ELECTROPHORETIC PATTERNS OF CERTAIN PROTEINS IN CAECILIANS (AMPHIBIA: GYMNOPHIONA)

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Abstract—1. A maximum of 15 loci from five genera of caecilians (Amphibia: Gymnophiona or Apoda) were examined. Species include Ichthyophis glutinosus, Geotrypetes seraphini, Caecilia occidentalis, Gymnopis multiplicata, and Dermophis mexicanus.  

2. The data indicate that Ichthyophis (family Ichthyophiidae) is less closely related than are the other genera (members of the family Caeciliidae). Dermophis and Gymnopis, which on other grounds are considered closely related, are no more similar to each other than are other caeciliid genera.

INTRODUCTION

In recent years there has been increasing interest in the amount of electrophoretic protein variation both within and between species and how this variation relates to ecological and evolutionary patterns within these groups. To date, however, there has been only one such report on caecilians (Amphibia: Gymnophiona or Apoda) by Nelson & Guttman (1973), who looked at serum proteins in Dermophis costaricensis. This study presents work involving five caecilian genera: Ichthyophis, Geotrypetes, Caecilia, Gymnopis, and Dermophis. The junior author was fortunate to have these diverse genera alive in the laboratory at one time, so the unusual opportunity to run this preliminary survey and to evaluate our techniques as applied to caecilians was seized.

MATERIALS AND METHODS

Plasma and tissue extracts were taken from the following: three Ichthyophis glutinosus (Peradeniya, Ceylon [Sri Lanka]); two Geotrypetes seraphini (Tafo, Ghana); one Caecilia occidentalis (Popayan, Colombia); two Gymnopis multiplicata (Puerto Viejo, Costa Rica and Isla del Canas, Costa Rica) and three Dermophis mexicanus (San Francisco, Guatemala). The specimens are part of M. H. Wake's personal collection; plasma and tissue extracts are deposited in the collection at the Museum of Vertebrate Zoology, University of California, Berkeley.

Whole blood was collected in heparinized pipettes from an aortic nick and centrifuged at 600 rev/min for 10-15 min; plasma was frozen at -15°C until use. Whole heart and liver were also frozen at -15°C until use. Tissues were prepared for electrophoresis according to the methods of Selander et al. (1971).

Enzyme systems, and the buffers used, include: (a) serum proteins, esterases, leucine aminopeptidase (LAP) and indophenol oxidase (IPO) using a lithium hydroxide buffer; (b) 6-phosphogluconate dehydrogenase (6PGD) and a-glycerophosphate dehydrogenase (aGPD), using a Tris-maleic buffer; (c) peptidase, using the Peulik buffer system; and (d) malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH) and lactate dehydrogenase (LDH), using a Tris-citrate (pH 7.0) system. This Tris-citrate electrode buffer was 0.14 M Tris-0.04 M citric acid. The gel buffer was a 1:14 dilution of the electrode buffer. All other buffers and all staining solutions are taken from Selander, et al. (1971). It was found that long term storage of tissues or of tissue extracts, even at -15°C, destroyed LDH activity.

A note should be added about nomenclature. If an enzyme was coded by two different loci, these were numbered according to electrophoretic mobility, with the faster locus designated as 1.

For each pair of species, the number of shared alleles was calculated by determining the number of loci which share the same allele out of the total number of loci examined. Enzyme systems in which it was impossible to determine homology, such as the esterases, were not included in the calculations.

RESULTS

A maximum of 15 loci was examined in each species. We do not have data on a few proteins for all species studied and these will be noted in the description of the results.

Esterases (Fig. 1a): Ichthyophis and Gymnopis both had three esterase loci. Esterase-1 in Gymnopis showed some subbanding.

Geotrypetes, Caecilia, and Dermophis all had two esterase loci; Geotrypetes may have a third locus of intermediate mobility between esterases 1 and 2, but this is uncertain because of difficulty in scoring. The Dermophis esterase-1 locus also showed subbanding in some cases.

