Phylogenetic relationships of the Brasilian leptodactylid frog genera *Craspedoglossa, Cycloramphus* and *Zachaenus*[1]

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Summary. Based on immunological comparisons of the serum albumins of *Craspedoglossa* and *Zachaenus* with antisera to albumin of several species of *Cycloramphus*, it is concluded that morphological and biochemical evolutionary rates have not been comparable within the study group. Taken together, the morphological and biochemical information demonstrate that while *Zachaenus* is a genetic member of the *Cycloramphus* lineage, it has attained generic status.

The relationships of the genus *Cycloramphus* to the leptodactylid genera *Craspedoglossa* and *Zachaenus* have been investigated by several authors using systematic comparisons of morphological features[3]. Until recently, authors have considered the 3 genera as distinct. Lynch[3] has synonymized *Craspedoglossa* with *Zachaenus*, while Heyer[4] has synonymized *Craspedoglossa* with *Cycloramphus* rather than *Zachaenus*. Both authors used morphological analyses to draw systematic conclusions[3]. Lynch's conclusions were based on a small sample of *Cycloramphus* species; Heyer's conclusions[4] were based on an analysis that involved all *Cycloramphus* species. In conjunction with a systematic morphological and biochemical study of the phylogenetic relationships among species of *Cycloramphus*[5], we are able to assess the extent of genetic differentiation between *Cycloramphus*, and *Craspedoglossa* and *Zachaenus*.

Materials and methods. Antisera to pure albumin was made according to established procedures[6] for *Cycloramphus sp. nov.* A (Brasil: São Paulo; Boracéia) and *C.sp.nov.* B (Brasil: São Paulo; near Iporanga). As sources of albumin, plasma or skeletal muscle preserved in a phenoxyethanol solution[7] were used. Micro-complement fixation studies with the albumins of all 3 genera *(Cycloramphus, Craspedoglossa, Zachaenus)* were performed[8] and results are reported as immunological distance units. For anuran albumins 1 unit of immunological distance between 2 species represents roughly 1 amino acid difference in the albumins of these 2 species[9].

Results and discussion. The table summarizes the results of tests with antisera to albumins of representative *Cycloramphus* relationships[5]. *Cycloramphus sp. nov.* A and *C. sp. nov.* B are an average distance of 48 units from one another. The 7 additional species of *Cycloramphus* are representative of the 18 recognized species of this genus and have distances ranging from 5–102 units to *C. sp. nov.* A and from 6–66 units to *C. sp. nov.* B.

In previous anuran studies, one finds distances from 0 to 120 units within a genus[10] and different genera typically differ immunologically by 100 units, although in some cases different hylid genera *(Acris* and *Hyla, Pseudacris* and *Hyla)* have been found to differ by as few as 45–60 units in their albumin immunological distances[11].

The results clearly indicate that the genetic differentiation of albumin between *Cycloramphus* and the other 2 generic units is of the same scale (or less!) than that found within the single genus *Cycloramphus*, and that all 3 generic units are part of the same evolutionary lineage.

These results can be interpreted biologically by 2 alternative hypotheses. Hypothesis 1: Morphological evolution and molecular evolution are different aspects of the same phenomenon and must be congruent. The best classification will be one that uses all available data. Hypothesis 2: Morphological evolution and molecular evolution can occur more or less independently[12]. The evolutionary change of amino acid substitutions in albumin is an approximately continuous process over time and is a good estimator of branching patterns as well as time elapsed since organisms last shared a common gene pool[13]. The evolution of morphology, because of its important phenotypic expression, is best able to infer systematic affinities.

Hypothesis 1 creates a systematic dilemma because the albumin differences place *Craspedoglossa* and *Zachaenus* within the *Cycloramphus* lineage, in contrast to the morphological evidence. The albumin data are not a single piece of information to be weighted along with the multiple morphological characters studied. Rather they represent potential variation at each of 580 different characters (amino acid residues) in the molecule. Thus the molecular data set contains more information than the morphological data set.

We favor hypothesis 2 for the following reasons. Previous studies[6,9–11] are consistent with an 'albumin clock' hypothesis, that is, differentiation of albumin is ubiquitous and relatively constant over time[13]. It is generally acknowledged that an organism's morphology can be almost unchanging over long periods of time even while molecular evolution is proceeding at expected rates[14]. Selection should operate more directly on the organism's gross morphology than on its albumin because the gross morphology directly interfaces with the environment. Thus selection can cause more erratic rates of morphological evolution than molecular evolution.

