as *D. brimleyorum* and the Crowley's Ridge population as a recent isolate from *D. fuscus* stock east of the current Mississippi River Alluvial Plain.

* * *

INTRODUCTION

Because of their distribution dusky salamanders are an ideal group for the investigation of serological differences between isolated populations. West of the Mississippi River populations of *Desmognathus* are found in the Ouachita Mountains of western Arkansas and eastern Oklahoma, Crowley's Ridge in eastern Arkansas, the Ouachita-Red River rolling lands of extreme southern Arkansas and Louisiana, and the Coastal Plain of Texas and Louisiana.

The Ouachita Mountain population is separated from the Crowley's Ridge population by the Arkansas River Valley and the former Mississippi

River Alluvial Plain. The western Kentucky population is currently separated from the Crowley's Ridge population by the Mississippi River and its Alluvial Plain. How long these populations of *Desmognathus* have been separated is not known. It appeared challenging to the authors to investigate whether factors promoting genetic divergence (gene mutation, isolation, genetic drift, etc.) have created distinct serological differences between the individual populations. Blood sera of three populations were compared by immunodiffusion and immunoelectrophoretic techniques.

MATERIALS AND METHODS

During February-April 1972, 103 dusky salamanders were taken from four collecting sites within three separate geographical populations. In the Ouachita Mountains, Arkansas, samples were obtained from two sites. Forty-four salamanders were taken from two creeks near Albert Pike Recreation Area, 9.7 km south, 17.0 km west of Norman, Montgomery County (T4S, R27W, Section 27 SW $\frac{1}{4}$). Thirty-one additional salamanders were collected 15 km (speedometer reading) northwest of Mena High School along Arkansas Highway 88, Mena, Polk County (T1S, R31W). The sample of six salamanders from Crowley's Ridge, Arkansas, was collected 1.6 km west of Birdeye, Cross County (T9N, R4E, Section 30, SE $\frac{1}{4}$). The Kentucky sample of 22 salamanders was taken at Ferguson Creek, 2.7 km east of Smithland along Kentucky Highway 70, Livingston County (Fig. 1). All salamanders were transported live to Milwaukee, bled and the serum collected and frozen. Representative specimens were then preserved and deposited in the Milwaukee Public Museum (MPM 11144-11149, 11156-11181) herpetological collection. Blood was obtained by dissecting the pericardial cavity, making an incision in the ventricular wall and drawing blood into a 0.5 ml syringe. After clotting, serum samples were obtained by centrifugation and each population sample was separately pooled.

A New Zealand white rabbit was used as the antibody source. A mixture of equal parts of *Desmognathus* serum (Ouachita population) and Freund's complete adjuvant was injected intramuscularly into three sites: both thighs and the back of the neck. An injection of one ml, divided among the three sites, was given once a week for three consecutive weeks and once again after 50 days had elapsed. Seven days thereafter a Ring test was performed. The highest titer reached was 1:16,000. A 0.2 ml injection was given to maximize titer. The rabbit was bled seven days later. The blood was allowed to clot, ringed and placed under refrigeration overnight. After centrifugation for 15 min, 30 ml of serum were obtained and subsequently frozen until used.

The immunodiffusion technique of Ouchterlony (1958) and the immunoelectrophoretic method of Grabar and Williams (1955) were employed for serum analysis. Approximately 20 μ l of serum for each population and 40 μ l of antiserum were placed in individual wells.

