

# Evolutionary Relationships in the *Bufo punctatus* Group

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**ABSTRACT:** A detailed investigation of populations of four species of Southwestern anurans (genus *Bufo*) demonstrated that *Bufo debilis*, *B. retiformis*, *B. kelloggi* and *B. punctatus* constitute a natural species group to be known as the *punctatus* group. Of the four species, *B. punctatus* Baird and Girard is the most similar to a common ancestral stock which probably existed during Mio-Pliocene time as the fossil species *Bufo suspectus* Tihen.

Reciprocal hybridization tests indicated that (1) more hybrid development occurs when crosses within the group involve *B. punctatus* as one parental species, (2) the other three members of this group are more strongly isolated genetically from each other than was first assumed from their general appearance and behavior, and from former investigations, and (3) the genetic compatibilities between members of the group are in the following order of increasing divergence from the ancestral stock: *B. punctatus*—*B. kelloggi*—*B. debilis*—*B. retiformis*.

Species divergence on a morphological basis could only be convincingly demonstrated through qualitative characteristics. Morphological differences were of smaller magnitude than previously suspected, and were found to agree favorably with the results of hybridization.

Sound spectrographic analysis revealed further evidence for including these four species in the same species group. Call frequency depends on body size, and the relationship of snout-vent length to the frequency of the basic harmonic of each call was found to be the same for each species.

The major habitat of each species in the Southwest is identified and relationships are discussed with respect to the ecology of each. The evolution of the *B. punctatus* group has been associated with the evolution of modern derivative communities of the Madro-Tertiary Geoflora within which each of the four species occurs today.

## INTRODUCTION

The *Bufo punctatus* group as herein conceived is comprised of four closely related species, *B. debilis*, *B. kelloggi*, *B. retiformis*, and *B. punctatus*. These toads are found primarily in the southwestern United States and northern Mexico. For more than a century their evolutionary relationships and taxonomic status have been variously interpreted.

*Bufo punctatus* Baird and Girard, sometimes referred to as the red-spotted toad or the desert toad, is widely distributed in western North America and has been well known since the time of its description.

Many authors have regarded *B. insidiosus* Girard (1854) as a synonym of *B. debilis* Girard (1854), but Taylor (1938) maintained *B.*

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*debilis* and *B. insidiosus* as distinct species and described *B. kelloggi* as another species of this group.

Sanders and Smith (1951) considered both *B. insidiosus* and *B. kelloggi* to be subspecies of *B. debilis*, and described a fourth member of the group as *B. d. retiformis*. Savage (1954) elevated *kelloggi* and *retiformis* to species rank and retained *B. debilis debilis* and *B. debilis insidiosus* as subspecies, an arrangement followed by Bogert (1962) and most subsequent authors.

In this investigation, possible evolutionary relationships between the four species (*B. punctatus*, *B. debilis*, *B. retiformis* and *B. kelloggi*) were analyzed by means of data obtained from hybridization experiments and studies of their structure, habitat and behavior, with special reference to the mating call.

#### METHODS AND MATERIALS

*Hybridization.*—Specimens for hybridization were collected from the following localities:

*B. debilis.*—1.0 mi S of Cochise Junction on Highway 666, Cochise Co., Arizona; and Elfrida, Cochise Co., Arizona.

*B. retiformis.*—2.2 mi E of Sells, Pima Co., Arizona; and 35 mi E of Ajo, Pima Co., Arizona (Tracy's Trading Post).

*B. kelloggi.*—22.5 mi W, 14.0 mi W, and 20.0 mi S of Hermosillo, Sonora, Mexico.

*B. punctatus.*—Lower Sabino Canyon, Santa Catalina Mountains, Pima Co., Arizona; and 1.1 mi W of Dragoon, Dragoon Mountains, Cochise Co., Arizona.

All crosses were made during the summers of 1961, 1962, and 1963. The animals were brought into the laboratory and injected with 0.5 cc Antuitrin S manufactured by Parke-Davis and Company. Eggs were stripped from the females approximately six hours after injection.

Testes extracted from the males were macerated with teasing needles in approximately 10 cc of pond water. Fifty to sixty eggs were then stripped into the sperm suspension, and after one hour the fertilized eggs were transferred to 10" × 16" white enamel pans until hatching. They were then transferred to other enamel pans in lots of approximately 40 or 80 individuals.

*Laboratory Maintenance of Larvae.*—The tadpoles were fed lettuce that had been boiled for approximately 30 minutes. Each pan was cleaned daily and fresh pond water added when needed.

In 1962 the tadpoles were maintained at a water temperature that ranged from 24 to 28 C. In 1961 and 1963 water temperatures varied more widely (ca. 22-30 C).

The embryos were staged daily and the percentages of fertilization, hatch, and metamorphosis were recorded.

After metamorphosis, the animals were transferred to aquaria containing about 300 cc of water; the aquaria were sloped to allow the recently metamorphosed larvae to move freely to and from the water. After tail absorption, the animals were taken out of the aquaria and placed in terraria. The dirt-filled bottom of each terrarium was

kept moist. Juveniles at this time were fed *Drosophila* and a small amount of water was maintained in a petri dish on the floor of each terrarium.

*Analysis of Mating Calls.*—Tape recordings of male calls were made during the summers of 1962 and 1963. All calls of *B. kelloggi* were recorded on a Nagra III portable tape recorder at a tape speed of 15"/sec, *B. debilis* calls were recorded on a Midgetape 500 at 33¼"/sec, and the calls of *B. retiformis* and *B. punctatus* were recorded on both machines.

Sound spectrograph reproductions were made on a Sonagraph (Kay Electric Co.) which has a scanning width of four inches over two frequency bands (0-6 kc and 6-12 kc). The sound spectrograph was calibrated for frequency with an audio-frequency generator, and for time by measuring the revolution time of the drum with a stop watch.

The calls were analyzed for frequency, pulse rate, duration, and structure. All frequencies were determined from sections. Pulse rates were counted from sonograms produced by playing the calls at one fourth or one half normal recorded speed. Durations were determined with a stop watch. Water and air temperatures were taken with each recording (Schultheis rapid-adjusting thermometer). Relative humidity was measured with a Bendix psychrometer.

*Analysis of Structure.*—The following morphological characteristics were measured: snout-vent length, length of tibia, length of humerus, length of femur, length of ulna, distance from angle of jaw to tip of snout, interorbital distance, length of eyelid, length of hind foot, nasal width (distance between external protuberances of nasal bones), width between outer edges of postorbital crest, width of parotoid, length of parotoid.

Females of each species were measured in the same manner, except that the parotoid gland was not measured. Mean, standard error, and range were determined for the measurements of each species for both males and females.

Skulls were prepared by the method of Sanders (1953), which involves decapitating the preserved specimens and periodically immersing the head in approximately 5 cc of Clorox to loosen adhering tissue. Skin, muscle, and connective tissue were then pulled away from the skull with forceps.

A minimum of eight skulls of each species was prepared and the following measurements were made with a stage micrometer fitted to a binocular scope:

1. Width of nasals—greatest distance between lateral edges of nasals.
2. Width of frontoparietals—greatest distance between lateral edges of frontoparietals at the posterior margin of the orbits.
3. Distance between jaw angles—measured from a dorsal position, and consisting of the greatest distance between the posterior tips of the quadratojugals.
4. Length of frontoparietals—greatest length of inner margin of fronto-

parietals; anterior limits were taken as the angles of greatest curvature of the inner margin.

5. Length of nasals—anterior tip of nasals to angle of greatest curvature of the inner margin.

6. Length of parasphenoid.

7. Width of parasphenoid.

8. Total skull length.

9. Interorbital distance—distance between proximal margins of orbits at point of suture between nasals and frontoparietals.

The data derived from measurements of females, males, and skulls of males, were reduced to the following statistics: mean, standard error, student's *t*, ratios of all combinations of variables per individual, mean ratio, standard error of ratios, and student's *t* for the ratios.

Qualitative anatomical characteristics of the skulls were also noted.

