

RELATIONSHIPS, ZOOGEOGRAPHY AND SPECIATION MECHANISMS OF FROGS OF THE GENUS *CYCLORAMPHUS* (AMPHIBIA, LEPTODACTYLIDAE)

W. Ronald Heyer

Linda R. Maxson

Abstract

Genetic relationships among Cycloramphus species are studied using the micro-complement fixation technique of albumin analysis. The data matrix is most complete for the fuliginosus group, within which are two clusters of closely related species: boraceiensis-dubius-semipalmatus and asper-dusenilutzorum-mirandaribeiroi-rhyakonastes. These clusters have three interesting properties: (1) There are no morphological features that define either cluster; (2) The two clusters are not closely related to each other; and (3) Each cluster occurs in adjacent, restricted areas.

Application of the albumin clock hypothesis to the data indicates that speciation within the genus has occurred from at least the Eocene, right through the Pleistocene, with most speciation occurring in the Miocene.

Most species have very restricted geographic ranges, within the middle and southern Atlantic Forest Morphoclimatic Domain. Furthermore, most species are found within the Atlantic Forest vegetation itself.

Various zoogeographical schemes are discussed in terms of Cycloramphus species relationships and distributions. We conclude that the allopatric model of speciation is the most plausible hypothesis for understanding the evolutionary zoogeography of Cycloramphus. The following points are critical components of the model in terms of Cycloramphus.

A) There have been at least three kinds of events that have acted as distributional barriers within contiguous Cycloramphus populations: (1) Uplifting of coastal mountains throughout the Cenozoic; (2) Worldwide climatic changes, particularly in the Pleistocene; (3) Local catastrophes.

B) Most of the morphological adaptations at the species level appear to be in response to selection pressures associated with different stream types, deteriorating climates, or premating isolating mechanisms.

C) The key to the allopatric model of speciation for Cycloramphus is the small sizes of geographic areas of isolation and speciation. The scale is local areas of high relief. Each local area had a unique history in terms of geological formation, hydrology, extent of Atlantic Forest cover during cooler and more arid times, and colonizations, adaptations, and extinctions of Cycloramphus populations. No single zoogeographical pattern is apparent. Local patterns of distribution and relationships predominate. The very local effect is thought due to the extremely specialized stream splash zone larvae of Cycloramphus.

Introduction

The Atlantic Forest Morphoclimatic Domain (Ab'Sáber, 1977a) supports a rich and endemic biota of plants (Soderstrom and Calderón, 1974) and leptodactylid frogs (Heyer, 1975) in contrast with the reptile fauna, which is neither especially rich nor endemic (Vanzolini, pers. comm.). The Atlantic Forests were sampled early in the history of Brasil by early explorers and settlers who travelled and settled in the coastal areas of Brasil. While the richness of the biota gives promise of rewarding study through understanding and extending evolutionary and zoogeographic theory, detailed studies of the Atlantic Forest biota are rare (e.g., Jackson, 1978). Most of our data and discussion focus only on the stream associated species of *Cycloramphus*. We have been able to find but one study of Atlantic Forest fish or aquatic insect distribution patterns that is appropriate for comparison with our distribution patterns (Garavello, 1977).

The paucity of detailed studies of the Atlantic Forest biota may be explained, in part, by two major factors. First, the morphoclimatic domains of Brasil have been mapped and defined only recently (Ab'Sáber, 1977a), although the distinctiveness of the areas has been long recognized. We have found that Neotropical frog distributions correlate well with the morphoclimatic domains in Brasil (Heyer and Maxson, 1982a, 1982b). Prior to the general availability of the morphoclimatic domain information, animal distributions often were correlated with vegetation distributions. The vegetation of eastern Brasil is a complex mosaic and any maps of vegetation lack the important generalizations found in the geomorphologically defined maps. The second point is that the taxonomy and systematics of the Atlantic Forest biota are poorly understood. The basic question of how many species of a given genus occur in eastern Brasil is unanswered for most of

the biota. This is true for South American frogs in general. Even for those genera for which the number of species are known, the distributions, patterns of variation, and relationships among included species are usually unknown.

The preceding companion paper on systematics, variation patterns, and distributions of *Cycloramphus* species provides the data needed for the analysis of the zoogeographic and speciation patterns that is the focus of this paper. In this paper, data are first presented on the relationships among the species of *Cycloramphus*; these data are then used to develop both zoogeographic and speciation mechanism scenarios. Our discussion of zoogeographic patterns includes brief statements on the assumptions we use, the general setting, and an analysis of the species distribution patterns. The section on speciation mechanisms develops from the discussions of relationships and zoogeography.

Relationships

One of the primary emphases of the entire study was to gather data from which genetic estimates of relationships could be determined. The methodology used to gather these data is the quantitative immunological micro-complement fixation (MCF) technique for analyzing evolution in the serum protein albumin. Since the MCF data are interpretable in a general time framework, these data have important zoogeographic implications.

MATERIALS AND METHODS

The albumin samples derive from serum or muscle tissue of 15 *Cycloramphus* species. Live specimens were chlorotoned or pithed, and bled from the femoral arteries

using heparinized capillaries. Plasma was obtained by centrifugation. Plasma was mixed (1:1) with phenoxyethanol solution (PPS, Karig and Wilson, 1971) and maintained without refrigeration in the field for rare specimens. In the case of small or rare species where plasma could not be obtained, the skin was removed from skeletal muscle which was then covered with PPS for shipment to the laboratory. Voucher specimens used in this study are deposited in the Museu de Zoologia da Universidade de São Paulo and the National Museum of Natural History, Smithsonian Institution, Washington, D.C.

Albumin was purified from plasma or plasma + PPS by single step polyacrylamide gel electrophoresis (Maxson *et al.*, 1979). Antisera to pure albumins were prepared in female New Zealand White rabbits as follows. Rabbits received an initial intradermal injection of Freund's Complete Adjuvant with albumin solution (1.2:1), followed at seven weeks by a second intradermal injection using Freund's Incomplete Adjuvant and albumin. Both injections were distributed between two sites on the rabbit's haunches. Three weeks later one ml of albumin solution was given intravenously in the marginal ear vein. This was repeated one week later. Rabbits were bled from the marginal ear vein one week following the second intravenous injections. An estimated total of 1-3 mg of albumin was administered per rabbit over the thirteen week immunization period. Three rabbits were used for each immunogen and individual antisera were tested for purity by the criteria described by Wallace *et al.* (1973).

Individual antisera were pooled in inverse proportion to their MCF titers and all reported results were obtained with these pooled antisera. Reactivity was measured by the quantitative MCF technique. Protocol for titering and MCF experiments is given in Champion *et al.* (1974).

Data are reported in immunological distance (ID) units, which are a measure of the sequence difference between the proteins compared (Champion *et al.*, 1974). For albumin it has been determined that one unit of immunological distance is roughly equivalent to one amino acid difference between the albumins compared (Maxson and Wilson, 1974).

Antisera were produced to albumin from several members of the same population for the following five species of *Cycloramphus*: *C. boraceiensis* (Brasil; São Paulo), *brasiliensis* (Brasil; Rio de Janeiro), *duseni* (Brasil; Santa Catarina), *lutzorom* (Brasil; São Paulo), and *rhyakonastes* (Brasil; Paraná). All antisera were judged to be directed predominantly to serum albumin by criteria described by Wallace *et al.* (1973).

RESULTS

Antisera titers ranged from a low of 2600 (*C. rhyakonastes*) to a high of 5500 (*C. brasiliensis*). The average titer of 3900 and slope of 400 is typical of that found in earlier studies of albumin evolution in amphibians (Maxson *et al.*, 1979). MCF reciprocal tests were performed on all ten species pairs and these data are reported in Table 1. The percent standard deviation

Table 1. Matrix of immunological distances among the albumins of five species of *Cycloramphus*.

Species Tested	ANTISERA				
	R	D	L	BR	BL
<i>C. rhyakonastes</i> (R)	0	6	6	54	71
<i>C. duseni</i> (D)	2	0	5	52	71
<i>C. lutzorom</i> (L)	0	6	0	50	74
<i>C. boraceiensis</i> (BR)	35	36	45	0	102
<i>C. brasiliensis</i> (BL)	86	85	66	102	0

from reciprocity as defined in Maxson and Wilson (1975) for these leptodactylid frogs is 36.9%, but this high value is an artifact of the very small distances measured between *rhyakonastes*, *duseni*, and *lutzorum*. When these values are disregarded, the percent deviation from reciprocity drops to a more typical 11.7%.

Data for the five reciprocal tests and for unidirectional tests with the other described species of *Cycloramphus* are reported in Table 2.

INTERPRETATION OF RESULTS

The use of MCF analysis of albumin for evolutionary study is relatively new. As more data are accumulating and being interpreted as a measure of genetic relationships and evolutionary time, questions regarding the data and their interpretation have also been raised (for example, see Radinsky, 1978, for questions and Carlson *et al.*, 1978 for a response, and Corruccini *et al.*, 1980 for further comments). It is necessary to indicate some of the limits of resolution of MCF and possible methodological problems that might influence the results.

A) The fact that a distance of zero is found between two albumins means the albumins are immunologically indistinguishable and have a high probability of having the same primary sequence. It does not mean that the two frogs necessarily share the same gene pool, that is, are conspecific. This is because of the stochastic nature of mutation. The probability of having no divergence between two albumins after a given time of gene pool isolation can be calculated using a Poisson distribution. With albumin having a mean amino acid substitution rate of roughly one per million years (Wilson *et al.*, 1977) there is a 37% chance ($P = \frac{e^{-\mu} \mu^k}{k!}$, where μ is the expected number of substitutions between two

species and $= 1$ and k is the observed number of substitutions and $= 0$) that even after one million years of isolated gene pools two species will have albumins of identical sequence. After two millions years of isolation ($\mu = 2$, $k = 0$) this probability of identical albumin sequence in "good biological species" drops to 14%.

B) The lower limit of MCF repeatability is ± 2 ID units (Maxson and Maxson, 1979). This ± 2 units is experimental error in the sense that if experiments were repeated, values would fall within this range. The upper limit of resolution is not well defined. At the maximum upper limit of 200 units, the rabbit antiserum may retain only a very few active binding sites in the reaction to the albumin being tested. This increases the variance of the estimate and requires that immunological distances between 120-200 units be interpreted conservatively.

C) There is the theoretical possibility that either a single amino acid substitution could be in a region where several antigen sites overlap or a single substitution could change the tertiary structure of the protein in such a way that the antiserum produced would be more different than expected from a single amino acid change in the antigen. Thus, a single amino acid substitution could give an unexpectedly high MCF value. This represents an intrinsic systematic error, in contrast to the experimental error of (B). This phenomenon has been demonstrated only for lysozyme, where particular substitutions in certain sequenced positions had greater MCF effects than in other known positions (Ibrahimi *et al.*, 1980). Whether this potential problem exists in MCF albumin analyses is not known. We do not think this is a general problem for the following reasons. Champion *et al.* (1975), Walker (1979) and Jukes (1980) have demonstrated in theory and verified with data that most amino acid substitutions occur outside active binding sites on a protein. A major change in tertiary structure

Table 2. Albumin comparisons among *Cyclorampus* species.
IMMUNOLOGICAL DISTANCE

Species	Anti-R ^a	Anti-D	Anti-L	Anti-BR	Anti-BL
<i>C. rhyakonastes</i> (MZUSP 57829-831)	0	6	6	54	71
<i>C. duseni</i> (USNM 217868-884)	2	0	5	52	71
<i>C. litorum</i> (MZUSP 57806)	0	6	0	50	74
<i>C. mirandaribeiroi</i> (USNM 327972-73)	2	8	1	54	71
<i>C. asper</i> (USNM 217844)	12	11	17	51	85
<i>C. valae</i> (USNM 218020-27)	28	27	24	49	68
<i>C. boraceiensis</i> (USNM 217948-956)	35	36	45	0	102
<i>C. semipalmatus</i> (USNM 217918)	35	37	48	5	93
<i>C. semipalmatus</i> (USNM 217929)	44	42	49	5	113
<i>C. semipalmatus</i> (USNM 217924)	41	41	46	5	105
<i>C. dubius</i> (USNM 217867)	37	37	45	1	106
<i>C. ohausi</i> (MZUSP 57816)	42	52	48	82	71
<i>C. eleutherodactylus</i> (USNM 217899)	38	38	40	48	81
<i>C. eleutherodactylus</i> (USNM 217897)	41	42	43	49	82
<i>C. granulosis</i> (USNM 217904) ^b	50	60	ND ^c	76	115
<i>C. stejnegeri</i> (USNM 208500)	46	ND	ND	84	107
<i>C. brasiliensis</i> (USNM 217856)	86	85	66	102	0
<i>C. fuliginosus</i> (USNM 217902)	120	113	104	145	116

^aThe five antisera used are the same as in Table 1.