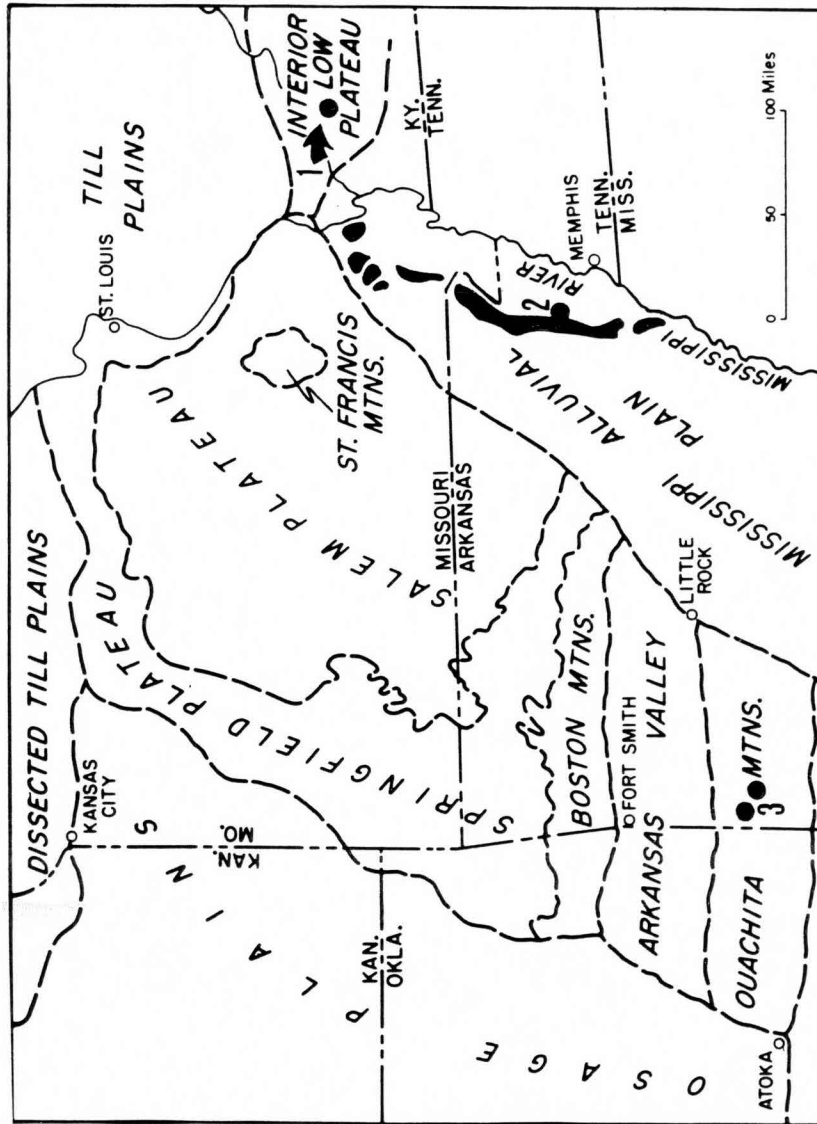


Fig. 1. A map (modified from Thornbury, 1965) featuring the Interior Highlands, Upland Tracts in the Mississippi Valley, including Crowley's Ridge (black) and other zoogeographic components that affect western *Desmognathus* distribution; (1) Livingston County, Kentucky, (2) Cross County, Arkansas, (3) Polk and Montgomery Counties, Arkansas. Collecting localities are indicated by solid circles.

RESULTS

Immunoelectrophoresis

All three populations had identifiable serum protein differences in the albumin region of the electrophoretic separation. Populations from Crowley's Ridge, Arkansas, and western Kentucky showed slight differences in the albumen patterns (Fig. 2). They were distinguishable from the Ouachita Mountain population by different pre-albumins and albumins.

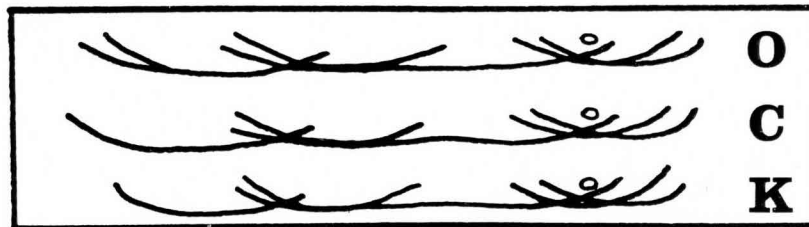


Fig. 2. Sketch of immunoelectrophoretic patterns illustrating population serum protein differences. Ouachita Mountain samples are indicated by O. Crowley's Ridge by C and western Kentucky by K. Ouachita samples had six distinguishable bands including two in the albumin region. Crowley's Ridge samples had a longer albumen band than did those from western Kentucky.

Gel Diffusion-Modified Ouchterlony

The results obtained with immunoelectrophoretic techniques were corroborated by gel diffusion analysis. The sera of Crowley's Ridge and western Kentucky populations were easily distinguishable from that of the Ouachita populations. A small spur was formed when the serum from the western Kentucky population was reacted against that of the Crowley's Ridge population, utilizing the anti-Ouachita serum. Thus, these populations could be readily distinguished (Fig. 3).

By the above technique, serum could be identified as belonging to each population. This conclusion was tested by having a colleague label known pooled serum samples A, B and C and then submit them to us, as unknowns, for identification. In all cases identification was correct.

DISCUSSION

The systematics of the genus *Desmognathus* has been controversial. The Ouachita Mountain population has been considered a full species, *Desmognathus brimleyorum*, by Stejneger (1895), and a subspecies, *D. fuscus brimleyorum*, by others (e.g., Conant, 1958). *Desmognathus fuscus* from western Kentucky and western Tennessee were described as a new subspecies, *D. f. conanti*, by Rossman (1958). The *Desmognathus* from Crowley's Ridge have received little attention.

