

NATURAL HYBRIDIZATION AND ITS EVOLUTIONARY IMPLICATIONS IN GUATEMALAN PLETHODONTID SALAMANDERS OF THE GENUS *BOLITOGLOSSA*

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ABSTRACT: The Guatemalan and Mexican plethodontid salamanders *Bolitoglossa franklini* and *Bolitoglossa resplendens* are allopatric except in an area of habitat disturbance near Volcán Tajumulco, in western Guatemala, where they hybridize. However, morphological and electrophoretic analyses suggest that hybridization is relatively limited, with most hybrids ascribable to early backcross generations. The hybridization has a pervasive effect on the morphology of *B. resplendens*, whereas *B. franklini* has a far more stable phenotype and can accept a larger dose of alleles of *B. resplendens* without apparent morphological change. Extent of hybridization is underestimated if morphological criteria are used. This is the first documented case of hybridization in tropical salamanders and is the result of secondary contact of genetically and ecologically well differentiated species.

Key words: Amphibia; Caudata; Plethodontidae; *Bolitoglossa*; Hybridization; Allozymes; Electrophoresis; Morphology; Guatemala; Mexico

SPECIATION in plethodontid salamanders has been studied in detail, especially in certain groups in the United States (e.g., Highton, 1962; Highton and Webster, 1976; Larson and Highton, 1978; Stebbins, 1949). These studies have demonstrated geographic variability in morphology and allozymes, and have led to the conclusion that time, distance, and isolation are the primary elements in speciation. Allopatric speciation (Mode Ia of Bush, 1975) seems to have been the rule in plethodontid salamanders, and isolating mechanisms may involve both ecological and behavioral characteristics (Arnold, 1977). The several documented instances of hybridization (Brown, 1974; Duncan and Highton, 1979; Highton and

Henry, 1970; Peabody, 1978) have been interpreted as resulting from secondary contacts of species in which isolating mechanisms either have not yet become fully established or have broken down as a result of some environmental perturbation.

Plethodontid salamanders are rich in number of species in the New World tropics especially in Mexico and Central America (Wake, 1970). The few studies of geographic variation in tropical salamanders have documented the allopatric patterns of distribution within species groups. These patterns are similar to those seen in more northerly regions. Species ranges are smaller in the tropics, but speciation probably has followed the

TABLE 1.—Summary of comparative data for two species of *Bolitoglossa*.

Species	Body form and coloration	Range	Elevation	Habitat	Microhabitat
<i>B. franklini</i>	Gracile; black with silvery vermiculated dorsal pattern	Pacific coastal slopes of southeastern Chiapas and western Guatemala	1800–2700 m	Cloud forest	Bromeliads; in and under bark of logs and stumps
<i>B. resplendens</i>	Stout; black with brick red band, often interrupted and with irregular borders	Inland highlands of southeastern Chiapas and western Guatemala	2600–2900 m	Forest-meadow edge	Under surface objects, in bromeliads, and in and under bark of logs and stumps

same general mode in both tropical and extratropical groups (Wake and Lynch, 1976). Here we report the first instance of natural hybridization in tropical salamanders, analyze its extent, its morphological expression, and its causes, and interpret these results in light of distributional and other data bearing on speciation events.

BACKGROUND

Bolitoglossa franklini and *Bolitoglossa resplendens* are members of a complex of mostly allopatric species distributed widely in Chiapas, Mexico, and Guatemala (Table 1). Two assemblages of nominal species are recognized in this complex: bright red and black salamanders of interior upland forests, and black salamanders marked with silvery-gray to greenish to dull orange pigment from Pacific coastal cloud forest habitats (Wake and Lynch, 1976). Members of the first assemblage include *Bolitoglossa lincolni* of the Cordillera de los Cuchumatanes and *B. resplendens* of the Mesa Central region of Chiapas, the Sierra de Cuicilo of extreme western Guatemala, and the San Marcos region, Guatemala. The second assemblage includes *Bolitoglossa nigroflavescens* of the Cerro Ovando region of Chiapas, *Bolitoglossa brevipes* of the extreme southeastern Sierra Madre of Chiapas, and *Bolitoglossa franklini* of the Pacific slopes of the coastal volcanoes from

Tacaná in the northwest to Atitlán in the southeast. Only in the vicinity of Volcán Tajumulco, near San Marcos, Guatemala, do the two assemblages meet (Fig. 1). However, even here the two species involved are ecologically distinct being separated by differences in elevation, major habitat type, and microhabitat (Wake and Lynch, 1976).