^bInsufficient antigen.

^cND = not done.

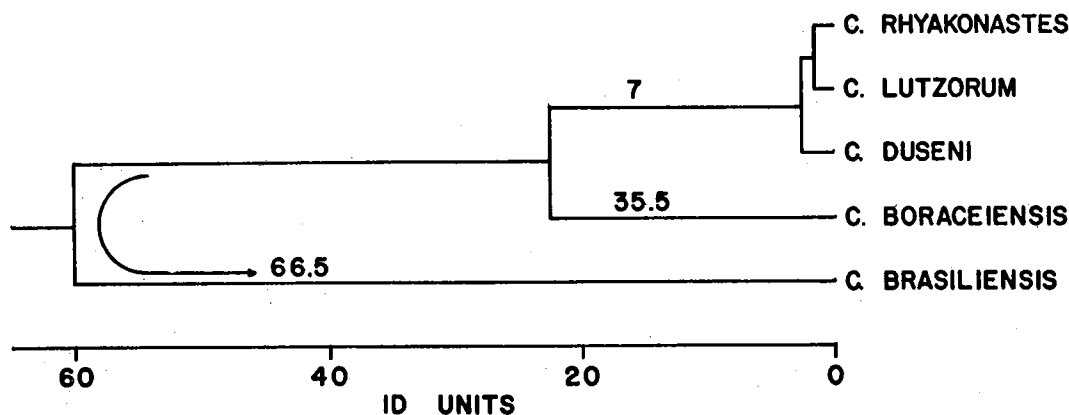


Figure 1. Genetic relationships among *Cycloramphus* species for which reciprocal immunological distance (ID) values are available.

would likely affect an active binding site and, thus, would not be expected to persist in nature. Against this general argument, however, it should be pointed out that albumin is generally thought to function as a packing molecule and that the exact shape of the molecule may not have much effect on its function. Thus, individual species data could occasionally have higher ID values than would be expected solely from the number of amino acid differences. We, therefore, base all interpretations within the context of the entire data set.

PHYLOGENETIC RELATIONSHIPS

The phylogenetic tree in Figure 1 was constructed from the average data matrix

in Table 3 (averages of tests from Table 1) by a method described by Farris (1972), but modified so that each monophyletic group of the tree utilizes only the cladistically closest outside lineage in determining its relative limb length. This procedure minimizes inference errors (Maxson and Wilson, 1975). The percent "standard deviation" as defined by Fitch and Margoliash (1967) as a measure of "goodness of fit" of the tree to the input data is 5.6%, indicating a good fit of the tree to the data. The percent error evaluation of fit of data to a tree, favored for immunological data by Prager and Wilson (1976), is only 2.9%, which is considerably lower than that often seen in immunological studies of a wide variety of organisms (Prager and Wilson, 1978).

Only data resulting from reciprocal tests

Table 3. Matrix of average immunological distances used to calculate the phylogenetic tree in Figure 1 (INPUT) and distances calculated from Figure 1 (OUTPUT).

Species Tested	ANTISERA				
	R	D	L	BR	BL
<i>C. rhyakonastes</i> (R)	—	4	3	44.5	78.5*
<i>C. duseni</i> (D)	4.5	—	3.5	44	78
<i>C. lutzorum</i> (L)	3	5.5	—	47.5	70
<i>C. boraceiensis</i> (BR)	45	44.5	46	—	102
<i>C. brasiliensis</i> (BL)	76	75.5	77	102	—

*Upper right hand matrix = INPUT; lower left hand matrix = OUTPUT.

can be used to reconstruct detailed phylogenetic histories, but the data from unidirectional tests are often useful in making approximate assignment of species to the branches of the tree (Figure 1) on the basis of albumin similarities.

The following conservative conclusions are drawn from the data in Figure 1 and Table 2.

A) There are two closely related clusters of species: (1) *Cycloramphus asper, duseni, lutzorum, mirandaribeiroi, rhyakonastes*, and (2) *Cycloramphus boraceiensis, dubius, semipalmatus*.

B) The remaining relationships are not close and are not easily interpretable at present.

DISCUSSION

One aspect of the data presented in Figure 1 is noteworthy. The ID's are asymmetrical between the lineages of *C. boraceiensis* and *C. duseni-lutzorum-rhyakonastes*. One of the major assumptions in the interpretation of the albumin data is that amino acid substitutions occur stochastically at a more or less constant rate. Thus, accumulations of differences should accrue at the same mean rate in lineages that have separated, but will still exhibit a variance. The data under discussion indicate a three or four fold difference, however. If the Sarich and Cronin (1976) correction is applied to the data, there is still a three fold difference. We use the more conservative original data to illustrate the greatest possible magnitude of the differences. These results have at least two explanations. First, the albumins could have evolved at the observed rates in the two lineages. Nevertheless, such a disparity in rates of MCF data is still within the 95% confidence limits of expectation in a random process. This is assuming that the accumulation of

ID units follows a nearly Poisson distribution, where the variance is approximately twice the mean (Nei, 1977). Second, the data for *C. boraceiensis* could represent an intrinsic systematic error with associated increased variance leading to unexpectedly high ID values as discussed previously. At present, we do not have the data to determine which alternative represents the correct explanation, or whether these two represent the only possible explanations. Additional data are needed to resolve this problem.

Morphological data presented in the preceding companion paper reveal four groupings: (1) the *bolitoglossus* group, (2) the *fuliginosus* group, (3) the *granulosus* group, and (4) the *ohausi* group. The morphological data did not allow further clusterings of relationships within groups. Complete comparison of morphological and genetic estimates of relationships are not possible at this time as we do not have a complete genetic data matrix available. As indicated above, phylogenetic relationships can be calculated in detail only where reciprocal cross reaction data are available. Antisera were produced only for species within the *fuliginosus* group. Thus, no definitive statements can be made concerning the relationships among the groups. The available data are not easily interpretable. *Cycloramphus eleutherodactylus, granulosus, ohausi, stejnegeri*, and *valae* are clearly not closely related to members of the *fuliginosus* group. However, some within group distances exceed some between group distances. These data indicate that either intrinsic systematic type errors exist in the *C. brasiliensis* and *fuliginosus* data or the *fuliginosus* group is paraphyletic.

Cycloramphus ohausi has a stronger MCF reaction to the members of the *fuliginosus* group that are geographically most distant. This trend is in the opposite direction from that seen in a member of the *granulosus* group, *C. valae*. In order to determine with precision the relationships of *C. ohausi*, antiserum would have to be

prepared for this species, which forms a monotypic morphological species group.

Three antigen samples were tested for species of the *C. bolitoglossus* group. Two geographic samples were tested for *C. eleutherodactylus*: one from São Paulo, Boracéia, the other from Rio de Janeiro, Teresópolis. The third sample, *C. stejnegeri*, gave ID values falling within the same range of values as measured for all the other species of *Cycloramphus*. There is thus no biochemical evidence for considering *Craspedoglossa* a separate genetic lineage from *Cycloramphus*. This conclusion is consistent with that presented in the morphological companion paper. It is important to note, however, that *Zachaenus parvulus* is also a genetic member of the *Cycloramphus* lineage but represents an apparent instance where morphological evolution has occurred much more rapidly than albumin evolution (Maxson *et al.*, 1981). There are three species of the *bolitoglossus* group for which no albumin samples are available.

Albumin antigen samples were tested for two of the three species of the *C. granulosa* group. *Cycloramphus granulosa* demonstrates large ID values to all antisera tested, indicating that there are no close relationships with *granulosa* and the species for which antisera are available (*fuliginosa* group members). The ID values for *C. valae* indicate that there is a closer relationship between *C. valae* and the geographically nearest members of the *fuliginosa* group than to the geographically more distant ones. The data suggest that the *granulosa* type morphology (fringed toes; granular, non-tuberculate dorsal skin) may have arisen independently in separate geographic areas from the *fuliginosa* type of morphology (webbed toes; tuberculate, non-granular dorsal skin), and that *C. valae* may be more closely related to certain geographically proximate members of the *fuliginosa* group than it is to *C. granulosa*. At present, the critical materials to deter-

mine the relationships among the species of the *granulosa* group are not available.

At the time the data were analyzed, we had no albumin sample for *C. bandeirensis*, which may be an annectant form between the *fuliginosa* and *granulosa* groups.

The most complete data on within group genetic relationships are from the *fuliginosa* group. Eleven species are recognized in this group for which antigen samples are available from 10 species and antisera samples from five. Samples for three individual *C. semipalmatus* are included; two from São Paulo, Boracéia and one from São Paulo, Paranapiacaba. The one way tests give identical reactions and demonstrate the small degree of experimental error in the technique. The two very closely bunched clusters of *C. boraceiensis-dubius-semipalmatus* and *C. asper-duseni-lutzorum-mirandaribeiroi-rhyakonastes* pointed out previously are not closely related to each other immunologically. The immunological relationships of *C. brasiliensis* and *fuliginosa* to each other and to other species of *Cycloramphus* are very different from those proposed on the basis of external morphology. *Cycloramphus brasiliensis* and *fuliginosa* are very similar morphologically; based only on the morphological data, the two are clearly each other's closest relatives within the genus. Within the *fuliginosa* cluster, *brasiliensis* and *fuliginosa* are part of a pattern in which morphological character states are distributed in a mosaic. The albumin data indicate that *C. brasiliensis* and *fuliginosa* are not genetically closely related to each other nor to any other species of *Cycloramphus* tested. Either there is a major incongruence of morphological and biochemical data or one of the data sets is in error. We do not think there are errors in the data sets. Previous workers have pointed out the similar morphologies of *C. brasiliensis* and *fuliginosa*. Perhaps one or more amino acid substitutions resulted in major tertiary structural changes of the albumin molecule resulting in inflated

MC'F ID values. The consistently high ID values for *C. brasiliensis* and *fuliginosus* indicate that this explanation may be a plausible one. However, such a change should not result in an inflation of more than 10-25 ID units. The magnitude of difference seen between the ID values for both *brasiliensis* and *fuliginosus* clearly is greater than this possible intrinsic systematic source of inflated ID values. Actual close relationships of *brasiliensis* and *fuliginosus* would require one of two explanations. First, the albumins of each species must have undergone major tertiary structural reorganization caused by one or two amino acid substitutions after the two species became separate lineages. We think this explanation highly improbable because single amino acid substitutions rarely result in major structural change in protein. Second, amino acid substitutions must have taken place at a faster rate in these two lineages than is true for the majority of lineages. Obviously, to completely rule out these explanations, the best resolution would be to sample another biochemical system that would give an independent test of genetic relationships. It is difficult, if not impossible, to gather data to provide an independent test of relationships from external morphology. Karyotype data would not resolve the question posed, although they might prove interesting. We conclude that the morphological and albumin data sets are both valid and incongruent. The interpretative consequences of this conclusion are that (1) *C. brasiliensis* and *fuliginosus* are both very old species that speciated early in the history of the genus, (2) they have been under strong stabilizing selection for external morphological features, while (3) stochastic amino acid substitutions have accumulated in the albumins at a more or less constant rate. The two species may in fact be most closely related to each other within the genus, but have been separate lineages for a very long time.

The analysis of relationships leads to one general question and two conclusions.

The question is whether the different morphological types reflect relationships or whether they have arisen independently from a *fuliginosus* type of morphology in separate geographic areas. The albumin data suggest the latter explanation is plausible. Further albumin analysis will clarify this issue, which has important evolutionary consequences in understanding the zoogeography of the genus. Efforts currently are being made to gather the necessary albumin samples.

The first conclusion is that the characters of external morphology that differentiate among members of the *C. fuliginosus* group are not indicative of genetic relationships. The main morphological characters separating species within this group are characters of size, degree of toe webbing, and dorsal texture. Extreme states of all these characters are to be found within the *C. boraceiensis-dubius-semipalmatus* or *C. asper-duseni-lutzorum-mirandaribeiroid-rhyakonastes* clusters; no morphological character states distinguish between these clusters.