Several populations of *Desmognathus* have been studied previously by

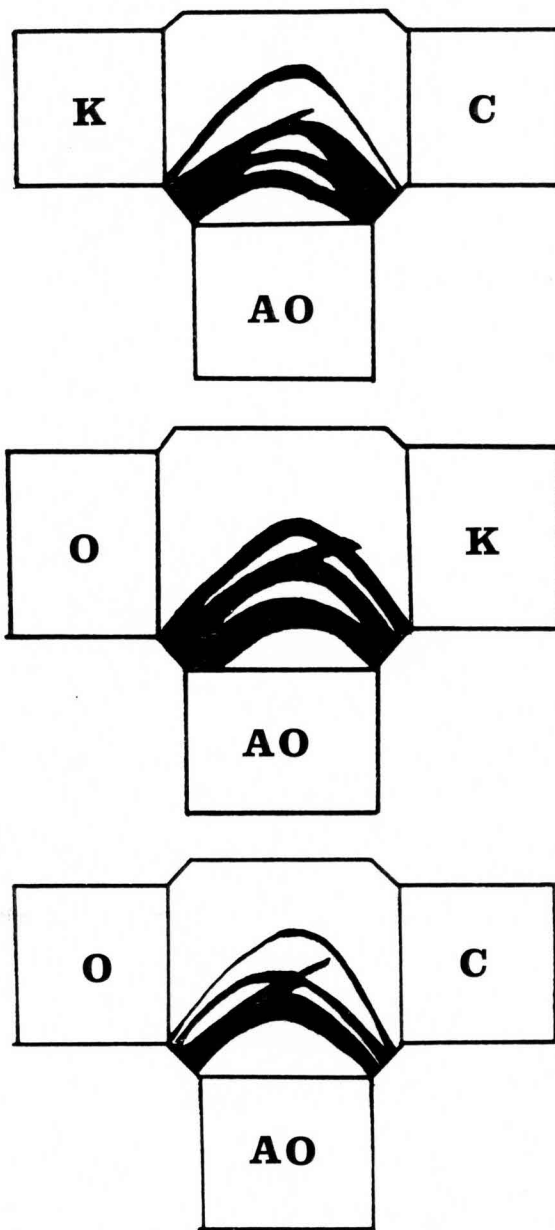


Fig. 3 Illustrations of precipitin reactions of pooled serum samples in Type III Ouchterlony plates. Ouachita Mountain serum is indicated by O. Crowley's Ridge by C, western Kentucky by K, and Anti-Ouachita by AO.

electrophoretic techniques. Shontz (1968) compared hemoglobins and isoenzymes of numerous species of eastern *Desmognathus*. The findings of Means (1974) indicated a complexity of relationships within *D. fuscus*. Hinderstein (1971) sampled "*D. fuscus*" from four sites, including Edmonson County, Kentucky, and Montgomery County, Arkansas. There was considerable variation in the lactate dehydrogenase patterns between all populations.

In this study the different bands of precipitation and spurs obtained when the sera of the populations were cross-reacted suggest genetic differences within the separate populations. These differences would reside in those genes which code for the pre-albumin and albumin proteins. All three populations are distinguishable by differences that appear in the albumin region of electrophoretic migration.

The populations from the Ouachita Mountains, Crowley's Ridge and western Kentucky are antigenically distinguishable from each other. Furthermore, the western Kentucky and Crowley's Ridge sera demonstrate closer affinities to each other than either do to the Ouachita serum.

Certain limitations should be applied to the interpretation of these findings. The limited population from Crowley's Ridge (six specimens) may not reflect the heterogeneity of the population. Unfortunately, the current status of Crowley's Ridge *Desmognathus* populations is dismal (D. B. Means, pers. comm.) and the subpopulation we sampled may be extinct. None of the latter have been observed since 1972. Further, these data are the result of but one antiserum, and cross reactions could not be run because of insufficient quantities of serum from the Crowley's Ridge population.

The establishment of the Crowley's Ridge population

During the Wisconsin Epoch there was a cooler world climate. Large amounts of water were incorporated within glaciers resulting in lowered sea levels. The Ohio and Mississippi Rivers presumably had greatly reduced flow as compared with pre or post-glacial volumes. This would have been an opportune time for eastern *Desmognathus* to cross what was then the Ohio River Channel and invade Crowley's Ridge. Sometime between 17,000-11,500 years ago glaciers began retreating rapidly and the Ohio and Mississippi Rivers enlarged, rushing great quantities of water to the Gulf of Mexico (see Wright, Stein, Farrand, Evenson, Andrews, and Emiliani, 1976, for discussion). These torrential rivers would have isolated Crowley's Ridge *Desmognathus* from others. A more recent event, which would have caused isolation, occurred sometime between 6,000 and 2,000 years ago. During this period the course of the Mississippi River changed from west to east of Crowley's Ridge (Fisk, 1944). Thus, the combined flows of the Ohio and Mississippi Rivers became a barrier to east-west movement. This relatively brief isolation is consistent with the minor serological variation indicated by this study.

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