If hypothesis 2 is accepted it means that while the branching pattern of the lineages can be recovered from the albumin data, the classification scheme cannot. The latter must be obtained from the morphological data and be superimposed upon the branching pattern. The morphological evidence suggests that *Zachaenus* be maintained as a distinct genus. For the present, we conclude that selection, relatively rapidly, has produced the distinctive *Zachaenus parvulus* morphology from the generalized *Cycloramphus*

Comparisons of albumins of *Cycloramphus, Craspedoglossa* and *Zachaenus* species

Species tested	Immunological distance to *Cycloramphus*	
	sp. nov. A	*sp. nov.* B
Cycloramphus		
sp. nov. A	0	45
sp. nov. B	50	0
sp. nov. C	54	6
sp. nov. D	49	24
asper	51	17
brasiliensis	102	66
eleutherodactylus	100	43
ohausi	82	48
semipalmatus	5	46
Craspedoglossa		
stejnegeri	>84	46
Zachaenus		
parvulus	98	27

morphology and the morphological gap between *Zachaenus* and *Cycloramphus* is sufficiently large that they should be maintained in separate genera. This decision rests on the recognition of genera as adaptive units[15], not strictly time differentiated units. This conclusion implies the time history of evolution is an additional and independently resolvable aspect which helps elucidate the classification but does not determine it.

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Behavioral differentiation between two species of cactiphilic *Drosophila*. 1. Adult geotaxis and phototaxis[1]

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Summary. Using Hirsch-Hadler geotaxis and phototaxis mazes, we measured the responses of 2 cactiphilic species of *Drosophila*, *D. mettleri* and *D. nigrospiracula*, to gravity and to light. Both species were found to be photonegative but they differed significantly from each other in their geotactic behavior. *D. mettleri* was geonegative while *D. nigrospiracula* was geopositive. This result was surprising in that natural populations show a contrasting vertical distribution with *D. nigrospiracula* located higher than *D. mettleri* on rotting saguaro substrates.

An interesting case of niche separation occurs between 2 cactiphilic species of *Drosophila* of the Sonoran Desert. The larvae of 1 species, *D. nigrospiracula*, utilize the necrotic tissue of cardon *(Pachycereus pringlei)* on the Baja peninsula and saguaro *(Carnegiea gigantea)* on mainland Mexico. *Drosophila mettleri* larvae live in the soil permeated with the fermenting juices from these rotting cacti[2,3]. While the larval niche separation appears quite complete, considerable overlap exists in the distribution of adult flies. Table 1 shows a typical distribution of adult males and females of both species were aspirated off a rotting saguaro near Tucson, Arizona in October of 1979. The saguaro trunk was necrotic from the ground to a height of about 3 m, and feeding sites were available over the entire area. While *D. nigrospiracula* adults prefer feeding sites located higher up on the cactus trunk, *D. mettleri* are found both on the cactus and on the soil below. *D. mettleri* females are found more often on the ground, frequently under loose rocks. The χ^2 statistic is extremely high indicating that the 2 species are quite different in their distributions. Considering males only from both species, an inhomogeneity χ^2 of 50.87 is obtained, compared to 84.77 for females. Females of *D. mettleri* and *D. nigrospiracula* are even more different than are the males. These data are similar to those reported by Heed[2].

The strong difference in vertical distribution between the 2 species suggested to us that gravity might be a factor in their niche separation. Geotaxis, the behavioral response to gravity, is easily measured in *Drosophila* using Hirsch-Hadler geotaxis mazes. The response of flies to another ecological variable, light, is measurable in a 2nd type of device, the phototaxis maze. This report concerns the geotactic and phototactic behavior of *D. mettleri* and *D. nigrospiracula*.

Hirsch-Hadler phototaxis and geotaxis mazes are described in detail in many studies[4–6]. Flies make a series of choices with respect to light or gravity and are assigned a score of 1 (highly photo-or geonegative) to 16 (highly photo- or geopositive). Photo- or geoneutrality is indicated by a mean score of 8.5. The flies used in these studies were descendents of a multi-female collection of *D. nigrospiracula* reared from a saguaro rot in May 1979. The *D. mettleri* came from a multi-female collection of individuals reared from soil beneath the same rotting saguaro. All stocks were maintained in the laboratory on banana media (University of Texas recipe) until tested in mazes in September, 1979. Males and females were separated under light ether anesthesia 2 days prior to testing. 3 replications were carried out for each sex of each species with each replication consisting of several hundred individuals.

The results of the maze experiments are presented in table 2. There were several striking features of the performance of these 2 species in the geotaxis mazes. First, in natural conditions, *D. nigrospiracula* are found in higher positions on the cactus than *D. mettleri*, which are primarily on or near the ground. If these 2 species were selecting their