The second conclusion is that the closest relationships within the genus *Cycloramphus* are found among geographically close species, including sympatric associations. The *C. boraceiensis-dubius-semipalmatus* cluster is restricted to a portion of coastal São Paulo (State). Likewise, the *C. asper-duseni-lutzorum-mirandaribeiroid-rhyakonastes* cluster has an essentially restricted distribution from the south coastal portion of the State of São Paulo to the north coastal portion of the State of Santa Catarina (outlying localities discussed later). Within each of these areas, these are the only species of the *C. fuliginosus* group that occur. This same pattern has also been found in the Andean frogs of the genus *Gastrotheca* (Scanlan *et al.*, 1980) and may be a general speciation pattern for Neotropical frogs.

Zoogeography

The zoogeographic analysis and discussion are based on three assumptions. The first is that morphological variation and species limits within the genus *Cycloramphus* have been analyzed accurately (companion paper). The second is that the relationships determined from MCF analysis of albumin represent the true relationships among species. The third assumption is that the distributions represented by the dot maps in the companion paper represent the total distributions of each species in terms of the scale used. The approach used herein is to accept these assumptions as true and to explore the zoogeographic implications.

Obviously, the zoogeographic interpretations developed for *Cycloramphus* will be only as reliable as our assumptions. Further field studies may uncover new species or more likely additional distributional data will modify the known distributions of the species; however, the current distributional data set is adequate to draw general as well as some specific conclusions. We anticipate additional distributional data to clarify, rather than modify, our zoogeographical discussion. The relationships used in the zoogeographic analysis are only those that fall within the error limits of the MCF technique. The weakness of the albumin based relationship data is that the matrix remains incomplete. The data are most complete for members of the *C. fuliginosus* morphological group; therefore, most of the zoogeographic discussion will focus on the stream associated members of the genus *Cycloramphus*.

BACKGROUND INFORMATION

Members of the genus *Cycloramphus* occur along the coastal strip areas of eastern Brasil from the southern part of the

State of Santa Catarina to the middle of the State of Espírito Santo. This distribution coincides well with the southern half of the Atlantic Forest Morphoclimatic Domain (Ab'Sáber, 1977a). Morphoclimatic domains, determined by geomorphological techniques, are defined by superposition of relief, soil, drainage, climate and vegetation. Our use of the term Atlantic Forests refers to the most visible aspect of the Domain, its vegetation. The Atlantic Forest Domain does not extend far inland (Figure 2) and its present relief consists of coastal lowlands and coastal mountain ranges. The topography of SE Brasil is extremely complex (Figure 3). Depending on latitude and topography, the transition between the Atlantic Forest Domain and adjacent domains may be abrupt or gradual with a complex of intermediate conditions, seen most visibly as transitional vegetation types. For example, the transition from the Atlantic Forest Domain to the Araucaria Domain in the State of Santa Catarina is sharp and abrupt. The Atlantic Forest Domain extends up to about 1200-1500m elevational level on the east facing slopes of the Serra do Mar. Just over the crest of the Serra do Mar, the Araucaria Domain occupies the plateau. Between the Atlantic Forest Domain and the Cerrado Domain in the region around Belo Horizonte and Ouro Preto, a transitional humid forest exists that differs in structural detail from typical Atlantic forest vegetation. This transitional humid forest occurs in a mosaic distribution with typical Cerrado vegetation. Further to the north in Pernambuco, there is a peculiar transition between the Atlantic Forest and Caatinga Domains, the agreste. In historical times, up until the 1600's, the Atlantic Forest Domain existed as a continuous tropical evergreen forest on coastal lowlands and mountains (Figure 2), with small mountain brooks locally abundant throughout much of the Domain.

The geological history of the Atlantic Forest Domain is long, complex, and not



Figure 2. Map of South America showing the Atlantic Forest Morphoclimatic Domain (black).

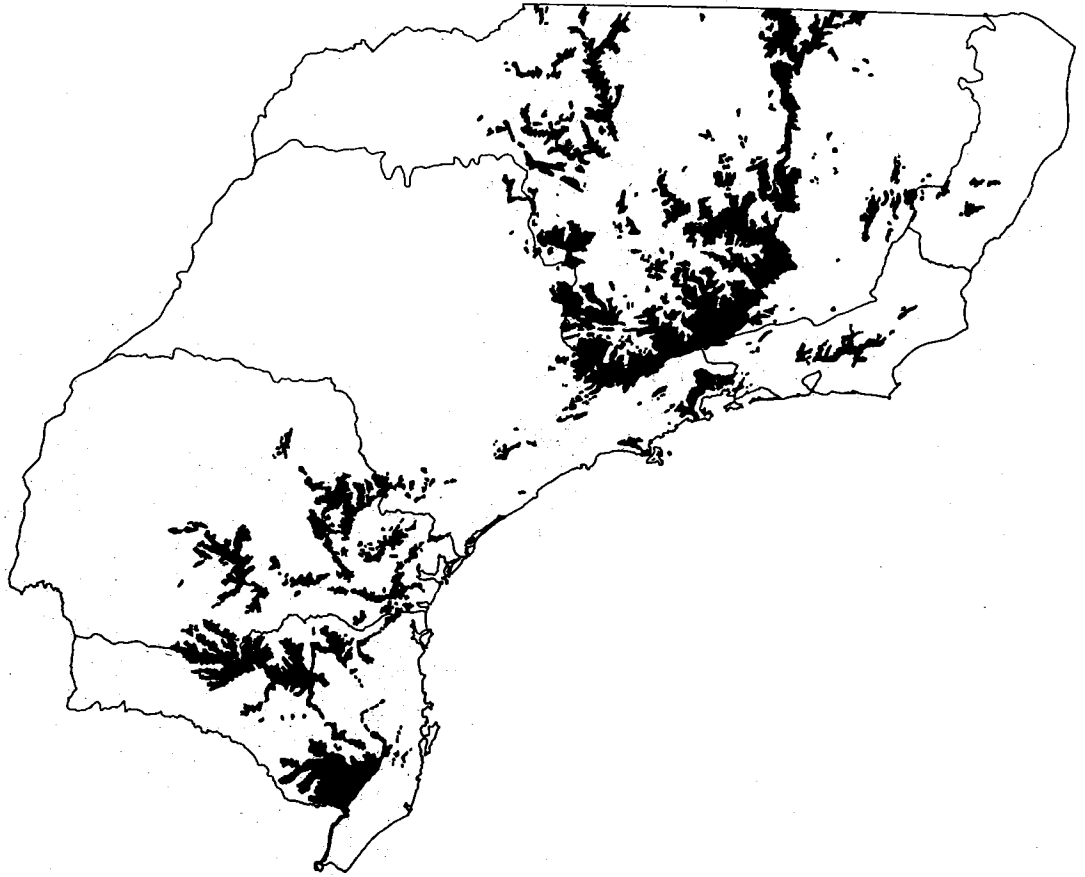


Figure 3. 1000 m contour map of southeast Brasil. Areas in black 1000 m or more in elevation.

fully understood. Part of the Domain occurs on the Brazilian Shield, with rocks dating to the Precambrian. The present relief of the Domain is almost certainly post-Cretaceous; the major features were established by the mid-Tertiary with some final uplifts occurring as late as the Quaternary (Almeida, 1976; Moreira and Camalier, 1977; Moreira and Lima, 1977; Suguio and Martin, 1976). The hydrography of the region changed as a result of major relief changes within the Domain. As a consequence, the major hydrological features are post-Cretaceous. Of importance to the zoogeography of *Cycloramphus*, the hydrography of the Domain has not been stable and changes in small stream distributions must have

occurred throughout the Cenozoic to and including the Quaternary (for general hydrological reviews, see Botelho, 1977 and Santos, 1977).

There have also been fluctuations in extent of the coastline. During more arid times, documented best in the Pleistocene, the coast line extended at least to the 60 m bathymetric contour line from the present coast. The effect of this would be to broaden the extent of the lowland coastland portion of eastern Brasil. At these times, islands such as Ilha Grande, Ilha de São Sebastião, and Ilha de Santa Catarina were part of the mainland.

Cycloramphus species are associated with three main habitat types: a small mountain

stream habitat, a general forest floor terrestrial habitat, and a forest floor fossorial habitat.

All stream-associated *Cycloramphus* occur in and along small, rocky, mountain brooks, 1/2-4 meters wide, with a flow that produces a rushing sound when water breaks over the rocks in the stream bed. Such streams do not occur in the coastal lowlands, but only in areas of sufficient topographic relief, such as in the Serra do Mar. Even within areas of steep topographic relief, streams are not uniformly distributed; some areas have many small streams, others very few. The abundance of small streams in the Atlantic Forest Domain is likely just past the maximum at present. Current worldwide climatic regimes result in a near peak of pluvial conditions with near maximal rainfall and ground water levels. During the glacial periods of the Pleistocene, the climate was drier and cooler in the region occupied today by the Atlantic Forest Domain (Ab'Sáber, 1977b). Glacial climates probably resulted in fewer mountain brooks and reduction of currently large streams to suitable *Cycloramphus* habitats. Thus, both the numbers of available streams and the geographic distribution of suitable streams differ between pluvial and glacial periods.

The forest floor habitat is found in areas covered by Atlantic Forest vegetation. As indicated previously, the Atlantic Forests were continuous within the Atlantic Forest Domain until the advent of Europeans in Brasil. During glacial maxima, however, the extent of the Atlantic Forest Domain was drastically reduced and fragmented (Ab'Sáber, 1977b, Jackson, 1978). The concomitant lowering of the sea level did not result in greater expanse of the Atlantic Forest Domain, as most exposed coastal lowlands did not appear to support Atlantic Forest vegetation (Ab'Sáber, 1977b).

The fossorial habitat is likely limited to a subset of the Atlantic Forests (discussed further in distribution section of this paper).

Otherwise the same statements apply as for the preceding paragraph.

A final piece of background information is the occurrence of local extinctions. Cruz (1974) documented one such catastrophe at Caraguatatuba which resulted from the torrential rains of 18 March 1967. An appreciable area near Caraguatatuba underwent extensive landslides and destruction. The destruction and removal of the fauna and flora from the region of the slide was complete. The process that results in these local extinctions is that water building up between the surface soil clays and the bedrock acts as a lubricant; at a critical point, heavy rains will cause extensive landslides. The magnitude of these extinctions is local, however. For example, the torrential rains of 18 March 1967 were more widespread than the area where the landslide occurred. Nevertheless, local extinction of *Cycloramphus* populations is a stochastic phenomenon, with possible effects at the species level over evolutionary time.

DISTRIBUTIONS

This section examines general distribution patterns, based on the maps of the preceding companion paper. In order to have a framework for discussion, a predictive composite distribution map was made for the stream associated species of *Cycloramphus*. The map is based on two assumptions. The first is that the species of *Cycloramphus* are limited to the Atlantic Forest Domain. The second is that mountain brooks occur where there is at least a 500 m change in elevation over a short area, indicated as closely packed contour lines on the millionth maps. The minimum of 500 m was chosen on the basis of examining the distribution of *Cycloramphus fuliginosus* in the city of Rio de Janeiro compared with a contour map. The resultant map is an area bounded by the limits of the Atlantic Forest Domain, within which are areas of sharp relief (Fig-



Figure 4. Predictive map of occurrence of stream associated *Cycloramphus* species. Area bounded by (approximate) limit of Atlantic Forest Morphoclimatic Domain within which are areas of sharp relief (stippled), where mountain brooks likely occur.

ure 4). The predictive map indicates (1) areas where no mountain brooks occur, and (2) areas where mountain brooks potentially occur. As discussed previously, small streams are patchily distributed within areas of sharp relief. One obvious feature drawn from the map (Figure 4) is that even if mountain brooks were abundantly distributed within the areas of sharp relief, the fauna associated with mountain brooks has a patchy, discontinuous distribution within the Atlantic Forest Domain.