General serum proteins (Fig. 1b): Three loci, which showed no polymorphism, were seen in Ichthyophis. Serum from only one specimen of Geotrypetes was available, so it is not known if there is any polymorphism in the two loci detected. Caecilia showed only one major band which had the same mobility as the single band seen in Gymnopis. Dermophis did show...
Fig. 1. Electropherograms of caecilian proteins.
(a) Esterases 1-3. Key to species identity: A1-A3 = Ichthyophis glutinosus; B1-B2 = Geotrypetes seraphini; C = Carcilia occidentalis; D1 = Gymnopis multipunctata from Puerto Viejo, Costa Rica; D2 = G. multipunctata from Isla del Cano, Costa Rica; E1-E3 = Dermophis mexicanus. This designation is followed in all electropherograms presented here.

(b) General serum proteins
(d) IDH 1 and 2.
(f) MDH 1 and 2.
(g) Peptidase. (h) 6-PGD. (i) LAP.

polymorphism for the fastest moving band, having fast, intermediate and slow alleles; the intermediate allele is of the same mobility as that of Gymnopis.

zGPD (Fig. 1c): No species showed polymorphism at this locus; the anodal bands seen in Gymnopis and Dermophis are probably subbanding.

IDH (Fig. 1d): There appear to be two loci involved. Ichthyophis, Geotrypetes, and Dermophis all showed no polymorphism at the faster locus. IDH-1, although in some cases subbanding was seen. IDH-1 of Carcilia showed only faint activity. The three-banded phenotype seen in Gymnopis may result from heterozygosity at this locus or the more anodal bands may simply be subbanding; because of the uniform staining intensity, the former hypothesis is favored.

The slower locus, IDH-2, could not always be detected which may reflect the sensitivity of this enzyme to long-term storage. The mobility at this locus was the same in all species except Ichthyophis in which the enzyme was of faster mobility.

IPO (Fig. 1e): Data are available for only Ichthyophis, Gymnopis and Dermophis. The three banded pattern shown by all specimens of Ichthyophis could be produced in two ways if the functional molecule is a dimer: (a) there are two alleles at one locus and these three individuals are heterozygous for these two alleles; or (b) the enzyme subunits are coded for by two different loci. Alternatively, if the molecule is a monomer there is a possibility that three independent loci are involved. More extensive population studies or breeding experiments are needed to distinguish among these alternatives.

The Gymnopis which showed a two-banded pattern may be a heterozygote. Dermophis showed no anodal band but did have a well-defined cathodally migrating band. It is not known if the anodal IPO activity was too weak and be detected in these samples or whether this cathodally-migrating protein is homologous to the anodally-migrating proteins of the other genera.
MDH (Fig. 1f): As with IDH, there appear to be two loci involved. Only *Ichthyophis* exhibited polymorphism at the fast locus, MDH-1, with a fast homozygote, a slow homozygote, and a heterozygote represented. None of the others showed any polymorphism at this locus, although a lot of subbanding was seen.

*Geotrypetes* exhibited polymorphism at the slow locus, MDH-2. One allele was of the same mobility as that seen in *Ichthyophis* and *Gymnopis* while the second was a slow allele. No MDH-2 activity could be detected in *Caecilia* (see discussion). The MDH-2 of *Dermophis* was of much faster mobility than that of the others.

Peptidase (Fig. 1g): Data are available for only *Gymnopis* and *Dermophis*; no polymorphism was seen in either one.

6PGD (Fig. 1h): Both *Ichthyophis* and *Geotrypetes* were polymorphic at this locus; none of the others showed any variation.

LAP (Fig. 1i): Data are available for *Ichthyophis, Caecilia, Gymnopis and Dermophis*. *Ichthyophis* apparently had two loci, the slower of which had the same mobility as that of *Caecilia*. The electrophoretic mobility was the same in both *Gymnopis* and *Dermophis*. No variation was seen.

**DISCUSSION**

Technical considerations. As noted above, we found that low temperature storage destroyed activity of LDH, an enzyme considered quite stable. Extracts electrophoresed within 24 hr of sacrifice of the animal did show activity while those stored for longer periods did not. It is possible that long-term storage also affected the activity of IDH-2, IPO, peptidase, and LAP, although sensitivity to storage may differ among the different species examined, and may explain the lack of data for some species at these loci.