*Quantitative Relationships.*—The relative positions of *B. debilis* and *B. retiformis* for each measured variable were determined by the use of the following formula (Hubbs, Hubbs and Johnson, 1943):

$$(M_x - M_2) / (M_1 - M_2) = \text{position (P)}$$

where  $M_1 = B. punctatus$ ,  $M_2 = B. kelloggi$ , and  $M_x = B. debilis$  or *B. retiformis*. The index (I) for *B. debilis* or *B. retiformis* was then expressed as 100 (P).

## RESULTS AND DISCUSSIONS

### GENERAL MORPHOLOGY

*Color Pattern.*—The toads of the *Bufo punctatus* group (Figs. 1, 2) are predominantly small with flattened heads and tuberculate skins. These species are brightly colored dorsally, and have white to cream

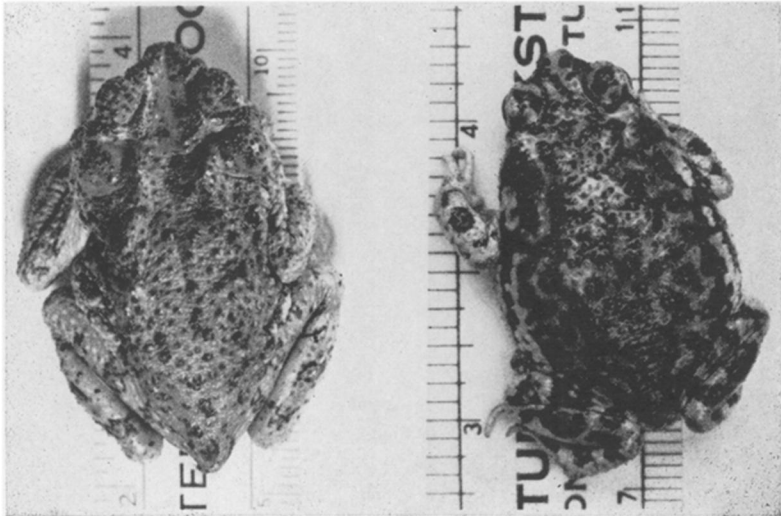


Fig. 1.—Adult representatives of two of the species of the *Bufo punctatus* group. *B. punctatus* (left), *B. kelloggi* (right).

venters which are frequently punctate. Males are smaller than females and have darkly colored vocal pouches. Pigmentation of the vocal pouch is considerably reduced in *B. punctatus*.

Ventral body spots are generally located in the pectoral region and are quite variable between different populations of the same species; they are most prominent in *B. punctatus* and *B. kelloggi*. The other two species of the group, *B. debilis* and *B. retiformis*, show considerable reduction of punctation in the pectoral region. In fact, this characteristic is generally absent in these two species except for a spot which occurs at the point of junction of the foreleg and body.

In *B. punctatus* the dorsal coloration is reddish brown to cream with small red spots which are usually on dermal tubercles. In *B. kelloggi* the dorsal coloration is a brown reticulate pattern on a lighter brown background. In *B. debilis* and *B. retiformis* the dorsum is predominantly green, broken by a black reticulate pattern, which frequently gives the effect of spotting in *B. retiformis*.

Coloration and color pattern are probably good indicators of phylogeny in this group. Savage (1954) states that the color pattern of *Bufo kelloggi* seems to represent the mode from which the coloration of the DEBILIS group was derived. Moreover, the striking green coloration in *Bufo debilis* and *B. retiformis* probably indicates a close genetic relationship between these species. The brown coloration and more blotched pattern of *B. kelloggi* may indicate its closer relationship to *B. punctatus* than to the other two species of the group.

*External Crests.*—Postocular and preocular crests are present, with postoculars extending around the anterior margin of the tympanum. These crests are obvious in *B. punctatus* and *B. kelloggi* but are re-

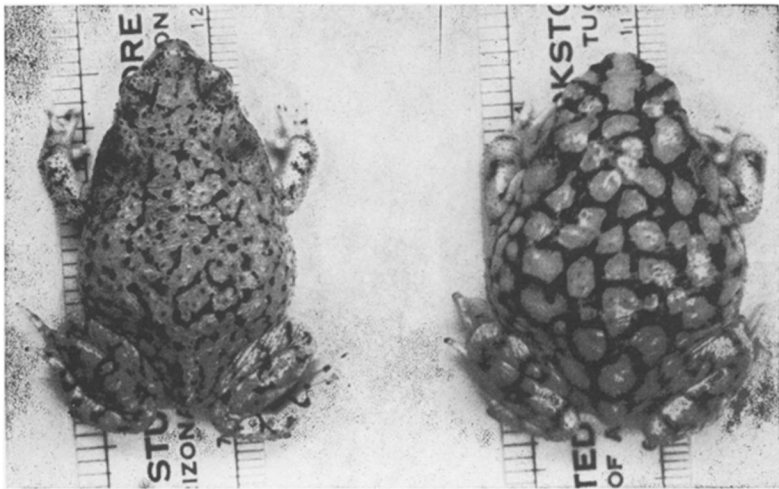


Fig. 2.—*B. debilis* (left) and *B. retiformis* (right). Note elongate parotoids and reticulate dorsal patterns.

duced in *B. retiformis* and *B. debilis*. The presence of a distinct cranial crest in *B. kelloggi* and a reduced crest in *B. retiformis* and *B. debilis* prompted Savage (1954) to state that the cranial crests in the DEBILIS group were primitive in nature and were indicative of the relationship of these species to a somewhat more generalized toad related to the present day *B. valliceps*. Cranial crests are certainly indicative of the direct relationship of *B. kelloggi* to a *B. punctatus*-like ancestor: *B. punctatus* may indeed be related to *B. valliceps* (Tihen, 1962a).

*Parotoid Glands*.—As noted by others, smallness of the parotoids also appears to be a primitive characteristic within this group. Again we assume that *Bufo punctatus* with small, round parotoids is the most primitive or oldest species within this particular group. *B. retiformis*, *B. debilis* and *B. kelloggi* exhibit rather elongate parotoids which are not as readily distinguishable from each other as they are

TABLE 1.—Means of ratios of body measurements of males of the species of the *Bufo punctatus* group

	<i>Bufo punctatus</i>	<i>Bufo retiformis</i>	<i>Bufo debilis</i>	<i>Bufo kelloggi</i>
<u>snout-vent length</u>				
parotoid width	10.98 ± 0.40	4.66 ± 0.10	4.25 ± 0.09	4.51 ± 0.15
<u>snout-vent length</u>				
postorbital crest— postorbital crest	2.87 ± 0.04	3.25 ± 0.03	3.05 ± 0.02	3.16 ± 0.03
<u>ulna</u>				
nasal	1.65 ± 0.03	1.52 ± 0.01	1.36 ± 0.02	1.28 ± 0.03
<u>humerus</u>				
parotoid length	2.85 ± 0.10	1.02 ± 0.03	0.95 ± 0.03	1.16 ± 0.03
<u>tibia</u>				
parotoid length	4.26 ± 0.14	1.63 ± 0.03	1.51 ± 0.03	1.77 ± 0.04
<u>tibia</u>				
nasal	2.56 ± 0.06	2.33 ± 0.02	2.17 ± 0.02	2.03 ± 0.03
<u>foot</u>				
parotoid width	2.08 ± 0.06	1.67 ± 0.06	1.87 ± 0.07	1.44 ± 0.06
<u>parotoid length</u>				
parotoid width	0.84 ± 0.02	1.67 ± 0.05	1.65 ± 0.02	1.38 ± 0.05
<u>parotoid length</u>				
length of eyelid	0.64 ± 0.03	1.64 ± 0.03	1.81 ± 0.04	1.35 ± 0.03
<u>interorbital distance</u>				
postorbital crest— postorbital crest	0.42 ± 0.01	0.43 ± 0.01	0.47 ± 0.01	0.45 ± 0.004

from the round bead-like parotoid of *Bufo punctatus*. This feature has caused many authors to disregard other characteristics of these species which obviously ally them to *B. punctatus*. When accurately measured and compared, the parotoid of *B. kelloggi* is more rounded in shape than that of *B. debilis* or *B. retiformis*, and hence more similar in form to the parotoid of *B. punctatus* (Table 1).