The heuristic value of the map was tested by superimposing individual species distributions. Species were divided into two ecological groupings for this purpose:

stream associated species and terrestrial species. Visual inspection of the map indicates a good correlation of predicted and actual distributions (Figure 5). Another way of examining the distributions is to compare the distributions with random distributions. In order to do this, the high relief areas within the Atlantic Forest Domain were calculated (stippled areas of Figure 4). These cover 18.9% of the total area of the Atlantic Forest Domain mapped. Knowing this, random (expected) distributions were calculated by determining the percentage of a given number of distribution dots (total localities for a given set) that would be expected to fall on the total



Figure 5. Composite distributions of *Cycloramphus* species superimposed on the predictive map. Circles = stream associated species, squares = terrestrial species. A circle or square may indicate more than one locality or species.

Atlantic Forest Domain area based on the percent of high relief area. For stream associated species ($N =$ number of map dots = 55), the observed (as individual dots taken from the maps of the preceding paper occurring on the stippled areas of the map of Figures 4 or 5) versus expected (random) numbers are: 41 versus 10; for terrestrial species ($N = 26$), 21 versus 5; for all species ($N = 81$), 62 versus 15. These comparisons are all highly significant statistically. The predictive distribution map (Figure 5) is a good first approximation of the actual composite distributions of the species of *Cycloramphus*. The distributions as analyzed contain one unexpected result.

The predictive map was based on assumptions for stream associated species and was not expected to account for terrestrial species distributions, but the terrestrial species distributions correlate as well with the areas of sharp relief as the stream associated species. The strength of the correlated distributions is such that they are probably real, but the influence of European colonization and settlement patterns in the area might also contribute to these distributions. Certainly today there is little forest left in the lowland areas of the Atlantic Forest Domain. Any forest associated organisms are found in areas of sharp relief where patches of forest still persist.

Distributional Limits

Two aspects are examined with respect to the factors that limit distributions. The first involves the factors that limit the collective species distributions. These will be discussed in terms of the major habitat associations of *Cycloramphus* species with small streams, the terrestrial forest floor habitat, and the forest floor fossorial habitat. The second aspect involves the factors that limit individual species distributions. In the following discussion, the individual species distributions will be categorized. The analysis of the factors producing the patterns is the subject of the section on zoogeographical hypotheses.

Cycloramphus species of the mountain stream habitats are associated with the brooks in all life stages — larvae, juveniles, and adult males and females are encountered in or along the streams. The predicted distribution map (Figure 5) explains the current distribution of the stream associated species well in terms of restriction to the southern half of the Atlantic Forest Morphoclimatic Domain and within that, to areas of sharp topographic relief. The single exception outside of the Atlantic Forest Domain (circle in State of Paraná in Figure 5) is the type locality of *C. duseni*. The material that Andersson used to describe this species was collected by the botanist Dr. P. Dusén from "Parana, Sierra do mar; Ypiranga, in crevices and cracks in the vertical cliffs along the railway, 3/9 1911" (Andersson, 1914, p. 2). The only Ipiranga found along a rail line in the maps and aids at hand is that figured (Figure 5, also companion paper, Fig. 42). However, there is also one Ypiranga next to the railroad station of Banhado, in the municipio of Morretes (P. E. Vanzolini, pers. comm.). The exact locality, within the Atlantic Forest Domain, will be treated in a separate paper. Within the Atlantic Forest Domain, distributions correlate well with regions of topographic relief, that is,

mountain brook distributions. The local circumstances for most localities lying outside predicted areas of occurrence are not known. One such locality has been recently visited, however. The locality of Joinville, Santa Catarina lies on coastal hills. No small suitable streams were located in or near the town. Of course, such streams would be unsuitable for *Cycloramphus* today, as the Atlantic Forests no longer occur in or near Joinville proper. But even historically, the probability that stream *Cycloramphus* occurred at Joinville is low. Joinville is the only large population in the region including the escarpment further to the west where *Cycloramphus* are found. It is likely that the locality of Joinville was used by a collector to either indicate a shipping point, or a locality which could be found on a map. This example may be indicative of other such localities. In part, the historical aspects involving use of locality shipping points rather than where the specimens were actually collected can not be distinguished, as the critical test of recollecting specimens from questionable lowland localities is impossible. The absence of stream associated *Cycloramphus* species from predicted areas of occurrence needs validation. Historically, collections have been spotty. With the current framework (Figures 4,5), a meaningful sampling program could be formulated to determine the actual distributions of the stream associated species. Stream associated *Cycloramphus* likely occur throughout the middle and southern extent of the Atlantic Forest Domain. The Domain does extend much further to the north than where *Cycloramphus* have been collected (compare Figures 2 and 5). With the exception of the Serra dos Aimorés on the Espírito Santo-Minas Gerais border, there are no areas of sharp topographic relief within the Atlantic Forest Domain north of the Rio Doce comparable to those indicated south of the Rio Doce. There is, thus, no suitable habitat for stream associ-

ated *Cycloramphus* further to the north than indicated in the predictive map (Figures 4, 5). This statement, based upon inspection of the millionth map contour lines, is supported in the field for those areas in southern Bahia where Werner C. A. Bokermann has worked (pers. comm.). The larval portion of the life cycle may impose the most distributional restrictions on the stream associated species. All of the known tadpoles are very similar in morphology and are adapted to a surface water film life style. The larvae do not occur in the main waters of the streams; rather, they are found on rocks or boulders constantly splashed by water or where water seeps as a film over a rocky outcrop. The larvae graze the algal film that grows under these conditions. Small, rocky streams occur outside the Atlantic Forest Domain, but *Cycloramphus* have not been collected from these streams. The Serra do Cipó and Serra do Espinhaço contain small streams which have other Atlantic Forest faunal species associated with them (for example, *Thoropa*, Bokermann, 1965 and Maxson and Heyer, 1982). The Serra do Cipó has been rather thoroughly sampled for frogs at this point, particularly through the efforts of W. C. A. Bokermann and I. Sazima. The streams of this area are quite seasonal and it is likely that a long term supply of exposed rock surface algae does not exist in sufficient amounts to support the larval phase of *Cycloramphus*. If this is true, then the larvae of *Cycloramphus* are more specialized than *Thoropa* larvae, which are also surface film adapted, but *Thoropa* occur more often in association with wet rock outcroppings rather than the splash zone portions of streams. In general, the very nature of seasonal streams would seem inconsistent with a specialized splash zone microhabitat. The small streams of Cerrado and Caatinga are seasonal in terms of flow, if not in occurrence. Only the humid conditions associated with the Atlantic Forest Domain produce a constant enough water flow that

results in a relatively constant splash zone. The closest geographic area where rainfall patterns could produce the splash zone stream microhabitat is the Amazonian Morphoclimatic Domain. However, the low topographic relief of the eastern and central areas of the Domain does not support small babbling brooks.

Members of the *Cycloramphus bolitoglossus* group are not restricted in their biology or habitat distributions to the immediate environs of streams. The terrestrial larvae, which may characterize the group (although known unequivocally for only one species, *C. stejnegeri*), have been found on the forest floor in moist microhabitat conditions (Heyer and Crombie, 1979). *Cycloramphus eleutherodactylus* individuals (and presumably *C. diringshofeni* individuals) are general terrestrial forest floor frogs. Individuals of *C. eleutherodactylus* are encountered sporadically on or just above the floor of the Atlantic Forest vegetation. It is likely that the moist microhabitat conditions required for development of the terrestrial eggs and larvae are restricted to humid forests, primarily those of the Atlantic Forest Domain. The records of *C. eleutherodactylus* from Ouro Preto, Minas Gerais (outlying square in Minas Gerais, Figure 5), prompted a transect trip to the area from Pico da Bandeira, Espírito Santo. The purpose of the transect was to see what transition breaks of landform and potential vegetation occurred between Pico da Bandeira and Ouro Preto. As indicated previously, the area around Ouro Preto has a humid forest, differing in detail from typical Atlantic Forest. But the essential features of the vegetation are those of humid forests. Members of the *C. bolitoglossus* group appear to be limited to the Atlantic Forests and certain transitional humid forests connectant with the Atlantic Forests in some areas. The terrestrial larvae of the *C. bolitoglossus* group likely require more stable humid conditions than the encapsulated larvae of the genus *Eleuthe-*

rodactylus. Between the Atlantic Forest Domain and the Amazonian Domain (the geographically most proximate area where the microhabitat requirements would likely be met) is a corridor of open, less humid formations. Members of the *C. bolitoglossus* group have not been collected in the Atlantic Domain in the State of Espírito Santo northward. There is no immediately apparent reason why this is so in terms of biological restrictions. A possibility is that there is some currently unknown ecophysiological restriction in effect, limiting the distributions to the more southern latitudes of the Atlantic Forest Domain.

Cycloramphus stejnegeri (and presumably *C. bolitoglossus* and *carvalhoi*) is a fossorial species, inhabiting burrows in the forest floor (Heyer and Crombie, 1979). *Cycloramphus stejnegeri* does not have obvious morphological adaptations for construction of burrows (such as seen in male *Adenomera* and certain male *Leptodactylus*); presumably *C. stejnegeri* occupies and maintains burrows made by other organisms. The distributions of the fossorial species would be limited to those subsets of the Atlantic Forests where moist burrows are available for occupation. Otherwise the same general distributional statements apply as in the previous paragraph.

There are four major patterns of individual species distributions.

1) Eleven species are known from a single area of sharp relief (single stippled area in Figures 4, 5): *C. asper*, *bandeirensis*, *carvalhoi*, *catarinensis*, *dubius*, *jordanensis*, *mirandaribeiroi*, *ohausi*, *semipalmatus*, *stejnegeri*, *valae*. Four of these species are known from more than one locality within an area of high relief.

2) Six species are known from adjacent areas of high relief, but have total distributions limited to small geographic areas: *C. bolitoglossus*, *boraceiensis*, *brasiliensis*, *diringshofeni*, *granulosus*, *lutzorum*.

3) Three species demonstrate discontinuous distributions. The type locality of

C. duseni has already been mentioned as in error. With that locality more coastal, the species has the same pattern as those species of Pattern 2 with one outlier population in the State of São Paulo. *Cycloramphus rhyakonastes* has been found at several localities within a single area of high relief (as in Pattern 1), with an outlier population far removed from the other known populations (companion paper, Figure 51) in the State of Rio de Janeiro. *Cycloramphus fuliginosus* is known from several localities scattered in different areas of high relief in the States of Espírito Santo and Rio de Janeiro. At present, there are no data to suggest that the discontinuous distributions are either errors of locality data or species identification beyond that already discussed for *C. duseni*. If such errors do account for the patterns seen, we would predict that the outlying localities of *C. duseni* and *rhyakonastes* would represent errors of locality data and that *C. fuliginosus* would be a composite species. The samples are small for all *C. fuliginosus* localities except for those within the city of Rio de Janeiro.

4) *Cycloramphus eleutherodactylus* is the only species with a widespread distribution pattern.

The overall picture is that most species of *Cycloramphus* have localized distribution patterns. There are no major faunal breaks that involve larger areas than single areas of high relief. There are many areas where 2-3 species of *Cycloramphus* occur together, but 100 kilometers distant the species involved are not the same. The most diverse area known is the Organ Mountains, where four species occur in the forests around Teresópolis. The northern and southernmost portions of the range appear to be less speciose, although these areas have not been intensively collected.

Historical Distributions

The immunological distance units of the

albumin data correlate well with time (see Wilson *et al.*, 1977, for a review). As indicated earlier, the mean amino acid substitution rate per lineage in albumin is one amino acid per million years. Using this correlation and calibration to estimate divergence times between lineages allows inferences about the history of speciation events in *Cycloramphus*. Although the data matrix is incomplete, speciation events apparently occurred from late Eocene (at the latest, perhaps extending as early as the Paleocene for *C. brasiliensis* and *fuliginosus*) right up through the Pleistocene (*C. boracei*-*dubius*), with most speciation occurring in the Miocene (most of the remaining data). Speciation events thus correlate with the formation of the mountain ranges of coastal Brasil throughout the period of active uplift. Most species of *Cycloramphus* predate the Pleistocene; their distributions would be affected by climatic changes during that period.