In most anatomical features *B. resplendens* and *B. franklini* are quite similar. The somewhat larger *B. resplendens* has slightly shorter limbs than *B. franklini* (see McCoy and Walker, 1966; and J. F. Lynch, unpubl. data); *B. franklini* is somewhat more gracile and active. The hands and feet of *B. franklini* have less well developed subdigital pads and slightly less webbing than in *B. resplendens*. The latter species has a shiny, jet-black ground color and a bright, brick-red to red-orange dorsal band. The dorsal band may be almost continuous or it may be interrupted by black spots ringed with bright orange to gold (Fig. 2A). Rarely, *B. resplendens* is almost black with a few large, reddish spots, or even less frequently the dorsal spots may be small and numerous. Characteristically 3 or 4 moderately large, irregularly shaped, reddish spots are present ventrally.

The black ground color of *B. franklini* resembles that of *B. resplendens*, but the dorsal coloration is a heavy vermiculation of mainly bright silvery pigment with

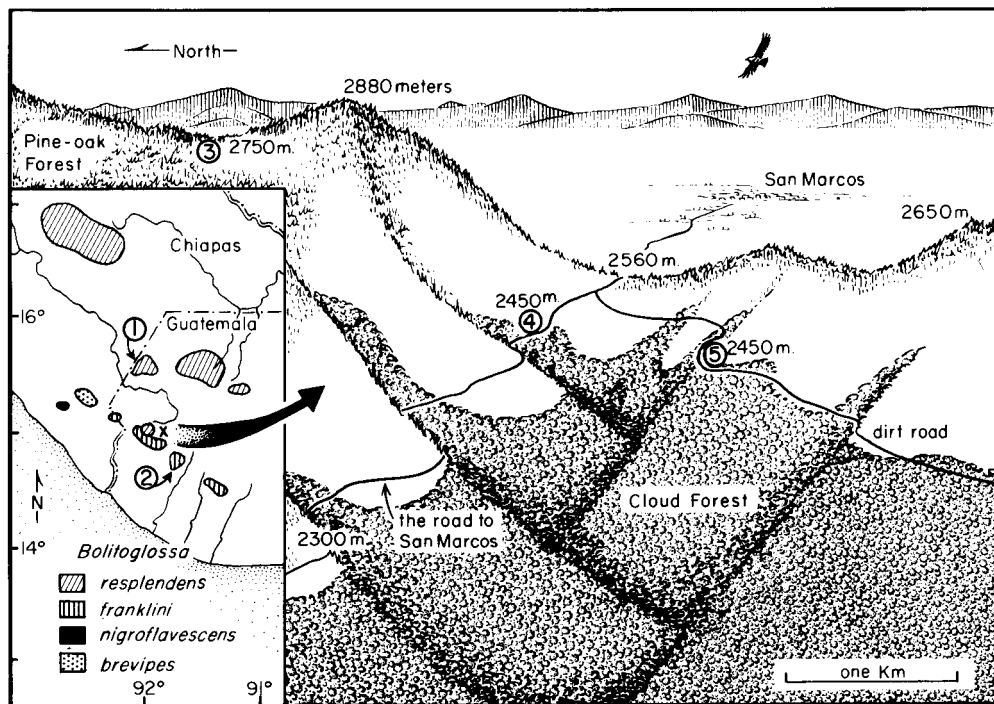


FIG. 1.—Hybrid zone between *Bolitoglossa resplendens* and *B. franklini* near San Marcos, Guatemala. The panoramic view is an enlargement of point x on the inset and illustrates a section of escarpment of the Guatemalan Plateau a few kilometers south of Volcán Tajumulco. *Bolitoglossa franklini* occurs in the cloud forest along the escarpment and meets *B. resplendens* where the latter penetrates fingers of cloud forest along corridors of disturbance such as roads. The inset illustrates the distribution of the four closely related members of the *Bolitoglossa lincolni* group of western Guatemala and Chiapas, Mexico. The circled numbers refer to the localities from which materials for the present study were collected.