*Eggs.*—The eggs of all of the species are surrounded by two gelatinous membranes. In *Bufo punctatus* the eggs are laid separately (Wright & Wright, 1949) whereas those examined of *B. kelloggi*, *B. debilis* and *B. retiformis* are deposited in what at first appears to be a jelly mass. However, close inspection reveals the presence of gelatinous strands of eggs which are clumped together due to the agglutinative quality of the outer membranes. This is not to imply that the eggs of these species exist in rosary-like strings as typified by the eggs of the species comprising the *B. americanus* group of toads. Rather, connections between the individual eggs are the result of protoplasmic extensions which could well be an artifact of agglutination. Blair (1959) reports that the eggs of *B. debilis* are laid singly rather than in strands. It would appear from this that the condition of the egg membrane at the time of deposition is variable within the species and that genetic similarities are present between *B. debilis* and *B. punctatus* for this structural characteristic.

The eggs of *B. punctatus* and *B. kelloggi* differ from those of the other two species by being darkly pigmented from the animal pole to approximately the meridional line. The eggs of *B. debilis* and *B. retiformis* are characteristically light cream in color with a meridionally located dark stripe (Fig. 3).

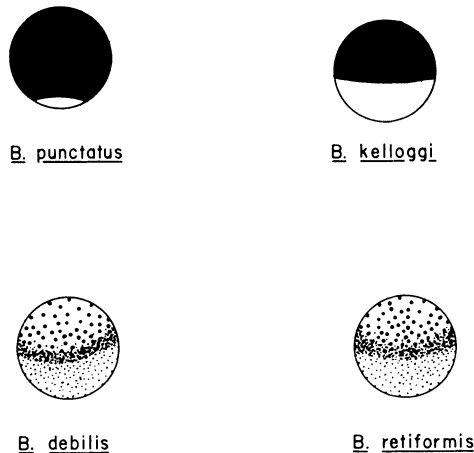


Fig. 3.—Eggs of the species of the *B. punctatus* group. Note the heavy pigmentation at the animal pole in *B. punctatus* and *B. kelloggi*, and the heavily pigmented meridional area in *B. debilis* and *B. retiformis*.

*Analysis of Structure.*—Tihen (1962a) examined the osteology of the *Bufo punctatus* group and placed the species of this group in the Caribbean section of the *B. valliceps* group. Blair (1963), in a general review of the external morphology of the North American toads, disagreed with the relationships proposed by Tihen (1962a), on the basis of the presence of a black throat skin in males of *B. debilis*, similar to the throat coloration found in the *B. americanus* group. Blair (1963) failed to observe the dark coloration of the throat of the male of *B. punctatus*, a gular pigmentation that is somewhat more reduced than in other species of the group.

The indices derived from the body measurements (Table 2), indicate that *Bufo debilis* and *B. retiformis* are more similar to *Bufo kelloggi* than they are to *B. punctatus*. The same relationships are shown by indices derived from the ratios of the body measurements (Table 3).

Analyses of linear measurements indicate that *B. retiformis* is close to *B. punctatus* in size. When measurements of the males of the four species of the group are plotted on the *B. punctatus* regression line of tibia on snout-vent length (Fig. 4), the samples of *Bufo punctatus* and *B. retiformis* overlap. The same situation occurs in the samples of *B. debilis* and *B. kelloggi*. However, no overlap occurs between the samples of *B. debilis* and *B. retiformis*. It thus seems that growth

TABLE 2.—Indices determined from the measurements of the males and females of *B. debilis* and *B. retiformis*, where *B. punctatus* = 100 and *B. kelloggi* = 0 on percentage scale 0.0 to 100.0

CHARACTER	MALES			FEMALES		% Difference between means of <i>B. punctatus</i> and <i>B. kelloggi</i>
	<i>B. debilis</i>	<i>B. retiformis</i>	and <i>B. kelloggi</i>	<i>B. debilis</i>	<i>B. retiformis</i>	
snout-vent	21.30	72.00	33.30	19.23	44.58	29.30
ulna	22.91	58.65	46.00	19.23	46.51	38.10
humerus	17.30	46.72	44.60	21.50	56.16	40.40
tibia	25.83	57.27	43.80	20.74	46.81	36.60
femur	25.53	66.16	42.60	16.35	52.43	39.00
foot	30.80	65.88	47.80	17.07	54.55	33.70
interorbital distance	39.17	53.80	32.50	26.77	23.32	33.40
angle of jaw to snout	20.77	50.39	36.70	17.66	36.40	36.00
postorbital crest—postorbital crest	22.68	50.75	28.60	19.31	36.35	37.00
nasal width	29.65	61.97	29.80	30.51	47.80	32.70
length of eyelid	8.55	54.01	35.00	10.43	40.65	35.40
parotoid length	-156.05	-221.50	25.80			
parotoid width	-19.47	148.95	17.50			
mean index	6.84	43.46	36.15	19.89	44.14	32.58



of extremities in the males of the entire group proceeds arithmetically with snout-vent change. On the other hand, when measurements of body extremities are expressed as a percentage of the snout-vent length, *Bufo retiformis* and *B. debilis* are similar in body proportions (Fig. 5).

On the basis of general morphology and those characteristics not related to size (*e.g.*, eggs, crests, color pattern), *Bufo kelloggi* is most similar to and obviously more closely related to *B. punctatus* than it is to *B. debilis* or *B. retiformis*. On the basis of size, one would expect a close relationship between *B. punctatus* and *B. retiformis* (Figs. 6, 7). However, size appears to be closely associated with adaptation at the specific level and is not a consistent evolutionary trend within the group. In view of the apparent ecological restrictions of these

TABLE 3.—Indices of the ratios of body measurements of males of *B. debilis* and *B. retiformis* where *B. punctatus* = 100.0 and *B. kelloggi* = 0.0

RATIO	<i>B. debilis</i>	<i>B. retiformis</i>	% difference between ratio of <i>B. punctatus</i> and <i>B. kelloggi</i>
<u>snout-vent length</u>			
postorbital crest— postorbital crest	34.89	-33.26	9.18
<u>ulna</u>			
nasal	21.66	65.06	22.90
<u>tibia</u>			
width of nasal	25.93	56.48	20.30
<u>interorbital distance</u>			
postorbital crest— postorbital crest	-47.03	56.45	8.17
<u>snout-vent</u>			
parotoid length	-20.81	-13.50	50.70
<u>humerus</u>			
parotoid length	-12.83	-8.50	59.40
<u>tibia</u>			
parotoid length	10.31	-5.80	58.50
<u>foot</u>			
parotoid width	66.32	35.47	32.50
<u>parotoid length</u>			
parotoid width	-119.57	-51.87	17.00
<u>parotoid length</u>			
length of eyelid	-65.89	-42.24	17.70
MEAN	-12.76	5.83	29.54

species it seems probable that characteristics related to size represent mutational differences which have occurred where environmental pressures could be met with a minimum of major evolutionary change. In other words, the ecological conditions that prevailed during the divergence of these species since the Miocene were relatively severe,

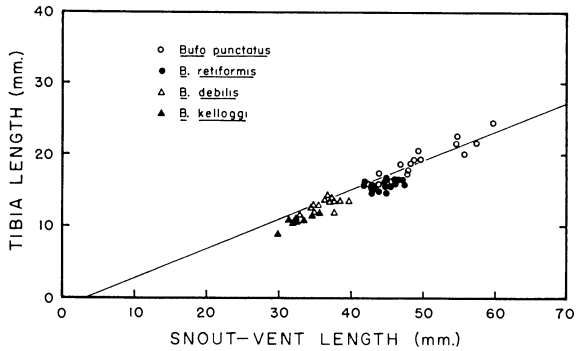


Fig. 4.—Comparison of tibia to snout-vent length of individuals of the *Bufo punctatus* group. The regression line is for a separate sample of *B. punctatus* where  $N = 10$ ,  $Y = -1.40 + 0.41 X$ , and  $r = 0.999$ .