The available data on uplift patterns and distribution of the Atlantic Forest Domain during glacial maxima are not precise enough to allow specific predictions. Specific uplift events and associated hydrological changes causing either species range expansions or their restrictions that could lead to allopatric speciation can not be made. The only generalization that can be made is that geological events occurring throughout the postulated times of speciation could have contributed to the isolation and subsequent speciation of *Cycloramphus* populations. Likewise, the available data on the distribution of the Atlantic Forest Domain during glacial maxima are too general to allow specific predictions on glacial maxima distributions of given species of *Cycloramphus*. Ab'Sáber (1977b) and Jackson (1978), using different approaches, produced maps showing the presumed distribution of the Atlantic Forests during the last major glacial maxima. The maps differ significantly in detail. However, both agree in that the Atlantic Forests were fragmented

into much smaller units during glacial maxima. In summary, there have been changes in *Cycloramphus* distributions caused (1) by topographic uplift throughout the history of the genus and (2) by climatic changes, most pronounced in the Pleistocene.

ZOOGEOGRAPHICAL HYPOTHESES

The purpose and organization of this section can be best presented in terms of an analogy of cloth and thread. What we wish to understand is the entire zoogeographic cloth. But the cloth was not made at a single time from uniform threads. Nor has the cloth simply been added to over time by weaving in different threads. Rather, parts of the cloth have been removed, some holes have been rewoven, but many have not been. The state of the cloth at this time is such that its history and present configuration will never be completely understood. What can be done is that some individual threads can be teased from the fabric for examination. Then an attempt can be made to see if some of the fabric can be discerned from examination of individual threads. What follows is, thus, not a logical series of zoogeographical hypotheses, but rather a collection of discussions, each with a different focus and each examining a part of the entire zoogeographic fabric.

Dispersal

Population dispersal is the movement of individuals into or out of the population area. It takes three forms: emigration — one-way outward movement; immigration — one-way inward movement; and migration — periodic departure and return. Dispersal is the means by which new or depopulated areas are colonized and equilibrium diversity is established (Odum, 1971, p. 200). Dispersal is thus a dynamic

component of current geographic distributions.

The point of discussion is whether current *Cycloramphus* distribution patterns are solely the result of time and dispersal. There are no data available to estimate emigration and immigration rates for any species of *Cycloramphus*; thus this point can not be definitively discussed. What can be said is that, if time and dispersal were the only factors in range formation and restriction, all species would have continuous ranges whose sizes we would predict from the estimates of age given by MCF data. Not all *Cycloramphus* have continuous ranges (e.g., *C. fuliginosus*, *granulosus*). Both *C. brasiliensis* and *fuliginosus* are very old species, but the range sizes are very different. *Cycloramphus eleutherodactylus* has a much larger range than either *brasiliensis* or *fuliginosus*, which are both much older than *eleutherodactylus* according to the MCF data.

In summary, the role of dispersal in accounting for present distribution ranges can not be definitively evaluated on the basis of available data other than to say that dispersal does not account for all distributional patterns seen in *Cycloramphus* species.

Ecology/History

Distributions can be predicted based on ecological characteristics. The general forest floor terrestrial species (*C. diringshofeni* and *eleutherodactylus*) would be expected to have distributions throughout the Atlantic Forest Domain. *Cycloramphus eleutherodactylus* has a broad range, but it does not extend throughout the Atlantic Forest Domain. Although the restriction is real, the exact limits are not known currently. Critical collections from the southern portion of the range are lacking. This also pertains to the northern limit, although a couple of negative data points are available. The anuran faunas near Santa Teresa, Espírito

Santo and Ilheus, Bahia are quite well known; *Cycloramphus eleutherodactylus* has not been collected from either.

Not enough is known about the kind of soil conditions required by semifossorial species of *Cycloramphus* (*bolitoglossus*, *carvalhoi*, *stejnegeri*) to make predictions on where these species would be expected to occur.

The stream associated species of *Cycloramphus* (*C. fuliginosus* and *granulosus* groups), are expected throughout those areas of the Atlantic Forest Domain where mountain brooks occur (Figure 4). This predicted distribution is true for the collective species ranges, but individual species distributions are all much smaller. Ecological factors explain local distribution well, but not species ranges.

Historical factors have to account for the restricted distributions seen. The hardest data to obtain are negative, in this case the documentation that a species does not occur in a certain area. Some negative data are available.

Portions of the Serra da Bocaina have been extensively sampled for frogs through the efforts of Adolfo and Bertha Lutz, Joaquim Venâncio, and W. C. A. Bokermann. Collectively, they and we found only one species of stream associated *Cycloramphus*. The diversity of the streams in the Serra da Bocaina appears as diverse to the human observer as the streams at other localities where two species occur together. The Serra da Bocaina stream species (*C. granulosus*) is found in waterfalls of the larger streams. There are no species in the smaller streams or along the non-falls portions of the larger streams. Historical factors must account for the presence of only one stream species in the Serra da Bocaina where two would be predicted on ecological grounds.

Two adjacent areas near Caparaó, Minas Gerais were sampled for riparian *Cycloramphus* in the Parque Nacional do Caparaó, Minas Gerais, including the Pico da Bandeira. Previous experience has shown that if stream associated species occur in a

region, they can be easily sampled. Predictably appropriate streams can be located in the daytime and searched at night. As all stages of the life cycle and all life activities apparently take place in the immediate stream environs, foraging individuals can be collected even if reproduction is not occurring. One biological clue to predicting stream appropriateness is that *Hylodes* and *Cycloramphus* species are often found in the same streams. *Hylodes* are diurnal and most are vocal. A stream where *Hylodes* can be heard during the day is likely to have *Cycloramphus* active at night. Near the Pico da Bandeira, a series of streams were searched for several nights within typical Atlantic Forest vegetation. Two species of *Hylodes* occurred in these streams, one in the smaller streams, one in the larger, as is characteristic. No *Cycloramphus* were found in these streams, where they would be expected based on ecological factors. Above the altitudinal limits of the Atlantic Forest vegetation, a single species of *Hylodes* and a single species of *Cycloramphus* were collected. This *Cycloramphus* distribution is unusual (discussed later). But of equal importance is the absence of a stream associated *Cycloramphus* within the Atlantic Forest Domain near Caparaó. This absence must have an historical, rather than ecological explanation.

Competition

The importance of competition in understanding species distributions is an open question. On the one hand are workers who think that competition is the strongest organizing factor in community composition, hence species distributions (for example, Diamond, 1975). Contrasting with this view, other workers find little evidence for competition explaining most species distributions (for example, Vuilleumier and Simberloff, 1980). Direct data on competition accounting for species distributions are scarce; there are none for the genus *Cyclo-*

ramphus. Patterns of co-occurrence do invite speculation, however. The critical question is whether present *Cycloramphus* communities are saturated to the point where competition is preventing invasion of additional species. If *Cycloramphus* communities are saturated, competition should produce parapatric distributions. If the communities are not saturated, competition is not a likely cause of the current distribution patterns seen.

There are several localities where two stream associated species occur together. As discussed in the companion paper, some of these sympatric species differ markedly in morphology and habitat usage, others do not. Of interest here is habitat usage. At most localities where two riparian species occur, the species occupy partially or entirely different habitats. The important exception is sympatric *C. dubius* and *semi-palmatus*; they are completely syntopic. Thus, two stream associated species of *Cycloramphus* can occur in exactly the same habitat. The patterns of co-occurrence thus suggest that throughout most (if not all) of the distributional range of *Cycloramphus*, competition is not implicated for the species distribution patterns seen and other explanations must be sought.

One outcome of competition is character displacement, resulting in different resource use such that competition no longer occurs. The morphological types as represented by the species groupings of *Cycloramphus* may interact with the environment in sufficiently different ways that there is no significant interaction among members of different morphological types. This would appear to be the case for the terrestrial *C. bolitoglossus* group compared with the stream associated *C. fuliginosus* and *granulosus* groups. That is, at least one member of each of these two groupings is likely to be found at any given locality that has mountain books. This pattern of co-occurrence is consistent with competition theory. The same kind of distribution pattern would be pre-

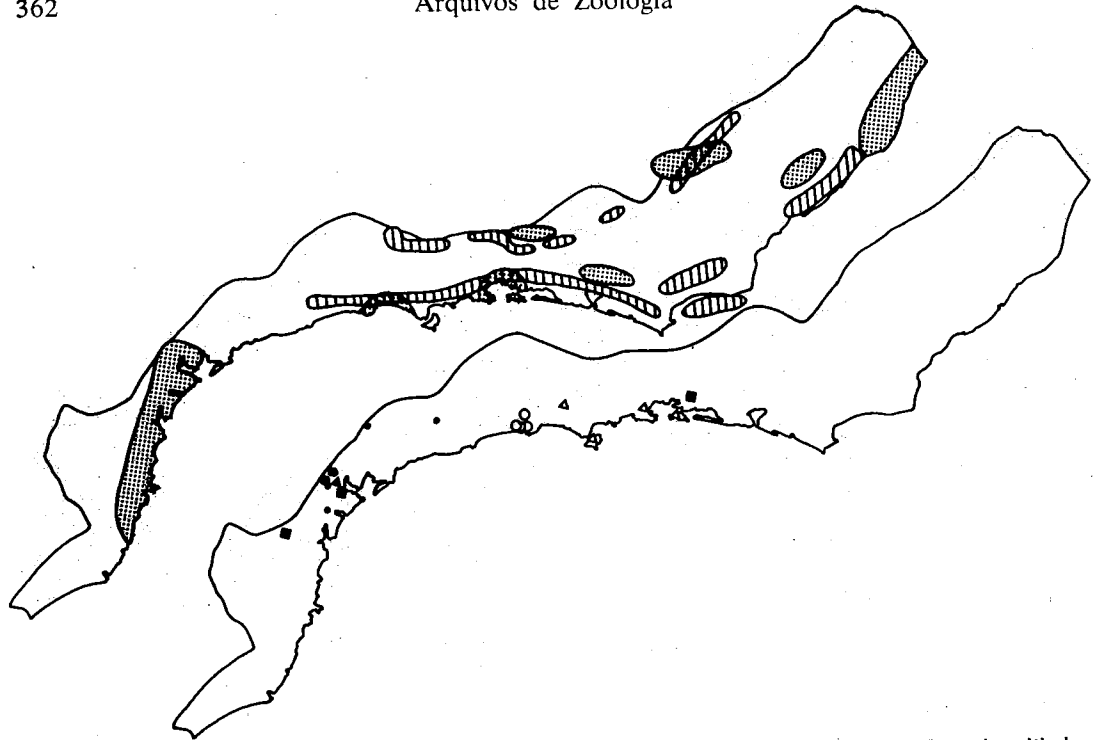


Figure 6. Pleistocene forest refugia and distributions of five species of *Cycloramphus* that likely arose in the Pleistocene. Stippling = refugia proposed by Jackson (1978); lines = refugia proposed by Ab'Sáber (1977b); boundaries of refugia approximate only. Open triangles = *C. boraceiensis*; open circles = *C. dubius*; closed circles = *C. lutzorum*; closed triangle = *C. miranda-ribeiroi*, closed squares = *C. rhyakonastes*, closed hexagons = *C. miranda-ribeiroi* and *C. rhyakonastes*.

dicted for the *C. fuliginosus* and *granulosus* groups. The morphologies of members of these two groups are sufficiently different that one would predict, on the basis of competition theory, to find members of each group co-occurring throughout the distributional range of the genus. This co-occurrence has not been documented for a single locality. In all known cases, either one or two members of the *C. fuliginosus* group occurs at a given locality, or a single member of the *C. granulosus* group, not both. The only possible exception is that museum specimens of *C. asper* and *C. catarinensis* collected in the 1920's bear the same, incorrect, locality data. Recently, *C. asper* was collected from near the presumed locality involved in Santa Catarina, but *C. catarinensis* was not. Members of the *C. fuliginosus* and *granulosus* group may actually represent ecophenotypes (in the

sense of Williams, 1972). That is, the webless, fringe-toed feet and granular dorsum of *C. granulosus* group members may be due to an evolutionary response to similar selective pressures acting on different lineages within the *fuliginosus* group. If this is the case, then the closest relatives of each of the *C. granulosus* group species would be found in geographically proximate *C. fuliginosus* group members, not with other members of the *C. granulosus* group. This explanation is not ruled out by the available data on genetic relationships.

Forest Refugia

The forest refugia hypothesis is based upon the fact that during the climatic fluctuations of the Pleistocene, the pluvial forests of South America underwent distributional expansions and contractions.