some highlights of dull golden orange in *B. franklini* (Fig. 2D). In some individuals the silvery color is dulled by infusion of the black ground color. A complete band of silver color occurs rarely. In some other instances the dorsal pigmentation is broken into numerous small, irregularly shaped spots. The venter is unmarked by large spots, but usually numerous, diffuse iridophores give the venter a slightly grayish cast (for more detailed color descriptions see McCoy and Walker, 1966, and Schmidt, 1936).

STUDY LOCALITIES

These species are restricted in distribution, and specimens are difficult to collect due to extensive habitat destruction. Samples were obtained from 5 localities

in western Guatemala (Table 2), 4 of which were chosen to serve as outside reference samples for analysis of the hybridization we suspected at locality 5. As will be shown below, hybrid individuals also proved to be present in samples from localities 3 and 4. The three localities with hybrids are in the vicinity of San Marcos, Guatemala. Locality 1 was our reference sample for *B. resplendens* and is about 63 km NNE of locality 5. Locality 2 was our reference sample for *B. franklini* and is about 25 km SE of locality 5 (Fig. 1).

The distribution and ecology of *B. franklini* and *B. resplendens* near San Marcos have been described by Wake and Lynch (1976). The two forms behave as ecologically distinct species. *Bolito-*



FIG. 2.—Photographs of *Bolitoglossa* from vicinity of San Marcos, Guatemala. Scale is 25 mm. A. *Bolitoglossa resplendens* from an area near San Marcos, Guatemala (locality 3) where some hybrid animals have been found. This individual is morphologically and genetically pure according to the criteria of this paper. B. An individual with a pattern like that of *B. resplendens*, but with the coloration of *B. franklini*. This is a probable backcross to *B. resplendens* (electrophoretic phenotype $a'a'bb'cc$). C. An individual with a pattern like *B. franklini* but with coloration of *B. resplendens*. This is a probable backcross to *B. resplendens* ($a'a'bb'cc$). D. An individual which fits all morphological criteria of *B. franklini*, but is genetically an indeterminate hybrid ($a'a'bb'cc'$).

glossa franklini is found in cloud forest mainly at elevations between 1800–2500 m, but locally it occurs as high as 2700 m. In contrast, *B. resplendens* occurs in the drier oak-pine-cypress forest and at the edge of clearings at the upper edge of the cloud forest between elevations of 2600–2900 m. We know only one locality (number 5) where the two species come into extensive contact. Elsewhere animals usually have been assigned readily to one or the other species on morphological grounds. Our locality 4 is a section of road along which *B. resplendens* is

placed altitudinally by *B. franklini*, but the two have not been taken in micro-sympatry. As long ago as 1971 a few (~10) among several hundred specimens from near locality 4 were noted to have characters of both *B. franklini* and *B. resplendens*, and hybridization was suspected (field notes of J. F. Lynch, Mus. Vert. Zool.).

The present study was initiated when individuals of intermediate morphology were encountered at locality 5 in a protected valley where a narrow finger of cloud forest penetrates surrounding oak-

pine forest. A dirt road cuts through this patch of cloud forest at an elevation (2450–2480 m) where the forest is about 300 m wide. In the middle 150 m segment of this road we found several apparently intermediate salamanders in a disturbed microhabitat (the road bank).

