IMMUNOLOGICAL COMPARISONS OF CAECILIAN ALBUMINS (AMPHIBIA: GYMNOPHIONA)

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IMMUNOLOGICAL COMPARISONS OF CAECILIAN ALBUMINS (AMPHIBIA: GYMNOPHIONA)

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ABSTRACT: Analysis of the relationships of six genera of caecilians (Amphibia: Gymnophiona) by microcomplement fixation techniques gave immunological distances of from 70 units to 210 units for *Dermophis* to other genera. The times of divergence indicated for these genera are consistent with those postulated on zoogeographic grounds.

DURING the past several years, biochemical methods have proved valuable as tools in the study of the evolutionary history of groups of organisms. One such technique is starch gel electrophoresis which is of particular value when comparing closely related species. However, for studies of intergeneric or interfamilial relations, comparisons of single proteins, such as albumin or transferrin, using the technique of microcomplement fixation have proved of greater value (Sarich et al., 1973).

The caecilians are a group of limbless, blind, burrowing or swimming amphibians that are pantropical in distribution. Since they are not often collected, relatively little is known about them. It is believed that the group occurred on the hypothetical ancient continent Gondwanaland more than 120 million years ago and its present pan-

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tropical distribution is the result of the breakup of the southern supercontinent, with subsequent radiation on the continental segments (Savage, 1973). Estes and Wake (1972) described the only known fossil of the group from the Paleocene of Brazil. The morphology of the fossil vertebra indicates that the species was limbless and elongate, as are modern species.

The relationships among the four families of caecilians (Ichthyophiidae, Caeciliidae, Typhlonectidae [Taylor, 1968] and Scolecomorphidae [Taylor, 1969*a*]) and among genera within families are not clearly settled. Because we had tissue or blood samples from six genera representing two families, we undertook a comparison of albumins using the method of microcomplement fixation in an effort to examine the relationships among the six genera.

MATERIALS AND METHODS

Plasma and/or tissue extracts were available from the following: *Ichthyophis glutinosus* (Peradeniya, Ceylon [Sri Lanka]), *Geotrypetes seraphini* (Tafo, Ghana), *Boulengerula boulengeri* (Usambara Mts., Tanzania), *Caecilia occidentalis* (Popayan, Colombia), *Gymnopis multiplicata* (Puerto Viejo and Isla del Canas, Costa Rica), and *Dermophis mexicanus* (San Francisco, Guatemala). Specimens will be deposited in the Museum of Vertebrate Zoology, University of California, Berkeley.

Whole blood was collected in heparinized pipettes from a nick in the aorta and centrifuged at 600 rpm for 10–15 min; plasma was frozen at –15°C until used. Heart and liver were stored at –15°C until used; both tissues were ground together, according to the methods of Selander et al. (1971), to form a tissue extract.

Serum albumin from *D. mexicanus* was purified by preparative polyacrylamide gel electrophoresis (Wallace and Wilson, 1972). Antisera to whole *Dermophis* serum and to purified *Dermophis* albumin were produced by immunizing three Dutch belted rabbits for each group according to a published immunization schedule (Wallace et al., 1971). The purified albumin was considered free of impurities since it gave a single precipitin arc when tested against antisera to whole sera in immunoelectrophoresis. Antisera to pure *Dermophis* albumin from individual rabbits were pooled in inverse proportion to their microcomplement fixation titers (Prager and Wilson, 1971); the pooled antisera had a titer of 1/15,000. Species comparisons were made using the technique of microcomplement fixation; results are expressed as immunological distances (Champion et al., 1974).

TABLE 1.—Immunological distances (IDU) from *Dermophis mexicanus* to five species of caecilians. See text for calculation of divergence time.

Taxa	IDU	Divergence time ($\times 10^6$ years)
Dermophis mexicanus	0	0
Gymnopis multiplicata	70	41
Caecilia occidentalis	97	57
Geotrypetes seraphini	168	99
Boulengerula boulengeri	178	105
Ichthyophis glutinosus	210	124

RESULTS

Immunological distances from *Dermophis mexicanus* to the other five species are given in Table 1. Unfortunately, there was not enough material available to prepare antisera to the albumins of the latter five species; for this reason, reciprocal tests could not be done.