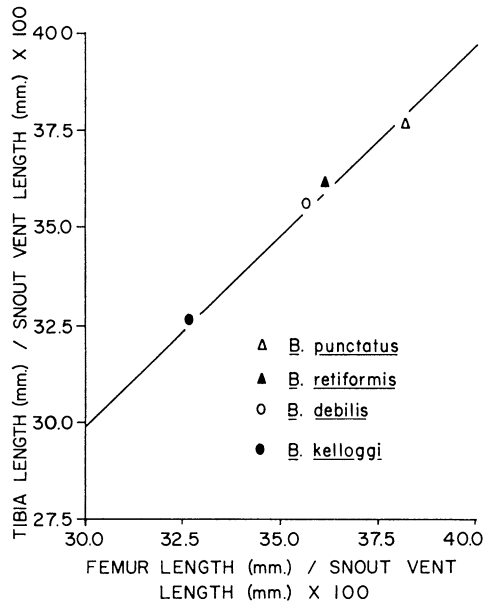


Fig. 5.—Regression of mean ratio, tibia and femur length, expressed as a percentage of snout-vent length. Regression equation,  $Y = -0.04 + 0.983 X$ , where  $r = 0.996$ ,  $P = 0.01$  and  $N = 4$ .

but the structural changes necessary for their continued maintenance within their specific environments appear to have been by comparison uncomplicated.

#### CRANIAL MORPHOLOGY

In general, the skulls of the species of the *B. punctatus* group are flattened in the vertical axis with prominent nasals which flare laterally (Figs. 8, 9). The skulls of *B. punctatus* and *B. kelloggi* are usually heavily ornamented with dermal bone which sometimes obscures the lateral edges of the frontoparietals and gives rise to the inconspicuous cranial crests found in these species. The parasphenoid extends anteriorly to the level of junction of the palatine with the sphenethmoid; its lateral wings extend almost to the lateral edges of the pterygoid. The squamosal is rounded at its suture with the prootic in *B. debilis* and *B. retiformis*; it is usually straight in *B. punctatus* and *B. kelloggi*.

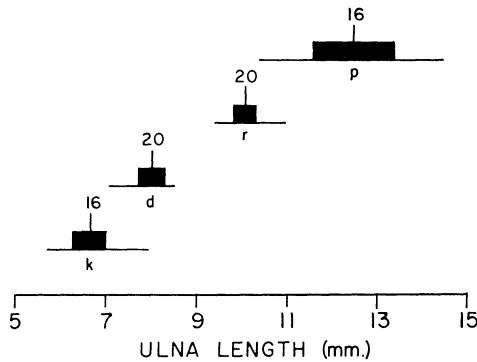


Fig. 6.—Frequency distributions of ulna lengths of *B. punctatus* (p), *B. retiformis* (r), *B. debilis* (d), and *B. kelloggi* (k). Rectangles represent 95% confidence intervals; sample size is given above the mean.

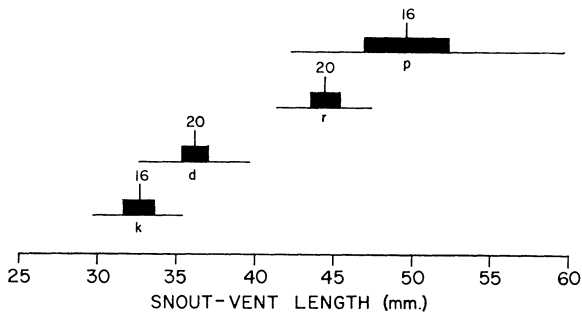


Fig. 7.—Frequency distributions of snout-vent measurements of the males of the four species; rectangles represent 95% confidence intervals. Number above the mean indicates sample size. *B. kelloggi*, *B. debilis*, *B. retiformis* and *B. punctatus* are indicated by k, d, r, and p, respectively.

*B. punctatus* usually has an extended flange on the posterolateral edges of the maxilla—a trait sometimes seen in the skulls of *B. kelloggi*, but not found in the skulls examined of *B. debilis* or *B. retiformis*.

To obtain quantitative values, nine measurements were made on the skulls of each of the four species (Table 4). The ratios of all possible combinations of measurements were also computed. These variables were then compared statistically in the manner described for the other linear measurements.

Of the 36 ratios computed, only one (total width/total length of the skull) was statistically different ( $p \leq .05$ ) for all species (Table 5). This ratio shows that the larger species within the group have

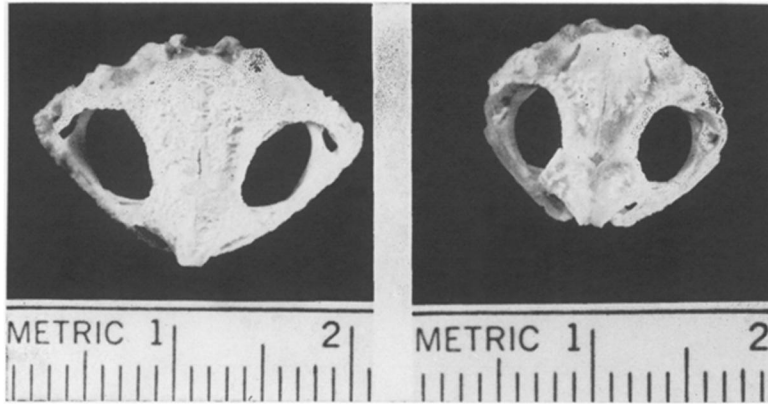


Fig. 8.—Dorsal aspect of the skull of *B. punctatus* (left) and of *B. kelloggi* (right).

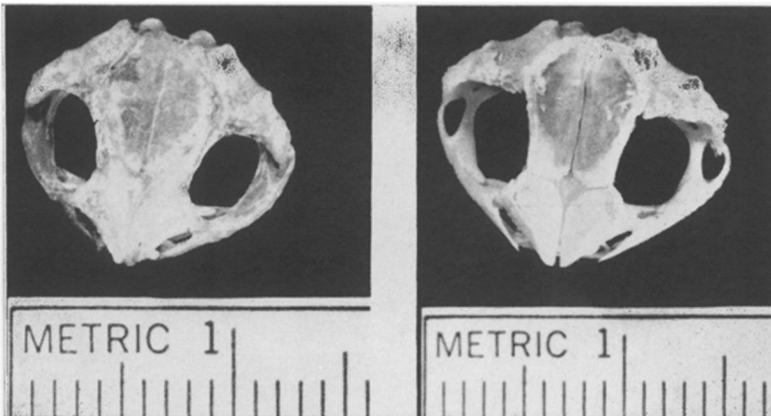


Fig. 9.—Dorsal aspect of the skull of *B. debilis* (left) and of *B. retiformis* (right).

proportionally wider skulls. This seems to be another growth phenomenon which is characteristic of the entire group rather than of any particular species within the group.