METHODS

In order to quantify the extent of hybridization at locality 5, a morphological hybrid index was devised based on coloration and proportional differences described above. A scale of 1 to 10 was established with 1 ("pure" *B. franklini*) having an undefined dorsal band, a vermiculated pattern or one with highly irregular spots on the dorsum, tiny spots (if any) on the venter, silvery-gray pigmentation, gracile body form, and irregularly shaped diffuse spots on the head. An individual scoring 10 ("pure" *B. resplendens*) had a discrete dorsal band or large regularly bordered dorsal spots, moderate to large ventral spots, distinctly reddish coloration, a stocky body form, and large discrete spots on the head. Living animals were each scored independently by two observers; the average of the two scores was used in the analysis below. Individuals scoring 5 and 6 were intermediate in all characters, or were like *B. franklini* in some characters and like *B. resplendens* in others (Fig. 2). There was high concordance in the scoring patterns of the two observers despite the fact that one is red-green color blind (this individual was included as a judge in order to emphasize the pattern and proportional features). Of those instances (9 of 63 cases) where a disparity in score of 2 or more occurred, all but one were attributable to differences in recording color quality. Morphologically pure *B. franklini* had scores of 1 and 2, and pure *B. resplendens* had scores of 9 and 10. Presumptive hybrids, based on morphological intermediacy, were expected to score between 3.5–7.5.

An electrophoretic analysis was undertaken for 111 specimens from all 5 local-

TABLE 2.—Collecting localities for *Bolitoglossa* used in the present study.

Locality number	Species and locality
1.	<i>B. resplendens</i> . Montañas de Cuilco, 1.5 km NE Peña Blanca, Dpto. Huehuetenango, Guatemala, 2750–2800 m elev.
2.	<i>B. franklini</i> . Slopes of Volcan Chicabal, 5 km SE San Martin Sacatepequez, Dpto. Quezaltenango, Guatemala, 2250 m elev.
3.	<i>B. resplendens</i> and hybrids. W slope of ridge 2 km W El Rincon, Dpto. San Marcos, Guatemala, 2700–2850 m elev.
4.	<i>B. franklini</i> , <i>B. resplendens</i> , and hybrids. Forest around Rta. Nac. 1, ca. 12 km W San Marcos, Dpto. San Marcos, Guatemala, 2400–2500 m elev.
5.	<i>B. franklini</i> , <i>B. resplendens</i> , and hybrids. Vicinity of Pojopom, 1–2 km WSW Esquipulas Palo Gordo, Dpto. San Marcos, Guatemala, 2450–2480 m elev.

ities. Freshly sacrificed specimens were dissected and samples of liver, kidney, spleen, stomach, heart, and body muscle were homogenized. Extracts were used either immediately or stored at -76°C . Combined mixed tissue extracts were subjected to horizontal starch-gel electrophoresis and results were interpreted following Selander et al. (1971).

We found 17 variable loci, but only 7 showed different frequencies between reference populations. These were: sorbitol dehydrogenase, *Sordh*, and isocitrate dehydrogenase, *Icd-1* (both using continuous triscitrate pH 8.0 and pH 7.0 buffer systems); peptidase-leucyl-alanine, *Pep* (discontinuous triscitrate-Poulik buffer); glucose phosphate isomerase, *Gpi* (continuous triscitrate pH 7.0 buffer); glutamate oxalo-transaminase, *Got-1* (lithium hydroxide buffer); 6-phosphogluconate dehydrogenase, *Pgd* (tris-maleic EDTA and NADP, pH 7.4, and triscitrate pH 7.0 buffers); and lactate dehydrogenase, *Ldh-1* (discontinuous triscitrate-Poulik buffer).

Carcasses of all specimens used in the study were fixed in formaldehyde, preserved in alcohol, and deposited in the Museum of Vertebrate Zoology.

