Journal of the Arkansas Academy of Science

Volume 33 Article 10

1979

Comparison of the Lens Protein Profiles of Three Species of Ozark Salamanders

James M. Britton
University of Arkansas, Fayetteville

A Comparison of the Lens Protein Profiles Of Three Species of Ozark Salamanders

JAMES M. BRITTON
Department of Zoology
University of Arkansas
Fayetteville, Arkansas 72701

ABSTRACT

The vertebrate lens has a high protein content (35%), 80-90% of which is composed of the soluble, lens-specific structural proteins, the crystallins. The lens protein profiles of urodelan species have been found to be qualitatively distinct. On this basis the lens proteins have been proposed as a measure of true speciation in urodelans.

This paper is the result of our effort to derive a technique for comparison of lens protein profiles on a purely qualitative basis without utilizing the complex methodology employed by previous workers. With this object in mind, the lens protein profiles of *Plethodon glutinosus*, *Ambystoma annulatum*, and *Ambystoma maculatum* were studied to determine the degree of difference actually observable on a purely qualitative basis, and thus the applicability of this technique to urodelan taxonomy. Definite observable differences were found in the lens protein profiles of all species; thus confirming the potential value of this technique to urodelan taxonomy.

The protein content of the vertebrate lens is very high (approx. 35%). The crystallins, soluble lens-specific structural proteins, comprise 80-90% of the total lens protein (Clayton, 1970). Investigations of the lens proteins of the various vertebrate classes have shown extensive differentiation at that level (e.g. Cobb et. al., 1968). Brahma and van Djoorenmaalen (1969) found distinct differences in the lens proteins of five phylogenetically distant species of anurans and urodelans.

McDevitt and Collier (1975), using cellulose acetate electrophoresis, examined the soluble lens proteins of 12 species of North American salamanders; 10 species of which were in the same family, the Plethodontidae. They were able, using qualitative differences in the electrophoretic profiles, to distinguish all of the 12 species, several of which are very close phylogenetically. Based on these findings they suggested the use of the lens proteins as a sort of "phylogenetic fingerprint" in distinguishing urodelan species. They further suggest that the lens proteins, recognized to be evolutionarily conservative, could be used as a measure of true speciation in urodelans. The potential of such a procedure in urodelan taxonomy, based as it often is on meristic criteria, is obvious.

This study was undertaken with the object of eliminating much of the complex methodology employed by previous workers so as to provide a simple, systematic, purely qualitative test of speciation in urodelans. Thus no attempt was made to quantify data as regards sample concentration or density of corresponding bands (some reference is made to density differences in certain regions of the total pattern). The lens protein profiles of three species of Ozark salamanders were studied to determine the degree of difference actually observable using such simplified methods.

METHODS

Salamanders were captured in the field during the month of November, 1978. *Ambystoma annulatum* were collected during their annual breeding migration crossing Highway 45 (Washington Co.) at a point approximately ten miles east of the Fayetteville campus of the University of Arkansas. *Ambystoma maculatum* and *Plethodon glutinosus* were collected in the area below the dam at Lake Wedington (Washington Co.), 13 miles west of Fayetteville on Highway 16.

After sacrificing the animals, the eyes were removed with the aid of scalpel and forceps and placed in a petri dish containing cold buffer (Tris HCl .05M pH 7.2). Lenses were then removed by gripping the eyeball in a small hemostat, slitting the wall and forcing the lens out with a blunt dissecting needle. The lenses were often extruded free of extraneous ocular material; any remaining extraneous material was removed with watchmakers forceps and dissecting needles. Due to the small amount of lens material provided by individual salamanders, the material for each species was pooled for use in electrophoresis. The pooled lenses were placed in cold buffer (Tris HCl .05M pH 7.2) and, if not used immediately, were stored at near freezing temperature until use.

Samples of the soluble lens protein for use in electrophoresis were obtained by preparing a homogenate of the lens material using ground glass - ground glass homogenization of the lenses in an amount of the above buffer approximately equal to the volume of lens material. The homogenate was then centrifuged and the supernatent collected. Samples of soluble lens protein thus obtained were refrigerated at 4° C until use in electrophoresis.

Cellulose acetate electrophoresis was chosen as the most suitable method for resolution of the protein samples because of the previously reported anomalous behaviour of the high molecular weight alpha-crystallins when polyacrylamide gel or other such "molecular sieve" methods are used (McDevitt, 1967). Gelman Sepraphore III cellulose polyacetate strips were pre-soaked for at least five minutes in Tris-Glycine buffer (0.1M pH 8.3). Lens protein samples were applied using a Gelman sample applicator, with the number of sample applications per strip varying from three to five. Electrophoresis was then performed using a Gelman Deluxe electrophoresis chamber and a Gelman power supply at a current of 1.25 to 1.5 m.a. per strip for 40-45 min.