Currently, the pluvial forests are near maximum distribution (man's intrusive effects aside). During glacial maxima, climatic conditions were generally drier and cooler; thus pluvial forest was fragmented and restricted to smaller areas, or refugia, where climatic conditions were still favorable (Ab'Sáber, 1977b). The forest refugia model makes the reasonable assumption that species associated with wet forests had distributions restricted to forest islands during glacial maxima, and that differentiation occurred in these isolated refugia (Haffer, 1969; Vanzolini and Williams, 1970). Vanzolini (1981a) has recently demonstrated that differentiation can occur rapidly in small refugia. The refugial model has been used recently to explain the geographic and species differentiation patterns of the lizard genus *Enyalius* in the Pleistocene (Jackson, 1978).

Three predictions can be made from the forest refugial model pertinent to understanding the zoogeography of members of the genus *Cycloramphus*.

Prediction 1. Speciation could have occurred as a result of isolation and differentiation of *Cycloramphus* populations in Pleistocene refugia. The albumin data are consistent with Pleistocene speciation for two species lineages: (1) *C. boraceiensis* and *dubius*, and (2) *C. lutzorum*, *mirandaribeiroi*, and *rhyakonastes*. The albumin data indicate that all other speciation events (for which data are available) are older than the Pleistocene.

The distributions of A'Sáber's and Jackson's proposed refugia do not coincide well either with each other or with *Cycloramphus* distributions (Figure 6). Ab'Sáber (1977b) and Jackson (1978) used different techniques to predict distributions of refugia. The poor coincidence of proposed refugia suggests that detailed geomorphological analysis of the area may result in a refinement of establishing precisely where refugia occurred during the Pleistocene. Until such studies are available, no de-

tailed comparisons of *Cycloramphus* ranges and Pleistocene refugia are possible. At this point, the refugial model may account for a few cases of speciation at most (two to four instances of speciation leading to five species with the MCF data at hand).

Prediction 2. Isolation of populations in different refugia could have led to intra-specific patterns of differentiation. The only species for which extensive geographic variation data are available is *Cycloramphus eleutherodactylus*. For this species, geographic variation in morphological characters is a fact. Neither is the pattern of variation known in enough detail nor the Pleistocene refugia sufficiently well defined to determine whether there is a coincidence of pattern of variation with refugial distributions. Isolation and differentiation of populations during the Pleistocene in refugia is certainly a plausible explanation for the pattern of variation seen in *C. eleutherodactylus*.

Prediction 3. Species distributions could be limited to Pleistocene refugial areas. Historically, species of *Cycloramphus* likely were limited to Pleistocene forest refugia and for whatever reasons, may have not expanded those distributions when the Atlantic Forest Domain distribution expanded during more mesic times. Due to the high number of *Cycloramphus* species with distributions restricted to a single area of sharp relief, coincidence of these distributions with Pleistocene refugia should be analyzed when the refugia are more precisely defined.

Until the refugia are more precisely defined, the refugial model will be untestable. At present the model remains a likely hypothesis which could account for several distributions, one pattern of intraspecific morphological variation, and very few speciation events.

Mountaintop Distributions

Most *Cycloramphus* species occur in the

Atlantic Forest vegetation. The few exceptions are species that occur at higher elevations, above the Atlantic Forests. Depending on latitude and local conditions, the Atlantic Forest vegetation occurs up to the 1400-1900m elevational level. The most striking example of a mountaintop distribution is *Cycloramphus bandeirensis*, occurring on the Pico da Bandeira, well above the altitudinal limits of the Atlantic Forest vegetation. Two hypotheses have been proposed to account for montane distributions in Brasil (Vanzolini and Ramos, 1977; Vanzolini, 1981b). "These may be either the result of speciation in situ, or relictual distributions from a previous colder and drier climatic episode" (Vanzolini, 1981b). As *C. bandeirensis* presumably has close relationships with other members of the genus occurring in the lower elevations of the Atlantic Forests, it probably evolved in situ. The mechanisms for this adaptation to a non-forested environment is likely that proposed by Vanzolini and Williams (1981) for the vanishing refuge speciation model.

The model starts with a widespread forest form at the humid peak of a climatic cycle. Deterioration of the climate brings about the dissection of the continuous forested areas by inroads of open formations. The forest isolates, initially large, progressively shrink in area. Some of them last the whole of the dry leg of the climatic cycle, constituting refuges in which forest fauna survives and undergoes geographic differentiation. Other forest isolates dwindle and finally vanish; normally the forest fauna would disappear with the refuge; but under certain circumstances, some such populations might survive, adapted to open formations and isolated from their relatives still faithful to the forest and inhabiting refuges. The populations surviving in open formations would be geographically isolated and

under strong selective pressures, i.e., in ideal conditions for fast differentiation (Vanzolini, 1982).

The application of the vanishing refuge model to *C. bandeirensis* results in the following scenario. The ancestor of *C. bandeirensis* occurred in the Atlantic forests in the region around the Pico da Bandeira. As the climate deteriorated, the forests dwindled until there was a forest island near the Pico da Bandeira. As this forest island deteriorated, local *Cycloramphus* were able to adapt to the changing environment and became adapted to the open formations. During a following wet cycle, the only open formation habitat to which the species was now adapted occurred at higher elevations. The species was now adapted to this open formation habitat; the open formation habitat was stable; thus the species is now restricted in its distribution to the montane open formation habitat and has not reinvaded the adjacent Atlantic Forests, which have re-entered the area. It is of note that no other lowland *Cycloramphus* reinvaded the lowland Atlantic Forests in this area. The one factor that must have allowed this turn of events to occur would be peculiarities of the local hydrology. The small streams in the Pico da Bandeira region must have had a rather continuous flow in spite of the fact that they occurred in open formation vegetation. This is the case today and it must have held during the dry cycle. This ties in with a previous supposition regarding the fact no *Cycloramphus* have been collected from the Serra do Cipó or Serra do Espinhaço. *Cycloramphus* are able to adapt to the general climatic features that occur at the Serra do Cipó and Serra do Espinhaço as evidenced by the ability of *Cycloramphus bandeirensis* to live in a similarly stressed environment (compared to the Atlantic Forests). The difference must be in the nature of the streams; seasonal at the Serra do Espinhaço and Serra do Cipó, relatively constant at the Pico da Bandeira.

The case for an in situ evolution of a montane species through the vanishing refuge model is clearest for *C. bandeirensis*. Two other species distributions may also be accounted for by this model. *Cycloramphus carvalhoi* has been taken only from Brejo da Lapa, Itatiaia. This is an area where a stranded lizard species occurs (Vanzolini and Ramos, 1977) as well as a distinctive frog fauna in general. The ecological and life history adaptations for *C. carvalhoi* are not known, but its distribution in a distinctive vegetation type above the elevational limits of the Atlantic Forest Domain, is suggestive that the species arose in situ from an ancestor that occurred in the Atlantic Forests themselves. The other possible species is *C. jordanensis*. The area around Campos do Jordão is a complex mixture of Araucaria and Atlantic Forest Domain elements. The area is not strictly Atlantic Forest vegetation, nor Araucaria Domain vegetation. No *Cycloramphus* have been collected from the strict Araucaria Domain. The restricted distribution of *C. jordanensis* suggests an in situ evolution to the habitat in which it occurs from an ancestor that occurred in the Atlantic Forests themselves. Again, local conditions in the higher altitudes may have maintained continuously flowing mountain brooks.

Summary

Events effecting the distributions of *Cycloramphus* (topographic uplifting, climatic cycles) have occurred throughout the Cenozoic. These same events likely were key factors in the speciation process, as speciation within the genus has occurred at least from the Eocene through the Pleistocene. Because of the extended time scale and relatively small total distributional range, time and dispersal rates probably have not limited distributions. All other factors being equal, *Cycloramphus* species should occur uniformly throughout the

Atlantic Forest Morphoclimatic Domain. All other factors are not equal, however. These other factors are discussed in terms of the major features of distribution demonstrated by *Cycloramphus* that must be explained to understand the zoogeography of the genus. These discussions are cast in terms of statements for ease of presentation; it must be kept in mind that they are hypotheses. Following are the distribution features we think important to explain for *Cycloramphus*.

1) Intraspecific morphological variation in *C. eleutherodactylus*. The refugial model can explain this variation due to isolation of *C. eleutherodactylus* populations in forest refugia and consequent differentiation within these isolated populations prior to their re-coalescence when the forest re-coalesced.

2) Island distributions. There do not appear to be any different zoogeographic factors involved other than those that effect the mainland *Cycloramphus*. The island stream associated species of *Cycloramphus* occurs on islands where there is sharp relief (see Figure 4) and is the same species that occurs on the adjacent mainland. All islands from which *Cycloramphus* have been collected were connected with the mainland 11,000 years BP (Vanzolini, 1973). Thus, all species distributions of *Cycloramphus* on islands were continental distributions in the recent past and demonstrate continental, rather than island, zoogeographic patterns.

3) Very local distributions. The available habitat for species demonstrating restricted distributions is patchy. This is seen most clearly for the stream associated species (Figure 4). These patches have had histories of long periods of isolation and periods when the climate changed and the habitat was not suitable for *Cycloramphus*. The zoogeographic histories of *Cycloramphus* probably took place at the level of these patches of habitats. Most recently, in the Pleistocene, there likely have been local extinctions of *Cycloramphus* in some of

these patches, which have not been successfully reinvaded by *Cycloramphus*.

4) Missing distributions. These distributions are difficult to document, but are real. The causes for these missing distributions may again be explained by the local level of zoogeographic events, the patches of available habitat. Some of these patches were populated by *Cycloramphus* in pre-Pleistocene times, but climatic fluctuations resulted in extinctions from some of these patches, which were subsequently not reinvaded.

5) Mountaintop distributions. The distributions of such species as *Cycloramphus bandeirensis* could be due to in situ evolution of the species as explained by the vanishing refuge model.

It is clear that no one explanation will account for all the zoogeographic patterns seen. Rather, as one would expect from a genus that has had a long history in association with a given area, there are several zoogeographic patterns that are due to different historical causes.

There are two zoogeographic patterns left for which there are not adequate data/hypotheses to fully explain them. The first is the question of whether certain morphological types are ecophenotypes, derived independently in situ, or whether the disjunct distributions of these species with similar morphologies represent a different kind of zoogeographic pattern. The second pattern is the disjunct intraspecific distributions of three species of *Cycloramphus*. The available data do not absolutely rule out whether either errors of locality data or interpretations of species limits are involved in the distributions for these three species. Even the patterns exhibited by those species occurring in adjacent areas of sharp relief should be re-examined with additional data to determine whether they are indeed the same species. For example, *C. granulatus* occurs in two adjacent, but disjunct habitats and shows some morphological differentiation. Genetic data and advertise-

ment call data are needed to establish whether the morphological differences are those of species level or intraspecific variation. The distribution of *C. brasiliensis* in both the Serra dos Orgãos and Serra da Mantiqueira needs similar validation or modification.

Speciation Mechanisms

THE PROPOSED MODEL

The allopatric model of speciation, which calls for ancestral species to be split into isolated populations, best explains the patterns of speciation as currently understood for stream associated members of the genus *Cycloramphus*. At present, there are too few data to include the terrestrial species in any general model. During the period of isolation, differentiation proceeds to a level that if and when the daughter populations come back into contact, they maintain the integrity of the separate gene pools. This model thus requires (1) some causal agent or event that isolates previously contiguous populations, and (2) differential selection pressures in the areas of isolation to produce separate differentiation patterns in the daughter populations and/or accumulation of genetic changes in isolation that may ultimately result in genetic incompatibility.

Three kinds of events have been identified that would act as distributional barriers within contiguous *Cycloramphus* populations. The first is the uplifting of the coastal mountains. Changes in the local topography would result in changes of stream distributions, flow and drainage patterns. A tectonic event might result in population isolation in some places and allow population expansion in other places. The second kind of event is worldwide climatic changes. These changes, particularly in the Pleistocene, resulted in alternation of cooler and

drier periods with warmer and wetter periods. These climatic changes caused the alternate contraction and expansion of the Atlantic Forest Morphoclimatic Domain with its associated flora and fauna. Members of the genus *Cycloramphus* have been historically associated with the Atlantic Forest Domain and would have experienced fragmentation, isolation, and some extinction of populations during the drier and cooler climatic periods. The third kind of event is local extinctions of populations due to landslides and other local catastrophes. Taken in concert, these three kinds of events have acted as isolating events throughout the Cenozoic.