RESULTS

The initial phase of this study was restricted to the three localities (3, 4, 5) in the San Marcos area. On morphological grounds 14 individuals scored in the range predicted for hybrids (3.5–7.5) and an additional 16 scored near that range (2.5–3.25, 8–8.75). All 30 individuals were from locality 5. However, many other individuals from locality 5 scored as pure *B. resplendens* or *B. franklini* on morphological grounds. Our impression in the field was that *B. resplendens* was exclusively present at the upper end of the road as it entered the cloud forest, while at the lower end of the road (deep in the cloud forest) only *B. franklini* was present. Our electrophoretic analysis disclosed some differences in frequency at certain electromorphic loci; these corresponded with our morphological classification. No fixed differences were found, however, and suspected hybrids were not unambiguously differentiated from parental classes. Even the supposedly pure samples from localities 3 and 4 could not be distinguished at the individual level by electrophoretic criteria. This surprised us, for our previous studies of tropical and temperate zone plethodontid salamanders led us to expect fixed differences between morphologically distinct species, as was the experience of another laboratory (Feder et al., 1978; Highton and Larson, 1979; Yanev, 1980). Frequently, even species of salamanders that are essentially identical morphologically show sharp genetic differentiation (Highton and Webster, 1976; Larson and Highton, 1978; Yanev, 1980). A possible interpretation of our finding was that natural hybridization between *B. franklini* and *B. resplendens* was more widespread than we had initially perceived in the San Marcos area, but we were unable to detect more than a fraction of it morpho-

logically. Accordingly we expanded our study to include more remote reference populations from localities 1 and 2.

Samples from locality 1 (which we consider to genetically pure *B. resplendens* for the purposes of this paper) and locality 2 (genetically pure *B. franklini*) showed fixed differences at three electromorphic loci. *Bolitoglossa franklini* was homozygous for a slowly migrating variant of *Icd-1* (designated *aa*), a slow variant of *Sordh* (*bb*), and a fast variant of *Pep* (*c'c'*). *Bolitoglossa resplendens* was homozygous for a fast variant of *Icd-1* (*a'a'*), a fast variant of *Sordh* (*b'b'*), and a slow variant of *Pep* (*cc*). The following electromorphic loci displayed important frequency differences: *Pgi* (*d'* .96, *d* .04 in *B. franklini*; *d'* .5, *d* .5 in *B. resplendens*), *Ldh-1* (*e* .96, *e'* .04 in *B. franklini*; *e* .5, *e'* .5 in *B. resplendens*), *Got-1* (*f* .62, *f'* .38 in *B. franklini*; *f* 1.0 in *B. resplendens*), *Pgd* (*g* .96, *g'* .04 in *B. franklini*; *g'* 1.0 in *B. resplendens*). The samples from localities 1 and 2 were quite distinct genetically with D (Nei, 1972) = .482 based on analysis of 18 electromorphic loci (8 monomorphic; 10 polymorphic).

We used these findings to predict electromorphic phenotypes of F_1 and B_1 individuals in the San Marcos area using expectations from simple Mendelian genetic crosses. By using only *Sordh*, *Icd-1*, and *Pep*, and assuming hybridization only when there was no alternative (individuals were scored as pure in ambiguous cases), we were able to sort our samples according to the outline in Table 3. The data from other loci were compatible with these findings.

An F_1 individual was expected to have the electromorphic phenotype *aa' bb' cc'*. In our sample from locality 5 we found three such individuals. However, the striking feature of this sample was the low number of parental types in relation to our expectations from morphological data. It appeared that most individuals were hybrids (either first generation backcrosses or more distant generation crosses; Table 3).

TABLE 3.—Assignment of individuals to presumptive classes. Localities 1 and 2 were by species definition; localities 3, 4, and 5 were by analysis of electromorphic data using 1 and 2 as standards.

Locality	n	<i>Bolitoglossa resplendens</i>	Backcross (to <i>B. resplendens</i>)	F ₁	Indeterminate hybrid	Backcross (to <i>B. franklini</i>)	<i>B. franklini</i>
1	7	7					
2	14						14
3	12	9	3				
4	15	3	3		1	5	3
5	63	9	20	3	16	11	4

We examined the three marker electromorphic loci for Hardy-Weinberg equilibrium. At locality 5 where we had an adequately large sample, we found strong, statistically significant ($\chi^2 = 5.05, 12.51, \text{ and } 8.21$ for the 3 loci, with 1 df) deficiencies of heterozygotes for *Icd-1*, *Sordh*, and *Pep*. Such deficiencies may indicate that free, random breeding was not occurring. An alternative interpretation is the possibility of a Wahlund effect (for example, pure individuals might migrate into the hybrid zone area, or some complex mixture of resident parental and hybrid populations may occur; Crow and Kimura, 1970). A further possibility is selection against hybrids.