The apparent absence of MDH-2 activity in *Caecilia* should also be considered. It can be explained in several ways: (1) it really is absent; (2) it is extremely sensitive and loses activity quickly; (3) it is of the same electrophoretic mobility as MDH-1. Such a situation is also found in MDH-2 of the lizard *Uta stansburiana*, where mitochondrial and cytoplasmic functions of cell hemogenates were electrophoresed separately (S. Y. Yang, pers. comm.). Avise & Kito (1973) suggested a similar hypothesis to explain the absence of one PG1 locus in five species of teleosts. Our data cannot distinguish between these hypotheses but further work should clarify the issue.

Genetic variation and distance. Studies of genic variation usually are concerned with intraspecific variability. Although there has been some extensive work with frogs (Desseru & Nevo, 1969) and salamanders (Hedgecock, 1973), there is no baseline for caecilians. The only work is that of Nelson & Guttmann (1973) who found seven general protein bands when they electrophoresed serum of *Dermophis costaricense* (*D. mexicanus*, Savage & Wake, 1972), while we could detect only two. The difference, however, may be the result of variation in techniques used. No estimate of genetic variability could be made because of our small samples of each species. When a large number of loci are examined, electrophoretic data can also be used to calculate genetic distances between species. No one, however, has attempted to determine genetic distance.

Sample sizes are too small to attempt calculations of genetic distance. A crude measure of this, however, would be the number of shared alleles. Such calculations show a range of 0 to 50% shared alleles for different species pairs (Table 1). These data should be interpreted with caution. The number of loci used was less than the total examined because (1) in some cases, such as the esterases and general proteins, it was impossible to determine which loci were homologous; and (2) data were not available for some loci in certain genera. Thus the actual number of loci used was small. Further, when working with organisms as distantly related as to be in different genera, it is not wise to assume that identical electrophoretic mobility implies identical molecules. It is quite possible that the actual number of shared alleles is much lower than indicated here.

Our samples, though small, reflect considerable taxonomic, morphological, and geographic diversity among caecilians. *Ichthyophis* is considered a primitive genus and is placed in the family Ichthyophiidae (Taylor, 1968). The genus is widespread throughout Asia; the species that we sample is Celanese. Our other four genera are members of the family Caeciliidae, a large, pantropical group. *Geotrypetes* is a viviparous West African genus; *Caecilia occidentalis* is a slender-bodied, extremely attenuate species of the genus *Caecilia* that occurs in Colombia, South America; *Gymnopis* and *Dermophis* are viviparous Central American genera that are considered to be closely related. In the light of this taxonomic, morphologic, and geographical diversity, it is of interest to assess the data to see what kind of diversity is revealed biochemically. As mentioned above, we cannot calculate genetic distance, but percentages of shared alleles are of interest. *Ichthyophis* shows low percentages of alleles shared with other genera. The African *Geotrypetes* and the New World *Caecilia, Gymnopis, and Dermophis* share several alleles and form a group correlated with their familial inclusion. *Dermophis* and *Gymnopis* show a low percentage of shared alleles, so close relationship of these genera is not supported biochemically.

We view these assessments with caution. However, we wish to consider that the biochemical data give some indication that *Ichthyophis* is more distantly related than are the other genera; members of the family Caeciliidae form an identifiable group; that *Gymnopis* and *Dermophis* are not more closely related than are other members of the family; and that *Geotrypetes'* long isolation on Africa from the Central
American genera does not seem to have resulted in biochemical differences that would indicate distance of relationship.

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REFERENCES


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Key Word Index—Caecilians; amphibians; Apoda; electrophoresis.

ERRATA

1. p. 473, para. 2, line 7. The specimens do not constitute a personal collection in the usual sense. They could not have been accessioned as living specimens; they will be deposited in various museums as soon as current work with them is terminated.

2. p. 473, para. 4, line 6: Poulik

3. p. 474, figure 1, g-i: D and E equal D1 and D2 of legend; F, G, and H equal E1, E2, and E3, respectively.