The ratio of frontoparietal width over length, a quantitative determination of a qualitative trait considered reliable by Tihen (1962a), is statistically unreliable as a character to be used in the separation of *B. punctatus* from the other species in the *B. punctatus* group. However, the mean of this ratio is quite distinct between the other species, *B. debilis*, *B. retiformis*, and *B. kelloggi*. Also *B. punctatus*

TABLE 4.—Means and standard errors (mm) for skulls of the species of the *Bufo punctatus* group

N	<i>Bufo punctatus</i> 8	<i>Bufo retiformis</i> 10	<i>Bufo debilis</i> 10	<i>Bufo kelloggi</i> 8
Measurement inter orbital width	4.96±0.10	4.59±0.10	4.36±0.09	4.02±0.12
angle of jaw—angle of jaw	19.18±0.46	15.18±0.16	13.18±0.18	12.10±0.35
width of frontoparietal	5.82±0.15	5.31±0.06	5.26±0.08	4.82±0.12
length of frontoparietal	7.75±0.24	7.45±0.12	6.39±0.15	6.70±0.26
length of nasal	3.90±0.18	4.19±0.11	3.77±0.08	3.32±0.14
total skull length	13.19±0.34	12.05±0.18	10.97±0.12	10.87±0.21
width of nasal	8.76±0.16	7.15±0.10	6.70±0.09	6.08±0.20
length of parasphenoid	8.32±0.15	7.15±0.11	6.16±0.09	6.14±0.16
width of parasphenoid	11.02±0.26	8.93±0.15	8.98±0.14	7.99±0.25

TABLE 5.—Means of the ratios of skull measurements of the species of the *Bufo punctatus* group

	<i>B. punctatus</i>	<i>B. retiformis</i>	<i>B. debilis</i>	<i>B. kelloggi</i>
width of frontoparietal	0.753±0.018	0.715±0.018	0.826±0.016	0.723±0.017
length of frontoparietal				
total skull width	1.456±0.021	1.260±0.013	1.201±0.010	1.114±0.024
total skull length				

is more similar to *B. kelloggi* with respect to this trait than it is to either *B. retiformis* or *B. debilis*.

The absence of the exposure of the sphenethmoid between the frontoparietals and nasals is supposedly conspicuous in *B. punctatus* with respect to the other species in this group (Tihen, 1962a). As evidenced by the skulls that we examined, this does not seem to be the case. The sphenethmoid is frequently exposed in each of the species—a trait which depends largely upon the amount of dermal ornamentation of a particular skull (see Figs. 8, 9).

Of the species discussed here, *B. kelloggi* seems to be the closest relative of *B. punctatus*. This is indicated by the shape of the frontoparietal, and by other less obvious morphological characteristics such as the shape of the suture line between the prootic and squamosal and the presence of a laterally extended flange on the posterior border of the maxilla.

The skulls of *B. retiformis* and *B. debilis* are quite similar in appearance. However, the skull of *B. retiformis* is much larger than that of *B. debilis* and there is more dermal ornamentation in the latter species.

In summary it can be stated that the quantitative morphological

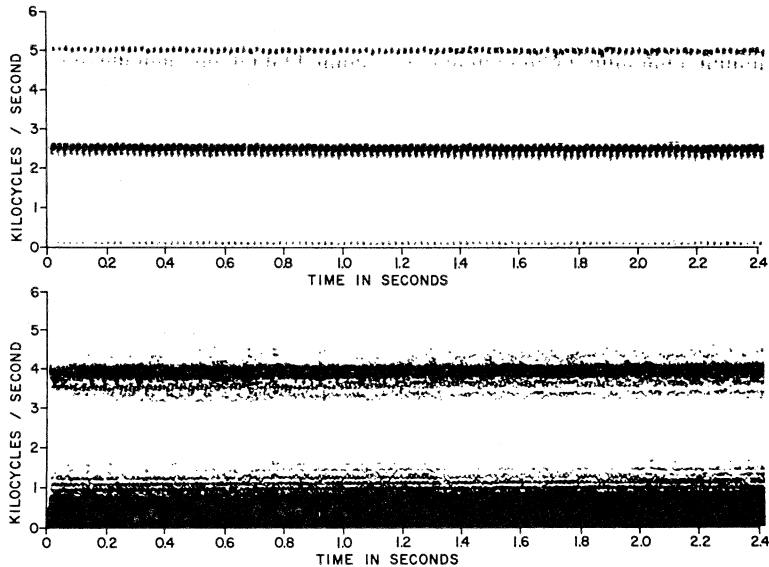


Fig. 10.—Sound spectrograms of the calls of *B. punctatus* (above) and *B. kelloggi* (below). Lowest dark lines in both cases are caused by background noises. In *B. punctatus* both primary and secondary harmonics are visible. In *B. kelloggi* the dominant harmonic occurs at approximately 4 kc. The secondary harmonic in the call of *B. kelloggi* occurs above 6 kc and is not shown in this figure.

characteristics of the skulls of these species are indicative of group affiliation. Those characteristics which are treated qualitatively have probably resulted from species divergence within the group and can therefore be used to show interspecific relationships.

#### MATING CALLS

*Analysis of Sonograms.*—In structure, the calls of the *Bufo punctatus* group are very similar (Figs. 10, 11). Each call consists of two dominant frequency bands and several lesser harmonics. The lower band is referred to as the fundamental and from it the emphasized frequencies given in Tables 6 and 7 were determined. The pulses in each species tend to fall into groups of two, with the frequency of the first part of each pair being slightly lower than the second.

Major differences in the calls of the species of this group can be noted from the sonograms and in the field. In the call of *B. retiformis* there is an initial sharp chirp, which due to its short duration could hardly be termed a "rising crescendo" as described by Savage (1954). The distinctiveness of the call of *Bufo punctatus* is as obvious to the ear as it is when subjected to sonographic analysis; it has a melodious, somewhat ventriloquistic, floating sound which does not seem harsh or sharp as do the calls of other members of this group when heard from short distances. The calls of *B. debilis* and *B. kelloggi*, although

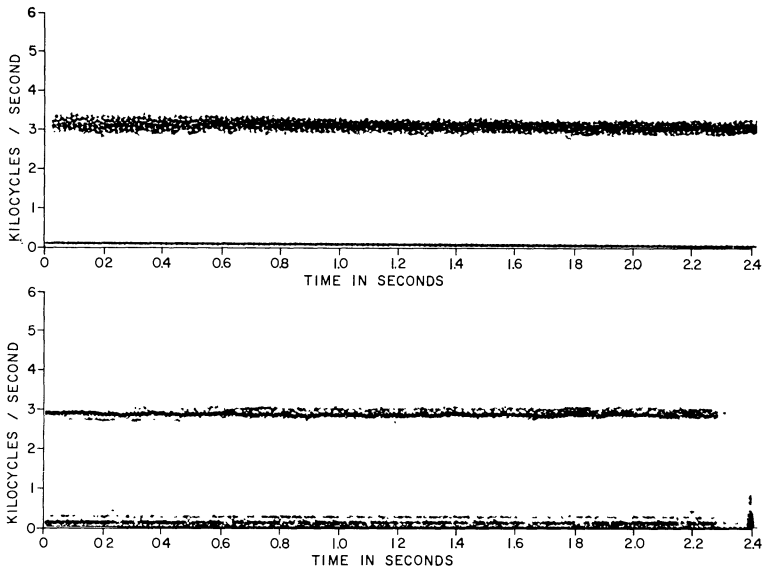


Fig. 11.—Sound spectrograms of the calls of *B. debilis* (above) and *B. retiformis* (below). Primary harmonics are shown for each species. Secondary harmonics in these particular calls occur at frequencies above 6 kc and are not shown in this figure.

TABLE 6.—Meristic features of the mating calls showing variation due to population differences and physical factors (water temperature, air temperature) of the environment. All values are means; range is also given for call duration. AT = air temperature; WT = water temperature; KC = kilocycles/sec; ST = surface temperature (soil)

	No.	AT	WT	Emphasized frequency	Pulse rate	Mean duration	Range
<i>B. punctatus</i>							
Tucson Mts., Pima Co., Ariz.	3	24.6	24.4	2.32	48.7	4.66	(2.0-6.0)
Scottsdale, Ariz.*	1	31.0	28.0	2.42	59.0	3.80	(2.9-7.0)
Austin, Texas*	3	24.0	26.0	2.59	52.3	8.26	(7.2-9.7)
<i>B. debilis</i>							
Cochise, Cochise Co., Ariz.	3	18.4	23.0	3.10	112.0	3.78	(2.65-4.70)
Benson, Ariz.	4	18.2	20.2	2.914	96.5	5.09	(2.4-10.35)
Valentine, Texas*	2	25.0	23.0	3.25	120.0	3.50	(2.6-4.9)
Silver, Texas*	2	23.0	23.0	3.12	122.0	4.00	(3.2-5.4)
Throckmorton, Texas	3	14.0	19.0	3.33	112.3	5.73	(4.1-7.3)
<i>B. retiformis</i>							
2.2 mi E Sells, Pima Co., Ariz.	4	26.0	25.2ST	2.65	212.0	3.01	(2.15-4.2)
35 mi E Ajo, Pima Co., Ariz.	3	26.8	25.4ST	2.81		2.44	(1.6-3.8)
<i>B. kelloggi</i>							
20 mi S Hermosillo, Son., Mexico	2	28.0	28.4	4.35(1)	136.0	3.20	(2.4-4.7)
18 mi W Hermosillo, Son., Mexico	7	27.2	27.2	4.001	131.7	3.39	(1.4-5.2)

\* Calls reported by Blair, 1956.



quite distinct when viewed in the light of sonographic analysis, often seem similar when heard in the field; they lack the slow pulsations observed in the call of *B. punctatus*.