However, despite the deficiencies of heterozygotes at individual loci for locality 5, the overall level of heterozygosity (in all loci) was high and variation was great. This seemingly paradoxical pattern resulted from the presence of a number of highly heterozygous individuals, combined with individuals with relatively low heterozygosity. To examine this further we used our samples from localities 1 and 2 to establish a genetic hybrid index. This was used to record heterozygosities in hybrid and non-hybrid classes. Each electromorphic variant for our three marker loci in *B. franklini* was given a -1 score, and each variant in *B. resplendens* was given a $+1$ score. A pure *B. franklini* thus scored -6 and a pure *B. resplendens* $+6$. An F₁ and also certain B₁ and later generational crosses scored 0. Additionally, B₁ and later generational backcross individuals scored $+6, +4, +2, 0, -2, -4, \text{ or } -6$. Odd scores (Fig. 3) ap-

peared only because four individuals in the hybrid zone displayed rare alleles not found elsewhere (for a discussion of rare alleles in hybrid zones see Sage and Selander, 1979). We plotted number of individuals by number of heterozygous loci for three samples (Fig. 4). The mean number of heterozygous loci (of 17) per individual was 2.0 for locality 1, .9 for locality 2, and 2.3 for localities 3, 4, and 5 combined. The variability rather than the mean value distinguished the last sample. The maximum number of variable loci was 3 for the reference populations, but was as high as 6 in the three San Marcos populations. However, a substantial number of homozygous individuals occurred in our San Marcos populations (13%).

Morphological and electromorphic (genetic) hybrid indices were compared for the three samples from the San Marcos area (Fig. 3). An asymmetrical relationship was found. Only 7 of 36 (19%) morphologically pure *B. franklini* were also genetically pure, but 15 of 22 (68%) morphologically pure *B. resplendens* were also genetically pure. Some individuals that were quite similar in morphology to *B. franklini* were more similar genetically to *B. resplendens*. In contrast, some individuals that scored as genetically pure *B. resplendens* were rather like *B. franklini* in morphology (score as high as 7 in one instance). Six of the 9 individuals in the genetically intermediate class (0) scored as pure *B. franklini* using morphological criteria, and none scored as pure *B. resplendens*.

The two scorers differed substantially

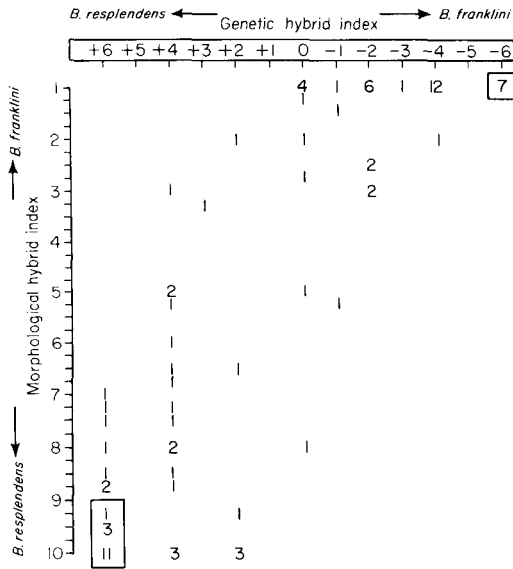
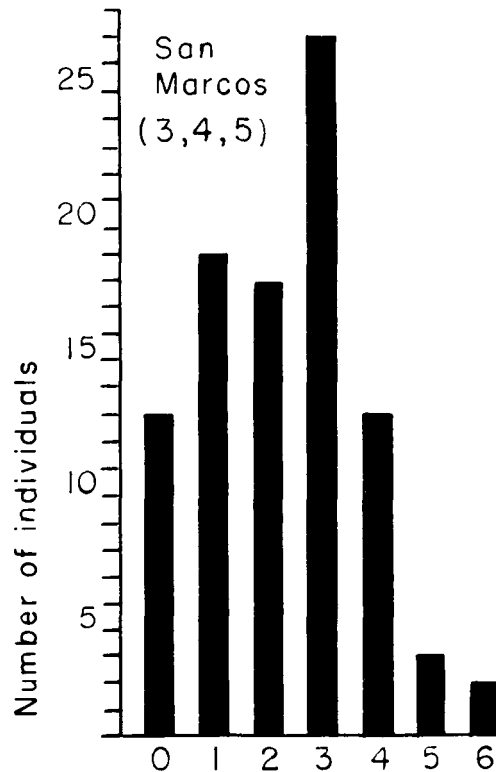