Following electrophoresis, strips were stained in a solution of 0.2% Ponceau S in 3% trichloracetic acid for at least five minutes; then destained in several rinses of 5% acetic acid until no background stain remained. The following results were based on the results of eight electrophoresis trials utilizing lenses from a total of 19 salamanders (eight *P. glutinosus*, seven *A. annulatum* and four *A. maculatum*).

RESULTS

The crystallins have been separated into three heterogenous groups; alpha, beta and gamma in order of decreasing molecular weight and mobility in an electric field (Clayton, 1970). Using cellulose acetate electrophoresis and companion immunoelectrophoresis of protein fractions obtained by column chromatography of whole lens protein samples, McDevitt and Collier (1975) were able to determine the distribution of alpha, beta, and gamma in the total electrophoretic pattern (Fig. 1). As they themselves emphasized, such a crude separation is useful for discussion purposes only.

Photographs of the patterns produced by electrophoresis of the lens proteins of *P. glutinosus*, *A. annulatum*, and *A. maculatum* are shown in Fig. 2. Schematic representations of the banding patterns.

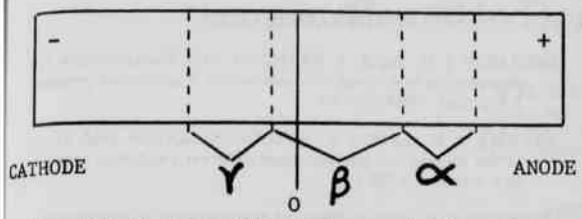

Figure 1. The distribution of the crystallins in a typical electrophoretic pattern (adapted from McDevitt and Collier, 1975).

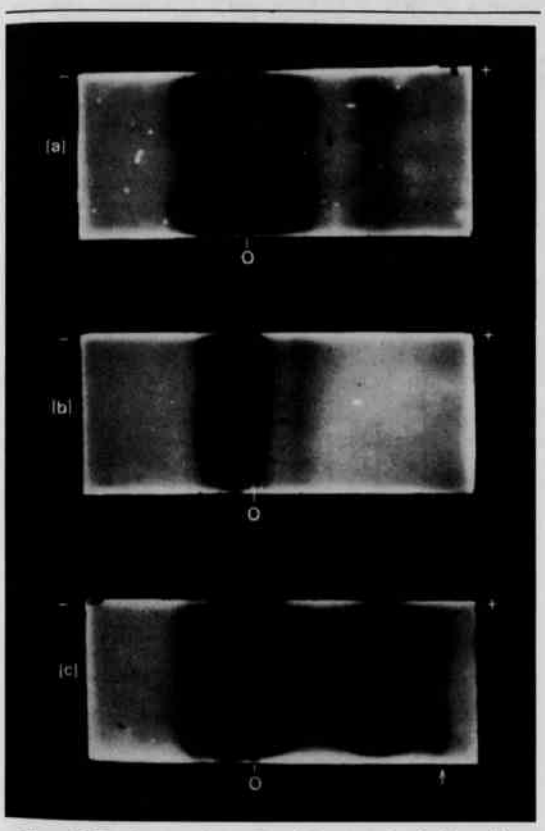

Figure 2. The lens protein profiles of three species of salamanders.
(a) *Plethodon glutinosus*
(b) *Ambystoma annulatum*
(c) *Ambystoma maculatum*

drawn to scale, are shown in Fig. 3. Readily observable differences can be seen in all areas of the patterns obtained for all three species.

The pattern for *A. annulatum* is distinctive in showing poor resolution and low band mobilities, with single alpha, beta and gamma bands. The patterns for *P. glutinosus* and *A. maculatum* appear superficially similar although clearcut differences exist in all areas of the patterns. In the far cathodal (gamma crystallin) region, resolution was better in the pattern for *P. glutinosus*, with three apparent bands opposed to two in *A. maculatum*. The nearest cathodal of the gamma bands of the two species appears to have similar mobilities. In the beta crystallin region, *A. maculatum* shows a very dense, slightly cathodal band absent in *P. glutinosus*. (Large amounts of beta