Blair (1956) describes the call of *B. debilis* as a "high frequency buzz, which can easily be confused with the call of the narrow-mouth frogs (*Microhyla*).” He states that the dominant frequency of the call of *B. debilis* is higher than the frequency of the call of any other toad in the Southwest. The data obtained by Blair (1956) from *B. debilis* at three localities in Texas agree favorably with those presented here for the same species from Cochise Co., Arizona, at a water temperature of 23 C (Table 6). The calls of *B. debilis* from Cochise Co. ranged from 3,138 to 3,024 cps. The call frequencies reported by Blair from the Valentine and Silver populations range from 3,300 cps to 3,065 cps, respectively. The calls reported here from Benson, Arizona, were recorded at water temperatures comparable to those in Blair's Throckmorton data, yet they differ markedly in both frequency and pulse rate.

The emphasized frequencies as presented by Bogert (1962) for *B. debilis* range from 3,500 to 3,900 cps. These values are almost out of the range shown here for the same species (Fig. 12). This demonstrates not only that the calls of individual toads within a local breeding population are extremely variable, but also that there can be a

TABLE 7.—Summary of call data, as graphed in Figures 12 and 13

	Frequency (kc/sec)	Pulse rate (pulse/sec)	Duration (sec)
<i>B. punctatus</i>			
(N)	7	7	7
maximum	2.700	53	9.7
minimum	2.262	46	2.0
mean	2.46 ± 0.06	51.7 ± 0.61	6.08 ± 0.87
<i>B. retiformis</i>			
(N)	7	4	7
maximum	3.007	220	4.2
minimum	2.714	204	2.2
mean	2.837 ± 0.04		2.78 ± 0.22
<i>B. debilis</i>			
(N)	14	14	14
maximum	3.600	128	10.4
minimum	2.795	84	2.6
mean	3.240 ± 0.03	110.2 ± 3.4	4.56 ± 0.4
<i>B. kelloggi</i>			
(N)	8	9	9
maximum	4.350	144	5.2
minimum	3.818	124	1.4
mean	4.044 ± .09	132.7 ± 2.98	3.28 ± 0.35

marked variation between the calls of conspecific animals from different geographic areas.

Obviously there is the possibility that wide variation in call frequency within a species is representative of subspecific (or ecotypic) variation. This is the case in *B. punctatus* (Table 6). Individuals of this species from the Texas population have call frequencies which are somewhat different from those of *B. punctatus* of the undescribed subspecies in Arizona, although not of the order of magnitude noted between the species of the *B. punctatus* group.

Blair (1956) suggests that "[*B. punctatus*] seems to have no close relatives among United States toads." However, the data presented here show that, in very important respects, *B. punctatus* has a life-form and mating call that are very similar to those of the species of Blair's supposedly distinct "*B. debilis* group."

Although "relatively little divergent" in Bogert's (1962) statement for his data, the mating calls of *B. debilis* and *B. kelloggi* are significantly distinct at the 95% confidence level with respect to both frequency and duration (Fig. 12). Further, it is to be emphasized that all species in this group differ in at least two characteristics of the calls.

In these species frequency varies inversely with body size (Fig. 13). This indicates that the vocal mechanism controlling call frequency has changed as a function of body size during the evolution of the group. This is what one would expect in view of the importance of size as an isolating mechanism in anurans (Lowe, 1954). A nocturnal anuran should have some vocal mechanism that corresponds to size, and especially in a closely related group such as the *B. punctatus* group where change in body size has been such a conspicuous aspect of its evolution.

Porter (1964) has stated that pulse rates of the calls of the species

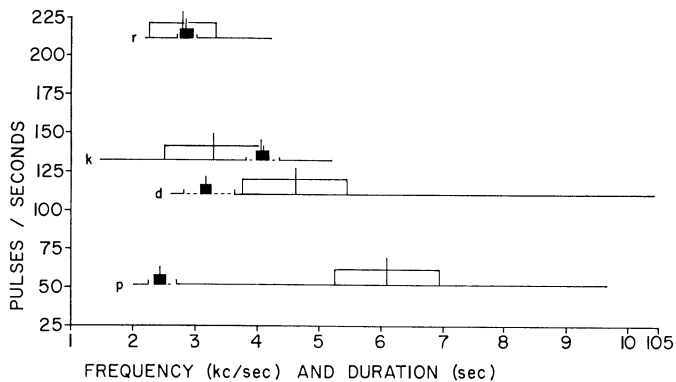


Fig. 12.—Comparison of call duration (open rectangles) and call frequency (solid rectangles) with mean pulse rate in *B. punctatus* (p), *B. debilis* (d), *B. kelloggi* (k), and *B. retiformis* (r). Data from Table 7.

of the *Bufo valliceps* group vary inversely with body size. This is not the case in the *B. punctatus* group (Table 7). Pulse rate should be dependent upon the tension of the vocal mechanism and the pressure of the air as it is released from the vocal apparatus. Where the call is an important isolating mechanism between two sympatric species such as *B. retiformis* and *B. punctatus*, where there is an overlap of the effective adult size (Fig. 7) and consequently the call frequency (Fig. 12), a second mechanism such as pulse rate which does not correspond to body size would have unquestioned selective potential.

Call duration, another easily measured characteristic of the anuran mating call, is probably in part controlled by body size. However, this is an aspect of the call that one would clearly expect to be affected by the momentary state or degree of excitement of the individual.

Until more evidence is accumulated, relatively little can be said with assurance about the importance of different characteristics of the call with respect to mate attraction and chorus formation. Bogert (1962) points out that pulse rate is probably more important as an isolating mechanism between the species of this group than are other quantifiable characteristics of the call such as duration and frequency. Our data show that the call frequencies of the species investigated are quite distinct at the 95% level of confidence although some overlap does occur, as would be expected in view of the frequency-body size relationship pointed out earlier. Call duration is more variable than frequency and pulse rate (Fig. 12) and is probably of lesser importance as an isolating mechanism. However, if this aspect of the call reflects the physiological state of excitement or "readiness" of the individual as proposed above, it could be a major selective influence within the species.

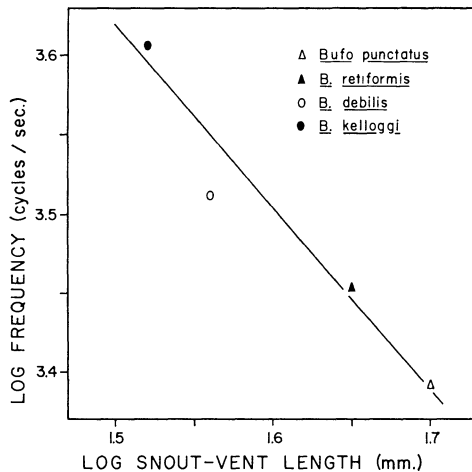


Fig. 13.—Regression of log mean call frequency on log mean snout-vent length where  $\log Y = 5.23 - 1.077 \log X$ ,  $r = 0.97$ ,  $p = 0.05$  and  $N = 4$ .