FIG. 3.—Comparison of scorings using genetic and morphological hybrid indices for individuals collected near San Marcos, Guatemala (localities 3, 4, 5). Individual scorings that are potentially pure *B. resplendens* and *B. franklini* are enclosed in boxes. All other individuals are considered hybrids.



(2 or more points) in only 9 of 63 cases. In two of these the color-blind individual failed to detect the almost diagnostic bright brick-red dorsal color of a genetically pure (by allozyme phenotype) *B. resplendens*. The remainder were hybrids (4 probable backcrosses to *B. resplendens*, 3 indeterminate).

DISCUSSION

The interspecific hybridization that we first detected in a few morphologically intermediate individuals from locality 5 proved to be more common than originally believed. Our electrophoretic analysis showed that only about 20 percent of the individuals from locality 5 can even conceivably be pure (using samples from localities 1 and 2 as standards), and there is a possibility that this figure is too high because of our inability to separate some backcrosses from parental phenotypes. Only a few potential F₁'s were found. There were heterozygote deficiencies for

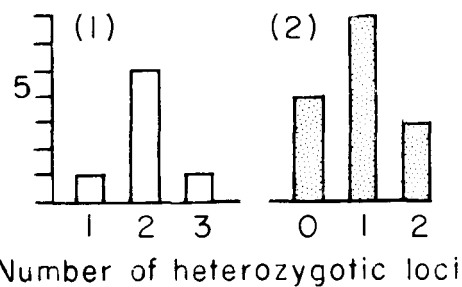


FIG. 4.—Number of heterozygotic loci per individual for 111 specimens of *Bolitoglossa* analyzed electrophoretically (based on 17 electromorphic loci). The top figure includes combined samples from localities 3, 4, and 5 (the hybrid zone). The lower figures illustrate the patterns in our reference populations 1 (*B. resplendens*) and 2 (*B. franklini*).

all three marker electromorphic loci. Only a few electromorphic phenotypes were found that were not expected from backcrosses. These results suggest that

free interbreeding between the two species is unlikely, but that a substantial amount of hybridization and backcrossing has probably occurred.

These are rather fecund species (Houck, 1977) and one or a few successful hybrid matings could have a profound and long-lasting effect in a restricted area. The 50 or so offspring expected in an F_1 hybrid generation from a single cross might remain in the area of hatching (these are terrestrial species with limited home ranges and low dispersal tendencies), and breed repeatedly with members of the two parental species. The two parental species would presumably have much higher densities than the F_1 's. Accordingly, there could be large numbers of first generation backcrosses, and in certain areas, such as in regions of local environmental disturbance, products of such crosses might actually dominate. Such may be the case in the vicinity of locality 5.

Periodic hybridizations might have occurred in the San Marcos area over a period of years. Hybridization probably is most likely in the upper cloud forest, near the upper elevational limit of *B. franklini*, and disturbance related to human activity may be an important contributing factor. Following some ecological disruption, the more ecologically generalized *B. resplendens* (an edge species) may have dispersed along habitat corridors into disturbed areas of cloud forest and there come into contact with populations of *B. franklini*. Because the generalized *B. resplendens* can use the more specialized microhabitats of *B. franklini* (see Wake and Lynch, 1976), the two species have the potential of limited microsympatry, and hybridization may occur under such circumstances.