Two kinds of adaptations (thus far identified) appear to arise in isolation. The first are adaptations to the predominant stream morphology occurring in the area of isolation. Streams are not uniformly distributed within areas of sharp relief. Also, within an area with streams, there may be a prevalence of one kind of stream over another in terms of width, extent of falls areas, flow, and size of boulders or crevices which serve as centers of *Cycloramphus* activities. The second kind of adaptations appear to be responses to deteriorating climate. In areas where the Atlantic Forest Domain disappeared due to climatic deterioration, *Cycloramphus* species either went locally extinct or adapted to the changing environment. In *Cycloramphus*, requirements of the larval stage are so narrowly defined that the likely usual course was extinction. However, local hydrological conditions in some montane areas permitted maintenance of the larval stage within an open formation setting. It is in these areas that *Cycloramphus* populations adapted to habitats associated with general deteriorating climatic conditions.

The outstanding feature of the allopatric model of speciation for *Cycloramphus* is the small sizes of geographic areas of isolation and speciation. The scale is local areas of high relief — those shown in Figure 4

would be an extreme example; the actual areas of speciation are likely smaller areas within those shown. Each local area had a unique history in terms of geological formation (when and how they were formed), hydrology, extent of Atlantic Forest cover during cooler and more arid times, and colonization, adaptations, and extinctions of *Cycloramphus* populations. No single zoogeographical pattern is apparent. Local patterns of distribution and relationships predominate. This very local effect results from some aspect in the life history of riparian *Cycloramphus* that confines occurrence to a very narrow and precisely defined microhabitat. The larval phase of the life cycle is the assumed limiting aspect, because the larvae are adapted to the wet surface covered rock splash zone of small brooks. Occurrence is, thus, limited to mountain brooks within areas of high relief and occurrence is discontinuous between areas of high relief.

EXAMPLES

The most recent speciation events are the clearest in terms of probable mechanisms. There are three such examples in the genus *Cycloramphus*.

The first example is the cluster *C. boraceiensis-dubius-semipalmatus*. *Cycloramphus boraceiensis* and *dubius* have allopatric (parapatric?) distributions in adjacent areas of sharp relief, mostly in the State of São Paulo (Figure 6). These two species are very closely related. The relationships of *C. semipalmatus* are a bit more distant, and its range overlaps those of the other two species. *Cycloramphus semipalmatus* is the most distinctive species in this cluster in terms of webbing and dorsal texture. The proposed speciation pattern is that *C. boraceiensis* and *dubius* represent the most recent example of allopatric speciation

resulting from isolation of a common ancestor in adjacent areas of sharp relief. These areas were probably Pleistocene forest refugia. The separation of the *C. boraceiensis-dubius* and *C. semipalmatus* lineages likely took place in the Pliocene, again by geographic isolation, but the distribution range has become considerably changed following range modifications during the Pleistocene. All three species have quite restricted distributions, fairly limited to the areas of origin.

The second example is the cluster *C. asper-dusei-lutzorum-mirandaribeiroi-rhyakonastes*. The most closely related species, which likely speciated in the Pleistocene, are *C. lutzorum*, *mirandaribeiroi*, and *rhyakonastes*. At present, the distributions of these species, as well as *asper* and *dusei*, are difficult to explain in detail in terms of the allopatric model of speciation. The areas these species occur in are exceedingly complex topographically. It is certainly possible that there were localized refugia within the areas of current distribution where differentiation led to speciation. The outlying localities of *C. rhyakonastes* in the State of Rio de Janeiro and *C. dusei* in the State of São Paulo, are difficult to interpret. These outlying distributions seriously violate the proposed model of local differentiation, speciation, and dispersal. Unfortunately, it may not be possible to determine whether these outliers represent areas of natural occurrence, for they have been altered by deforestation. Study of other groups predicted to have the same speciation pattern as *Cyclo-rampus* should clarify whether the proposed model is correct, or needs serious revision to incorporate the examples of outlying localities. Although the speciation details are not clear for this cluster, the general pattern of local, geographically proximate distributions among closely related species is evident (compare Figure 6 with Figs. 30 and 42 of the companion paper).

The third example is that of *C. bandeirensis*. In this case, differentiation is proposed to have occurred in a very restricted area through the vanishing refuge model of differentiation. This model, of adaptation caused by environmental deterioration would most logically place the differentiation of *C. bandeirensis* as a Pleistocene event. Unless the Atlantic Forest associated ancestor of *C. bandeirensis* has survived in a geographically proximate Pleistocene forest refugium, there will be no way to date the differentiation of *C. bandeirensis* to an open formation associate using biochemical means. The absence of *Cyclo-rampus* from the Atlantic Forests of Caparaó suggests that the ancestral stock of *C. bandeirensis* has not survived, except as *C. bandeirensis*. Thus, any biochemical comparisons with extant species may indicate the relationships of the Atlantic Forest associated ancestor, rather than the more recent differentiation into *C. bandeirensis*.

The three examples collectively demonstrate that, by and large, differentiation and speciation have been very local geographic events. There are no broad patterns of relationships within the Atlantic Forest Morphoclimatic Domain; the patterns are localized to areas of sharp relief within the Domain.

Two general kinds of adaptational complexes are involved in the differentiation leading to speciation in these examples. The adaptations in the first relate to different stream types. Characters of size, extent of toe webbing and microhabitat selection appear to be adaptations to particular classes of streams. Dorsal texture differences may also be of this type. This suggests that the areas where differentiation of each of the species occurred were characterized by a prevalence of different kinds of streams. The adaptations to different kinds of streams has resulted in the most distinctive features separating these species. The second set of adaptations relate to pre-mating isolating mechanisms. Dorsal texture may

be an adaptation of this type (see companion paper); advertising call certainly is. If dorsal texture differences are the result of adaptations to different kinds of stream environments, then the only observed adaptations that have evolved as strictly pre-mating isolating mechanisms are advertisement calls. The minor differences observed among available calls (for members of these three example clusters) suggest that either the observed differences are adequate to achieve pre-mating isolation or that selection for call differences may have been very recent.

The differentiation within these clusters occurs in the adult phase of the life cycle, not in the larval phase. Although few stream associated larvae are known, their similarities are striking. The constancy of larval form suggests that the larvae are so specialized that no flexibility to other adaptation is possible within the stream habitat environment. The ancestor of *Cycloramphus* likely had a larva that lived directly in the stream. The shift to a splash zone tadpole has been a one way route; the larvae are either too specialized or the stream anuran tadpole community is too packed to permit re-entry. Although details are lacking, the splash zone tadpole morphotype must in some way be pre-adapted to a terrestrial larval type. Certainly much of the respiration of splash zone larvae must take place at the air-body surface interface rather than the water-gill interface. Thus, the splash zone larva seems to be the limiting phase in the life cycle. The three historical outcomes of responses to changing stream conditions have been: (1) most commonly, local extinction; (2) adaptations of the adult stage to different stream types; and (3) at some point, the evolution of a terrestrial larva.

The patterns of relationships occurring in local geographic areas are discernible only with the genetic estimate data. As discussed previously, the morphological differences among stream associated *Cyclo-*

ramphus species present a confusing mosaic. No correlation of characters clearly demonstrates relationships. This mosaic of character state distribution is completely understandable in the context of the proposed model of speciation, because no widespread patterns are predicted. We wish to emphasize that without the genetic data the correct relationships would remain indeterminate.

ALTERNATIVE MODELS

The allopatric model best fits the available data in our opinion. For the sake of completeness, two other major speciation models, important in some other groups, should be briefly discussed.

The stasipatric model of speciation (White, 1978) involves chromosomal mechanisms of speciation. The model is most effective in explaining species of open formations where catastrophic selection occurs and distributions involving stable, narrow zones of hybridization between otherwise parapatric species pairs occur. There are no known instances of hybridization among *Cycloramphus* species and the major ecological associations are with closed, rather than open formations. The karyotype is known for only one species of *Cycloramphus*, *C. fuliginosus* (Bogart, 1970), so it is not known if there is karyotypic variation among members on the genus. We would not predict major karyotypic variation, although the presence of such variation would not in itself invalidate the allopatric model of speciation for *Cycloramphus*.

Clinal variation leading to parapatric or sympatric speciation is theoretically possible (Endler, 1977). There is a general cline in the portion of the Atlantic Forest Domain containing *Cycloramphus*. The Domain stretches across several degrees of latitude, ranging from a tropical climate in the north

to a subtropical climate in the south. The nature of this environmental cline is gradual. The clinal model of speciation is most effective where there is a sharp environmental break which would result in a step cline relationship. At the geographic extremes, *Cycloramphus* species are likely adapted to different environmental conditions, particularly involving temperature. The scale of the geographic ranges of most *Cycloramphus* species is so small that adaptations occur within regions that are climatically uniform. The only species with a wide distribution that might show incipient speciation through the clinal model is *C. eleutherodactylus*. The data (companion paper) do not indicate a clinal pattern of variation. Clinal variation can occur over small geographic areas, but for most *Cycloramphus*, distributions at the local level are discrete, not continuous, due to the discrete distributions of appropriate streams.

Thus, for *Cycloramphus*, the allopatric model of speciation is the strongest model. This is not to suggest that the allopatric model is the only model of speciation involved in Neotropical frogs. On the contrary, it is likely that speciation in Neotropical frogs has occurred via all vertebrate speciation mechanisms (Heyer and Maxson, 1982b).

PREDICTIONS

The following predictions develop from the assumption that allopatric speciation is the mode for members of the genus *Cycloramphus* and likely to be correct for other units of the Atlantic Forest Domain fauna.

The *C. granulatus* group morphology is hypothesized to be a result of ecomorphotypic adaptations rather than a result of a common evolutionary history. The closest relatives of *C. catarinensis* and *valae* should be with nearby *C. fuliginosus* group members, rather than with *C. granulatus*.

The disjunct distributions of three species of stream associated species of *Cycloramphus* (*duseni*, *fuliginosus*, *rhyakonastes*) are due to errors of either locality data or species limit interpretations.

It is difficult to imagine a more specialized, limiting, life history feature than a splash zone tadpole. Not only is the life history tied to streams, but even more restrictive, to specialized areas within streams. This narrow specialization, combined with a long evolutionary history in an unstable area (geologically and climatically), suggests that *Cycloramphus* exhibits an extreme example of speciation in very local areas. We predict that this same pattern would only be repeated in other groups which have life history features that limit their distributions to very patchily distributed habitats. Some other stream associated insect groups might be expected to show a pattern very similar to that seen in *Cycloramphus*. The areas of isolation and differentiation should be at a somewhat larger scale for fishes and frogs that have larvae that live in the waters of the streams. Thus, for groups that have occurred in the Atlantic Forest Domain throughout the Cenozoic, such as *Hylodes*, the total distributional range should be greater, individual species ranges should be larger, and there should be fewer species per comparable geographic region than for *Cycloramphus*. The first part of the prediction is true. *Hylodes* is known from the Serra do Cipó; *Cycloramphus* is not. The systematic chaos of *Hylodes* as presently understood precludes further verification of predictions. For stream frogs (with aquatic larvae) that have relatively short histories in the Atlantic Forest Domain, individual species ranges should be large and intraspecific variation should occur due to Pleistocene isolation of population units. The available data for *Centrolenella eurygnatha* are consistent with these predictions (Heyer, 1978) as are the data for the fish genus *Parotocinclus* (Garavello, 1977). At another level, frogs with

life histories not tied to patchily distributed habitats within the Atlantic Forest Domain, such as *Eleutherodactylus*, should have broader distributions both locally and geographically than either *Cycloramphus* or *Hylodes*. Further, there should be fewer species within the Atlantic Forest Domain per major lineage than for either *Cycloramphus* or *Hylodes*. Again, the current systematic understanding of *Eleutherodactylus* precludes testing the predictions at present.

Acknowledgments

Without field work, this paper would have been impossible to write. The cumulative effect of field observations has been critical. The opportunity to digest one field season's observations before embarking on the next allowed a different perspective for each subsequent trip; each trip attempting a sharper focus of the environment in terms of how *Cycloramphus* experience it. For sharing their observations in the field and making the field work possible, we thank Francisca Carolina do Val and P. E. Vanzolini. Others who have assisted in the field

work over the years are Ronald I. Crombie, Maria Christina Duchêne, Annelise Gehrau, Elena D. Heyer, Laura M. Heyer, Miriam H. Heyer, Eugenio Izecksohn, Kazumi Kanno, Frances I. McCullough, Oswaldo Peixoto, and Sergio Potch.