*Field Observations.*—It is assumed that one function of the anuran call is to provide a mechanism whereby spatial isolation of sympatric species can be readily acquired at the breeding site. The accumulation of individuals of both *B. retiformis* and *B. kelloggi* into their separate choruses was studied in the field at localities of sympatry in Sonora, Mexico, during the summer monsoons of 1961-1963. The principal area of study was immediately on the north and south sides of the Kino Bay Road between Hermosillo and 22.5 mi (by road) W of Hermosillo, where Lowe and Zweifel first discovered *B. kelloggi* and *B. retiformis* sympatric in 1958, as reported by Bogert (1962). We have verified repeatedly the findings of Lowe and Zweifel that calling and pair formation (amplexus) in *B. retiformis* ordinarily take place on dry, damp, or wet ground, very often at remarkable distances from the water's edge (up to ca. 18 m).

We also note *B. kelloggi* to be restricted to calling over a much narrower range of environmental temperature and humidity than *B. retiformis*, and further, that it calls predominantly during or immediately after a rain. All of this is to be expected in view of the more southerly thornscrub distribution of *B. kelloggi* and more northerly desertscrub distribution of *B. retiformis*. The range of ambient temperatures of calling males of *B. kelloggi* is 27.2-28.5 C for three summers. The range for *B. retiformis* for that same period is 26.2-30 C.

*Bufo debilis* was found to call at a somewhat lower temperature than the other species in the group. Lower temperatures are provided by the grassland climate and nature of the habitat. The minimum body temperature obtained from calling males of this species over six summers was 17.4 C (on wet soil at 17.8 C). The air temperature at points where males of this species were calling ranged from 16 to 20 C during the three-year period 1961-1963, and is exceeded only by our data for one year (1954), during which the maximum reached was 21 C.

At various localities in Cochise Co., Arizona, and in southern New Mexico, *B. debilis* is often observed to call at the bases (or butts) of bunch grasses in the usual Short-grass Plains habitat. At Benson, Arizona, the westernmost station investigated by us (Desert-Grassland), it was found calling up to 1 m away from the nearest vegetation. In "first-nighter" situations in general, individuals seem less careful regarding their exposure while calling. This species calls during or immediately after a summer rain on the wet ground or in shallow water of temporary rain-formed pools.

*Bufo punctatus* generally calls when out of the water, frequently on exposed boulders or rocks at the water's edge. Although large choruses have been found, it frequently calls while alone or in small groups of up to 10 or so males. In southern Arizona and adjacent areas the males of *B. punctatus* call where and when air temperatures range from 19 to 29 C, but seldom when water temperatures are in excess of 27-28 C.

The frequency of the call of *B. debilis* at Cochise and Benson,

Arizona, increases by 186 cps with a change in water temperature of 2.8 C (20.2-23.0 C) (Table 6). *B. retiformis* 2.2 mi E of Sells, Arizona, at a water temperature of 25.2 C has a mean call frequency of 2.85 kc. From this it is clear that if sympatric, and therefore at comparable body temperatures, the calls of these two species would be quite different. All of the species show some overlap with regard to call duration (Fig. 12), which probably varies with the physiological state of the organism as influenced by extrinsic factors.

In summary, it can be said that calls of the members of the *Bufo punctatus* group are quite similar in structure, but differ in two or more of the three major aspects of the call — frequency, duration, and pulse rate. Certain characteristics of the call such as an initial rise in frequency and the overall frequency can be used after some practice to distinguish the species in the field.

Within the group, pulse rate is probably the strongest species-specific characteristic (Fig. 12), and hence an isolating mechanism of primary importance. Frequency, on the other hand, is correlated with body size. Call frequencies would therefore be similar in those cases where there is similarity of body size between two species within the same group. Due to the importance of body size as an isolating mechanism (Lowe, 1954), frequency has developed as a concomitant secondary isolating mechanism in this group of anurans.

The behavior of the males of these species when calling is basically similar, except for the widely sympatric *B. retiformis* and *B. kelloggi*. *B. retiformis* tends to call at points away from the edge of the water, whereas *B. kelloggi* calls from the water or from its edge. *B. kelloggi* also seems to be restricted to a narrower range of environmental conditions for reproduction than is *B. retiformis*, and *B. debilis* calls at a considerably lower temperature than the other members of this group.

*Speculations on Origins.*—It certainly appears that the call of *B. punctatus* or of a *B. punctatus*-like species could well give eventual rise to those of *B. kelloggi*, *B. debilis* and *B. retiformis*. Furthermore, this would seem reasonable in a species existing in the formerly more widespread subtropical scrub of western North America which was evolving toward the Sonoran Desert of today (Axelrod, 1950). In this situation of increasing aridity and seasonal climatic extremes the anuran population might well have evolved toward smaller body size as an adaptation toward efficiency for a burrowing lifeform. Such a trend in body size alone would in turn give eventual rise to a species call of higher frequency.

During the development of the grasslands of middle latitudes of North America during Mio-Pliocene time, it appears that a small grassland species evolved in ecological as well as spatial isolation and it is likely that the resulting call did not diverge greatly from that of the parental species. This was the species represented by *B. debilis* of the Recent. For while the call of *B. debilis* presently found in grassland is distinct — with respect to pulse rate — from the call of *B.*

*kelloggi* which is found in the subtropical scrub of coastal Sinaloa and southern Sonora, the difference is by no means of the same order of magnitude as the difference exhibited between *B. debilis* and the other species in the group.

It appears that, as a result of progressively increasing aridity, a portion of this grassland population (at lower elevation and more westerly position) was later differentiated in western North America in an area of interspersed grassland and subtropical scrub which was destined to become the Sonoran Desert as we know it today (see Axelrod, 1950). *B. retiformis* undoubtedly evolved in this type of situation.

It appears certain indeed that *B. kelloggi* evolved in the coastal thornscrub, as represented on the Gulf Coast (Gulf of California), where it remains restricted today in modern derivative communities of the Madro-Tertiary Geoflora that are located on the Plains of Sonora-Sinaloa directly south of the Sonoran Desert. The geographic position and areal extent of the thornscrub (= Thorn Forest of Gentry, 1942) at that time is conjectural.

*Bufo punctatus*, which represents a close vertical derivative of the ancestral type (probably represented by the fossil *Bufo suspectus* Tihen), has become a reasonably successful species, well-adapted to arid and semiarid lands, yet more or less restricted to riparian and rocky, and/or flood-plain situations throughout its wide range across grasslands and deserts in peripheral communities of woodland and

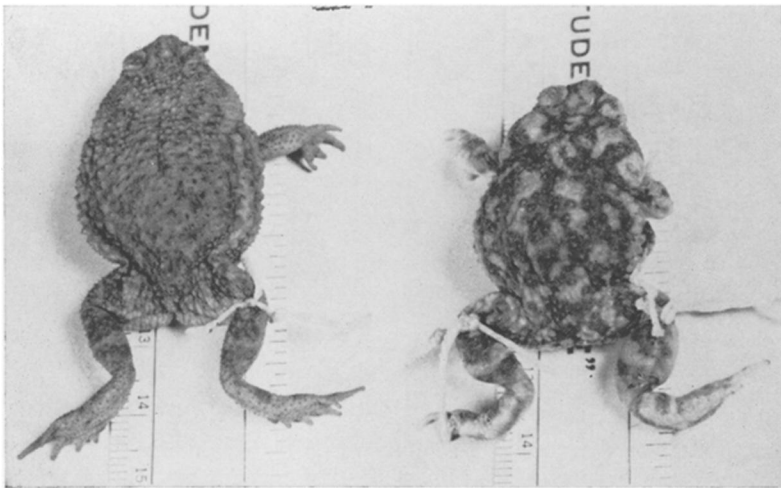


Fig. 14.—Left, adult female of *Bufo kelloggi* (UIMNH 40575), reported as intergrade between *B. kelloggi* and *B. retiformis* by Sanders and Smith (1958). Right, hybrid adult female, *B. kelloggi* × *B. retiformis* (MVZ 58793), collected by W. J. Reimer and R. G. Zweifel at 11 mi NW of Vicam, Sonora, Mexico (Reimer, 1955).

subtropical thornscrub. Today *B. punctatus* represents an ecologically more restricted relic of its more moist-adapted and undoubtedly more widely spread tropical or subtropical ancestor.