The two parental species are largely allopatric, even in the San Marcos area, as a result of different elevational distributions. Localities 3 and 4 are also in areas of disturbance near the elevational borders of the two species, and farming, sheep grazing, and woodcutting have

provided ecological conditions conducive to hybridization in the past. We found evidence of past hybridization in these areas, but the results are less dramatic than at locality 5, where hybridization seems to be occurring at the present time.

The asymmetrical relation between our morphological and genetic hybrid indices suggests either that *B. franklini* has a more highly canalized phenotype than does *B. resplendens*, or that the *B. franklini* morphology is strongly adaptive and subject to intense stabilizing selection in a more restricted microhabitat. *Bolitoglossa franklini* can accept a large dose of alleles of *B. resplendens*, but remain phenotypically stable. In contrast, *B. resplendens* becomes phenotypically like *B. franklini* following acceptance of relatively few *B. franklini* alleles. These points are illustrated in Fig. 2. An anecdote provides some additional support for this argument. Prior to our electrophoretic analysis we photographed 39 individuals from locality 5. These were assigned on the basis of general morphological features to three classes (2 pure, one hybrid). None of the 14 individuals identified as *B. franklini* proved to be genetically pure (there were 6 probable backcrosses to *B. franklini*, 2 probable F_1 's, 5 indeterminate hybrids, and 1 probable backcross to *B. resplendens*). Of the 11 presumptive hybrids, 1 was a probable F_1 , 2 were indeterminate hybrids, and 8 were probable backcrosses to *B. resplendens*. However, 11 of the 14 presumptive *B. resplendens* were pure and the remaining 3 were probable backcrosses to *B. resplendens*.

Our data suggest that *B. resplendens* is introgressing (Anderson, 1949) into *B. franklini*. The introgression has had little detectable morphological impact thus far; accordingly we initially underestimated the extent of hybridization. *Bolitoglossa resplendens* is an edge species, and initially it may benefit relative to *B. franklini* from habitat modification. *Bolitoglossa franklini* has a phenotype

more resilient to genetic introgression, but it is also more specialized ecologically. The disturbance that we postulate as the proximate factor leading to hybridization probably will result in local extinction of both species, and since this study was initiated local conditions have changed drastically.

Endler (1977) argued the difficulty of determining whether species interactions of the sort reported here result from primary *in situ* differentiation (i.e., parapatric speciation) or from secondary contact. Evidence of the predominance of allopatric differentiation and secondary contact in plethodontid salamanders is strong (Brown, 1974; Highton and Henry, 1970; Stebbins, 1949). Further, the separation of species in this group into series of differentiated cloud forest inhabitants of the coastal mountains, and relatively less differentiated inhabitants of the upland forests of interior mountains, suggests that a re-contact of coastal and inland units has taken place. Wake and Lynch (1976) have shown that the San Marcos area along the slopes of Volcán Tajumulco has the highest local species density for salamanders in nuclear Central America. The availability of a series of apparently ideal salamander habitats in this area suggests that it might be an appropriate setting for secondary contacts, especially in view of the existence of at least partial highland connections to interior mountain masses.

The two species considered here were recognized as being close relatives from the time of their first discovery (McCoy and Walker, 1966). They are components of a superspecies complex, and such complexes have typically offered challenges to taxonomists (Mayr, 1942). Despite the natural hybridization we report here, we prefer to recognize *B. franklini* and *B. resplendens* as distinct taxa on the basis of the well defined differences in distribution and ecology (elevation and habitat) that probably functioned as effective isolating mechanisms before human disturbance accelerated in recent years. The

deficiency of heterozygotes relative to Hardy-Weinberg expectations also argues for distinct species status.

Had our analysis been limited to morphological traits alone (as has been characteristic of most analyses of hybrid zones in the past) we would have erroneously concluded that hybridization was far more restricted. Epigenetic phenomena may effectively mask much of the genetic component of hybridization, and workers should be cautioned by our results to incorporate genetic analysis before drawing conclusions concerning the nature and extent of natural hybridization.

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