The concepts developed in this paper arise from past and continuing discussions with P. E. Vanzolini. We are certain that his contribution to this paper has not been fully documented in the text, which speaks for the completely free and open nature of his comments and insights.

Robert F. Inger, P. E. Vanzolini, and George R. Zug have critically reviewed the manuscript.

The following institutions and programs are thanked for their recognition that our evolutionary studies required two very different kinds of special financial support for field work and laboratory biochemical analysis work: Fundação de Amparo à Pesquisa do Estado de São Paulo; Museu de Zoologia da Universidade de São Paulo; I.E.S.P. Amazon Ecosystem Research Program, Smithsonian Institution; Director's Office, National Museum of Natural History; Fluid Research Award Program, Smithsonian Institution; U.S. National Science Foundation Grant 78-23396.

References

- Ab'Sáber, A. N., 1977a. Os domínios morfoclimáticos na América do Sul. Primeira aproximação. *Geomorfologia*, (Instituto de Geografia, Universidade de São Paulo) 53:1-23.
- Ab'Sáber, A. N., 1977b. Espaços ocupados pela expansão dos climas secos na América do Sul, por ocasião dos períodos glaciais quaternários. *Paleoclimas*, (Instituto de Geografia da Universidade de São Paulo) 3:1-19.
- Almeida, F. F. M. de, 1976. The system of continental rifts bordering the Santos Basin, Brazil. *Anais da Academia Brasileira de Ciências*, 48 (supl.): 15-26.
- Andersson, L. G., 1914. A new *Telmatobius* and new teiidoid lizards from South America. *Arkiv för Zoologi*, 9(3):1-12.
- Bogart, J. P., 1970. Systematic problems in the amphibian family Leptodactylidae (Anura) as indicated by karyotypic analysis. *Cytogenetics*, 9:369-383.
- Bokermann, W. C. A., 1965. Notas sobre as espécies de *Thoropa* Fitzinger (Amphibia, Leptodactylidae). *Anais da Academia Brasileira de Ciências*, 37:525-537.
- Botelho, C. Castro, 1977. Hidrografia. Pages 119-142 in *Geografia do Brasil, Volume 3, Região Sudeste*. Fundação Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro. 667 pp.
- Carlson, S. S., A. C. Wilson, and R. D. Maxson, 1978. Do albumin clocks run on time? *Science*, 200:1183-1185.
- Champion, A. B., E. M. Prager, D. Wachter, and A. C. Wilson, 1974. Micro-complement fixation. Pages 397-416 in C. A. Wright (ed.), *Biochemical and Immunological Taxonomy of Animals*. Academic Press, London. 490 pp.
- Champion, A. B., K. L. Soderberg, A. C. Wilson, and R. P. Ambler, 1975. Immunological compar-

- isons of azurins of known amino acid sequence. *Journal of Molecular Evolution*, 5:291-305.
- Corruccini, R. S., M. Baba, M. Goodman, R. Ciochon, and J. E. Cronin, 1980. Non-linear macromolecular evolution and the molecular clock. *Evolution*, 34:1216-1219.
- Cruz, O., 1974. A Serra do Mar e o litoral na área de Caraguatatuba — SP. Contribuição à Geomorfologia Litorânea Tropical. Universidade de São Paulo, Instituto de Geografia, Série Teses e Monografias, 11:1-181.
- Diamond, J. M., 1975. Assembly of species communities. Pages 342-444 in M. L. Cody and J. L. Diamond (eds.), *Ecology and Evolution of Communities*. Belknap Press of Harvard University Press, Cambridge. 545 pp.
- Endler, J. A., 1977. Geographic variation, speciation and clines. *Monographs in Population Biology*, 10:1-246.
- Farris, J. S., 1972. Estimating phylogenetic trees from distance matrices. *American Naturalist*, 106:645-668.
- Fitch, W. M. and E. Margoliash, 1967. Construction of phylogenetic trees. *Science*, 155:279-284.
- Garavello, J. C., 1977. Systematics and geographical distribution of the genus *Parotocinclus* Eigenmann & Eigenmann, 1899 (Ostariophysi, Loricariidae). *Arquivos de Zoologia*, 28(4):1-37.
- Haffer, J., 1969. Speciation in Amazonian forest birds. *Science*, 165:131-137.
- Heyer, W. R., 1975. A preliminary analysis of the intergeneric relationships of the frog family Leptodactylidae. *Smithsonian Contributions to Zoology*, 199:1-55.
- Heyer, W. R., 1978. Variation in members of the *Centrolenella eurygnatha* complex (Amphibia: Centrolenidae) from Serra do Mar and Serra da Mantiqueira, Brasil. *Papéis Avulsos de Zoologia*, 32(2):15-33.
- Heyer, W. R. and R. I. Crombie, 1979. Natural history notes on *Craspedoglossa stejnegeri* and *Thoropa petropolitana* (Amphibia: Salientia, Leptodactylidae). *Journal of the Washington Academy of Sciences*, 69:17-20.
- Heyer, W. R. and L. R. Maxson, 1982a. Distributions, relationships and zoogeography of lowland frogs: The *Leptodactylus* complex in South America, with special reference to Amazonia. Pages 375-388 in G. T. Prance (ed.), *Biological Diversification in the Tropics*. Columbia University Press, New York. 714 pp.
- Heyer, W. R. and L. R. Maxson, 1982b. Neotropical frog biogeography: Paradigms and problems. *American Zoologist*, 22:397-410.
- Ibrahimi, I. M., J. Eder, E. M. Prager, A. C. Wilson, and R. Arnon, 1980. The effect of a single amino acid substitution on the antigenic specificity of the loop region of lysozyme. *Molecular Immunology*, 17:37-46.
- Jackson, J. F., 1978. Differentiation in the genera *Enyalius* and *Strobilurus* (Iguanidae): Implications for Pleistocene climatic changes in eastern Brazil. *Arquivos de Zoologia*, 30:1-79.
- Jukes, T. H., 1980. Silent nucleotide substitutions and the molecular evolutionary clock. *Science*, 210:973-978.
- Karig, L. and A. C. Wilson, 1971. Genetic variation in supernatant malate dehydrogenase of birds and reptiles. *Biochemical Genetics*, 5:211-221.
- Maxson, L. R. and W. R. Heyer, 1982. Leptodactylid frogs and the Brazilian Shield: An old and continuing adaptive relationship. *Biotropica*, 14(1):10-15.
- Maxson, L. R., W. R. Heyer, and R. D. Maxson, 1981. Phylogenetic relationships of the Brazilian leptodactylid frog genera *Craspedoglossa*, *Cycloramphus* and *Zachaenus*. *Experientia*, 57:144-145.
- Maxson, L. R., R. Highton, and D. B. Wake, 1979. Albumin evolution and its phylogenetic implications in the plethodontid salamander genera *Plethodon* and *Ensatina*. *Copeia*, 1979:502-508.
- Maxson, L. R. and R. D. Maxson, 1979. Comparative albumin and biochemical evolution in plethodontid salamanders. *Evolution*, 33:1057-1062.
- Maxson, L. R. and A. C. Wilson, 1974. Convergent morphological evolution detected by studying proteins of tree frogs in the *Hyla eximia* species group. *Science*, 185:66-68.
- Maxson, L. R. and A. C. Wilson, 1975. Albumin evolution and organismal evolution in tree frogs (Hylidae). *Systematic Zoology*, 24:1-15.
- Moreira, A. A. N. and C. Camelier, 1977. Relevo. Pages 1-50 in *Geografia do Brasil, Volume 3, Região Sudeste*. Fundação Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro. 667 pp.
- Moreira, A. A. N. and G. R. Lima, 1977. Relevo. Pages 1-34 in *Geografia do Brasil, Volume 5, Região Sul*. Fundação Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro. 534 pp.
- Nei, M., 1977. Standard error of immunological dating of evolutionary time. *Journal of Molecular Evolution*, 9:203-211.
- Odum, E. P., 1971. *Fundamentals of Ecology*. Third Edition. W. B. Saunders Co., Philadelphia. 574 pp.
- Prager, E. M. and A. C. Wilson, 1976. Congruency of phylogenies derived from different proteins. *Journal of Molecular Evolution*, 9:45-57.
- Prager, E. M. and A. C. Wilson, 1978. Construction of phylogenetic trees for proteins and nucleic acids: Empirical evaluation of alternative matrix methods. *Journal of Molecular Evolution*, 11:129-142.
- Radinsky, L., 1978. Do albumin clocks run on time? *Science*, 200:1182-1183.
- Santos, R. S. Bezerra dos. 1977. Hidrografia. Pages 111-142 in *Geografia do Brasil, Volume 5, Região Sul*. Fundação Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro. 534 pp.
- Sarich, V. M. and J. E. Cronin, 1976. Molecular systematics of the primates. Pages 141-170 in M. Goodman and R. E. Tashian (eds.) *Molecular Anthropology*. Plenum Press, N. Y. 466 pp.
- Scanlan, B. E., L. R. Maxson, and W. E. Duellman. 1980. Albumin evolution in marsupial

- frogs (Hylidae: *Gastrotheca*). *Evolution*, 34: 222-229.
- Soderstrom, T. R. and C. E. Calderón, 1974. Primitive forest grasses and evolution of the Bambusoideae. *Biotropica*, 6:141-153.
- Suguo, K. and L. Martin, 1976. Brazilian coastline Quaternary formations — The States of São Paulo and Bahia littoral zone evolutive schemes. *Anais da Academia Brasileira de Ciências*, 48 (Supl.):325-334.
- Vanzolini, P. E., 1973. Distribution and differentiation of animals along the coast and in continental islands of the State of S. Paulo, Brasil. I. Introduction to the area and problems. *Papéis Avulsos de Zoologia*, 26(24):281-294.
- Vanzolini, P. E., 1981a. A quasi-historical approach to the evolution of reptiles in tropical geographic isolates. *Papéis Avulsos de Zoologia*, 34(19):189-204.
- Vanzolini, P. E., 1981b. A new *Gymnodactylus* from Minas Gerais, Brasil, with remarks on the genus, on the area and on montane endemisms in Brasil (Sauria, Gekkonidae). *Papéis Avulsos de Zoologia*, 34(29):403-413.
- Vanzolini, P. E., 1983. A note on Guiano-Brasilian *Polychrus*: Distribution and speciation (Sauria, Iguanidae). *Bulletin of the Museum of Comparative Zoology*, Harvard University, in press.
- Vanzolini, P. E. and A. M. M. Ramos, 1977. A new species of *Colobodactylus*, with notes on the distribution of a group of stranded microteiid lizards (Sauria, Teiidae). *Papéis Avulsos de Zoologia*, 31(3):19-47.
- Vanzolini, P. E. and E. E. Williams, 1970. South American anoles: The geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). *Arquivos de Zoologia*, 19(1-4):1-240.
- Vanzolini, P. E. and E. E. Williams, 1981. The vanishing refuge: a mechanism for ecogeographic speciation. *Papéis Avulsos de Zoologia*, 34(23):251-255.
- Vuilleumier, F. and D. Simberloff, 1980. Ecology versus history as determinants of patchy and insular distributions in high Andean birds. *Evolutionary Biology*, 12:235-379.
- Walker, I., 1979. The mechanical properties of proteins determine the laws of evolutionary change. *Acta Biotheoretica*, 28:239-282.
- Wallace, D. G., M. C. King, and A. C. Wilson, 1973. Albumin differences among ranid frogs: Taxonomic and phylogenetic implications. *Systematic Zoology*, 22:1-14.
- White, M. J. D., 1978. *Modes of Speciation*. W. H. Freeman and Company, San Francisco. 455 pp.
- Williams, E. E., 1972. The origin of faunas. Evolution of lizard congeners in a complex island fauna: A trial analysis. *Evolutionary Biology*, 6:47-89.
- Wilson, A. C., S. S. Carlson, and T. J. White, 1977. Biochemical evolution. *Annual Reviews of Biochemistry*, 46:573-639.