DISCUSSION

The immunological distance between *Gymnopis* and *Dermophis* is of the order seen between genera of other amphibians (for example, *Hyla* and *Acris* [Maxson and Wilson, 1975]) considered closely related on other grounds. For purposes of this discussion we have assumed that albumin in members of this order evolves at the same regular rate observed in most other groups (Maxson and Wilson, 1975), although this rate may be slow in groups such as birds (Prager et al., 1974). Albumin evolves at the rate of 1.7 immunological distance units per million years, and therefore behaves as an evolutionary clock according to Sarich and Wilson (1967). Immunological data indicate that *Gymnopis* and *Dermophis* diverged some 40 million yr ago, or at the beginning of the Oligocene. This is consistent with previous hypotheses for the history of these genera based on zoogeographic analysis. Savage (1966) concluded that the ancestors of the two Central American genera were isolated from the South American forms by the submergence of the Isthmian Link region in late Paleocene–early Eocene. North of the zone of submergence, various lowland units were isolated by embayments and/or climatic effects. Savage and Wake (1972) postulated that the *Dermophis* stock evolved in the Veracruzian center of differentiation and *Gymnopis* in the more southern Caribbean center (*terminology of* Savage, 1966). These centers were separated by a marine embayment through eastern Guatemala until mid-Pliocene times. The emergence of the Isthmian Link in the Pliocene and the removal of marine barriers in the early Pleistocene, coupled with the orogeny of the area and patterns of climatic change, provided lowland corridors for dispersal from the two centers. The present distribution of these genera involves three species of *Dermophis* occurring from southern Mexico to northern Colombia, and one species of *Gymnopis* from Guatemala to western Panama (Savage and Wake, 1972). It is of interest that *Dermophis* and *Gymnopis* are shown to be relatively closely related by this technique, for protein electrophoresis data alone indicate that these genera are no more closely related to each other than to *Geotrypetes* or to *Caecilia* (Case and Wake, 1975). Sarich et al. (1973) have demonstrated a close correlation between genetic distance (D) as measured by Nei, and immunological distance (ID). Since 1.0 D = 30 ID, a distance of 70 immunological units is equivalent to a Nei distance of 2.3 (on a scale of zero to infinity), and one would expect to see little or no electrophoretic similarity between these two genera.

The separation of *Caecilia* from the *Dermophis* stock some 57 million yr ago suggests divergence in the late Paleocene. This is supported by zoogeographic evidence, including information on the fragmentation of lower Central America at that time. The current taxonomic allocation of these genera poses problems in assessing their affinity and derivation. The distinctive morphology of *Caecilia* and the allied genus *Oscaecilia* prompted Taylor (1968) to suggest that they might comprise a separate family, and then to designate them a subfamily, the Caeciliinae (Taylor, 1969*b*). Under this system, the other subfamily, the Dermophiinae, includes Central and South American, African, and Indian genera. Taylor (1968) suggested that this was not a natural grouping, and morphological data collected by Wake support that view. We do not have biochemical information for other South American genera in order to test the divergence of Central American dermophiines and caeciliines from South American dermophiines, but would postulate, based on morphological evidence (Savage and Wake, 1972), that the caeciliine divergence is the older.

Geotrypetes seraphini, though in the same family as *Dermophis*, occurs in west Africa, and its ancestors also were presumably isolated from South American ancestors of *Dermophis* early in the Cretaceous by the breakup of Gondwanaland. That aspect of zoogeography and the immunological data support a time of divergence in the Middle Cretaceous. It should be noted, however, that Case and Wake (1975) found that electrophoresis of several enzymes indicates that *Geotrypetes* is as closely related to Central and South American caeciliids as the Neotropical genera are to one another. Further, Estes and Wake (1972) suggested that the South

American Paleocene fossil *Apodops* showed greater morphological affinity with the extant west African *Geotrypetes* than with species now inhabiting eastern South America. *Boulengerula boulengeri,* also in the same family as *Dermophis,* occurs in the mountains of east Africa. Zoogeographic and immunological data indicate that *Boulengerula,* like *Geotrypetes,* diverged in Middle Cretaceous time, suggesting an African ancestral stock. We await more living material in order to test the affinities of the several African genera.

Savage (1973) stated that the ancient caecilian stock must have occurred on Gondwanaland, and that the stock was separated with the fragmentation of Gondwanaland in the Early Cretaceous. *Ichthyophis glutinosus* occurs on the Indian subcontinent, which remained an island through part of the Tertiary, and where considerable adaptive radiation of caecilians occurred (Savage, 1973). *Ichthyophis* is placed in a separate family, the Ichthyophiidae, from the other genera tested (all members of the Caeciliidae) based on details of morphology and life history. Allozyme data (Case and Wake, 1975) also indicate that *Ichthyophis* is not closely related to the caeciliids. The IDU of 210 indicates a divergence in earliest Cretaceous. Therefore divergence of the ancestors of *Ichthyophis* in the Early Cretaceous is suggested zoogeographically, and supported by electrophoretic and immunologic data.

Little is known of caecilian relationships above the species level because of the relative paucity of specimens. With a recent increase in interest in their biology, however, more specimens are becoming available, and morphological, allozyme, and immunological techniques show great power, especially when employed concurrently, in aiding analysis of evolutionary relationships among these groups.

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