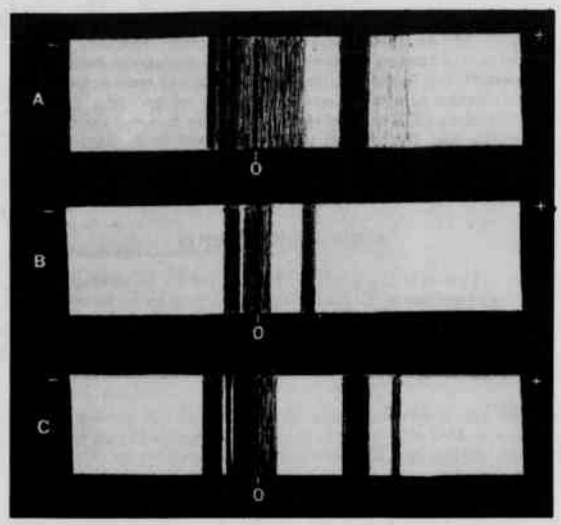

Figure 3. Schematic representations of the lens protein profiles of three species of salamanders.
(a) *Plethodon glutinosus*
(b) *Ambystoma annulatum*
(c) *Ambystoma maculatum*

crystallin are present in the same area in *P. glutinosus*, but the protein is diffuse.) Conversely, *P. glutinosus* shows an area of far anodal beta crystallin lacking in *A. maculatum*. The alpha bands of the two species appear to have identical mobilities; however, the clear presence of a pre-alpha (high mol. wt. alpha) band in *A. maculatum* (arrow, Fig. 2) distinguishes the two in this area. Although a pre-alpha band has been reported (McDevitt and Collier, 1975) in *P. glutinosus*, none could be resolved in these samples.

Resolution is generally best in the far cathodal (gamma crystallin) region of the total lens protein profile. The lack of resolution in the alpha, and to some extent in the beta, regions of the pattern reported by Brahma and van Djoorenmaalen (1969) is apparent in these samples. These resolution differences stem from the nature of the proteins themselves; gamma crystallins being single chain polypeptides with rather definite size, charge and mobility, and alpha and beta crystallins being aggregate proteins, with size, charge and mobility dependent upon a variable complement of subunits.

DISCUSSION

The potential value of a reliable test of speciation, especially in urodelans where meristic criteria are relied on heavily, is unquestionable. The lens proteins, which are direct gene products and therefore less susceptible to environmental modification than morphology, would be suitable for use in such a test. Their structural and non-enzymatic nature, by restricting observed differences to actual differences in protein structure, thus reflecting the actual gene makeup of the species with respect to lens proteins, also favors their use in such a test.

In reference to the species herein investigated, there is no difficulty distinguishing one from another on the basis of lens protein profiles alone. These results would tend to justify the conclusion of McDevitt and Collier (1975) that the lens proteins are potentially valuable in determining true speciation in urodelans. The apparent failure of this procedure to distinguish subspecies/intergrades and phases, as well as individual differences, as reported by McDevitt and Collier (1975) would be a further point in favor of the application of this procedure to taxonomic problems where the validity of a species is in doubt.

It is of interest here to note that study of the lens proteins could possibly indicate evolutionary trends in certain urodelans, the observed lack of banding and low mobility of *A. annulatum* being a case in point. During dissection, trends in morphology were noted in this species similar to trends noted by previous workers (e.g. Besharse and Brandon, 1974) in troglodytic and highly fossorial species. The possibility that the unusual pattern observed in *A. annulatum* is indicative of degenerative evolution of the eyeball is certainly worth further investigation.

ACKNOWLEDGEMENTS

I would like to thank Dr. W. C. Guest for his patience and assistance, C. Britton and C. Harding for transportation, and R. L. Boll and N. Hernandez for photographic assistance.

LITERATURE CITED

BESHARSE, J. C., and R. A. BRANDON. 1974. Postembryonic eye degeneration in the troglobitic salamander, *Typhlotriton spelaeus*. J. Morphol. 144(4):381-405.

BRAHMA, S. K. and W. J. VAN DJOORENMAALEN. 1969. Study of the soluble lens proteins from different amphibian species. Exp. Eye Res. 8:168-171.

CLAYTON, R. H. 1970. Problems of differentiation in the vertebrate lens. Curr. Top Devel. Biol. 5:115-180.

COBB, B. F., III, L. C. CARTER, and V. L. KOENIG. 1968. The distribution of the soluble protein components in the crystalline lenses of specimens of amphibians, reptiles, and birds. Comp. Biochem. Physiol. 26:519-528.

MCDEVITT, D. S. and C. R. COLLIER. 1975. The lens proteins of eastern North American salamanders and their application to urodelan systematics. Exp. Eye Res. 21(1):1-8.

MCDEVITT, D. S. 1967. Separation and characterization of the lens proteins of the amphibian, *Rana pipiens*. J. Exp. Zool. 164:21-29.