During the sympatric secondary contact of *B. kelloggi* and *B. retiformis* which persists today where desertscrub and thornscrub variously intermingle in southern Sonora, the much more distinctive call of *B. retiformis* (as compared to *B. debilis* which is not greatly different from *B. punctatus*), and its marked physical differentiation from *B. kelloggi* undoubtedly evolved further under subsequent selective pressures favoring such a definite character displacement (see Brown and Wilson, 1956). Occasional hybrids are still formed today in this area of secondary contact (Fig. 14).

#### HYBRIDIZATION

##### NATURAL HYBRIDS

The mating sites studied in the present investigation have been essentially unaffected by man-made ecological disturbance on a large scale where sympatry or approximate sympatry occurs. Of the four species involved, the following sympatric contacts exist today:

1. *B. punctatus* and *B. debilis*, over much of the range of *B. debilis*.
2. *B. punctatus* and *B. kelloggi*, in the northern part of the range of *B. kelloggi*.
3. *B. punctatus* and *B. retiformis*, over much of the range of *B. retiformis*.
4. *B. kelloggi* and *B. retiformis*, in the southern part of the range of *B. retiformis*.

In the first three cases the species remain essentially isolated through habitat selection. *B. punctatus* is largely restricted to rocky, riparian situations within the areas of the other three species. *Bufo retiformis*, *B. debilis*, and *B. kelloggi* are restricted for the most part within areas properly termed desertscrub, grassland, and thornscrub, respectively (see Lowe, 1964). The last case (*B. retiformis* and *B. kelloggi*) results in a fully sympatric contact on a microhabitat level, and reproductive isolation here seems to be maintained primarily through behavioral mechanisms.

In view of the wide areas of sympatry that are involved and the large numbers of specimens that have been collected, it is notable that extremely few putative natural hybrids or intermediates have been reported; these total approximately nine individuals (Smith and Chrapliwy, 1958; Riemer, 1955). In general, amphibian hybrids exhibit almost complete intermediacy (Twitty, 1936). We have examined the nine specimens noted above, and of these only one (MVZ 58793, collected by W. J. Riemer and R. G. Zweifel) appears to be intermediate (Fig. 14). The others are typical of *B. kelloggi* and show no resemblance to *B. retiformis* except through those morphological characteristics which warrant their allocation to the same species group. The specimen referred to as an intermediate (Fig. 14) exhibits several distinctive features that include an intermediate color

pattern, an intermediate head shape more similar to *B. retiformis*, and a scoliotic condition of the spine that frequently occurs in inter-specific anuran hybrids (Blair, 1963). This is indeed a hybrid and one of the specimens referred to by Bogert (1962) as *Bufo kelloggi*.

The virtually complete absence of natural hybrids in this case is taken as further evidence that reproductive isolation between these species is essentially complete and that isolation does not exist merely on some lesser level (*e.g.*, physiological inhibition of hybrid larvae due to temperature stress). Physiological differences between species segregate in the same manner as morphological ones (Volpe, 1952), but they may not effectively promote isolation between these particular populations for the following reasons: (1) no temporal difference between breeding seasons is present due to the necessity of breeding opportunism in arid and semi-arid monsoonal environments; (2) no thermal differences due to spatial separation in breeding aggregations (of a degree preventing hybrid development) have been observed nor, in fact, appear probable.

#### EXPERIMENTAL HYBRIDS

Whenever natural hybridization is limited, or where more quantifiable data are desired, laboratory hybridization can be reasonably employed as a "crude reflection of total genetic difference and hence of evolutionary affinity" (Blair, 1963).

The toads of the *Bufo punctatus* group were earlier referred to as the *debilis-punctatus* group (Camp, 1917) on the basis of skull morphology, and Group II (Baldauf, 1959) on the basis of general osteology. They have been referred to as the *Bufo debilis* group (Blair, 1963) and the Caribbean section of the *Bufo valliceps* group (Tihen, 1962a). These species have been previously investigated through hybridization by Blair (1963); he considered *B. debilis*, *B. retiformis* and *B. kelloggi* to be related to a "northern line" consisting principally of the *B. boreas* and *B. americanus* groups, and allocated *B. punctatus* to a "southern line" consisting principally of the *B. valliceps* group.

Hybridization as evidenced by results from crosses in this laboratory (Table 8) indicates reciprocal metamorphosis between all members of the *B. punctatus* group. In general, data on anuran hybridization

TABLE 8.—Results of laboratory hybridization during the summers of 1961, 1962, and 1963. Ad=adult, SAd=subadult, M=metamorphosis

Females	Males			
	<i>punctatus</i>	<i>retiformis</i>	<i>debilis</i>	<i>kelloggi</i>
<i>punctatus</i>		Ad	SAd	SAd
<i>retiformis</i>	M*		M	M*
<i>debilis</i>	SAd	M		M
<i>kelloggi</i>	M	M	M	

\* Data partly incomplete due to failure of laboratory temperature control.



where reciprocal metamorphosis occurs is taken as evidence for the allocation of species into species groups.

It appears obvious that the gametes of *Bufo punctatus* contain a general array of nuclear and cytoplasmic materials essential for development of the other species and hence exhibit a more generalized genetic composition. This is thought to be characteristic of a more primitive ancestral species in this particular genetic line. One would assume that an ancestral species would have a more diffuse or generalized genotype than a derivative which has evolved into a more restricted habitat, thereby narrowing its genetic assemblage; to this would be added the complication of new genetic material which

TABLE 9.—Results of crosses made during the summer of 1962 using *Bufo debilis* females

Cross	No. embryos	No. met.	% met.	Days to first met.	Days to last met.
<i>B. debilis</i> × <i>B. debilis</i>	25	..	..	..	..
<i>B. punctatus</i> × <i>B. debilis</i>	13	12	92.5	19	93
<i>B. retiformis</i> × <i>B. debilis</i>	11	1	9.1	66	..
<i>B. kelloggi</i> × <i>B. debilis</i>	6	1	16.7	70	..

TABLE 10.—Results of crosses made during the summer of 1963 using *Bufo retiformis* females

Cross	No. eggs	No. to reach stage 20	% stage 20	No. feeding larvae	% feeding larvae	No. met.	% met.
<i>B. retiformis</i> × <i>B. retiformis</i>	61	..	..	45	73.8	0.0	..
<i>B. punctatus</i> × <i>B. retiformis</i> No. 3	50	0.0	..	..	..	..	..
<i>B. punctatus</i> × <i>B. retiformis</i> No. 2	56	5	8.95	4	7.15	0.0	..
<i>B. punctatus</i> × <i>B. retiformis</i> No. 1	51	0.0	..	..	..	..	..
<i>B. debilis</i> × <i>B. retiformis</i> No. 2	100	13	13	9	9.0	0.0	..
<i>B. debilis</i> × <i>B. retiformis</i> No. 1	60	18	30.0	5	8.35	1	1.67
<i>B. kelloggi</i> × <i>B. retiformis</i> No. 1	193	17	8.60	14	7.07	0.0	..
<i>B. kelloggi</i> × <i>B. retiformis</i> No. 2	200	0.0	..	..	..	..	..

TABLE 11.—Summary of hybridization data for crosses made during the summer of 1961 using *Bufo kelloggi* females

Cross	No. eggs	% to reach blastula stage	% to reach yolk plug	% to reach neural crest	% to reach tail bud	% to hatch
<i>B. retiformis</i> male × <i>B. kelloggi</i> female						
a	103	100	53.4	21.4	9.7	..
b	49	100	95.9	61.5	53.1	46.9
c	47	100	70.2	27.6	19.1	..
<i>B. debilis</i> male × <i>B. kelloggi</i> female						
a	88	100	79.5	36.1	9.1	..
b	43	100	79.1	60.4	48.8	..
c	45	100	73.3	15.5	11.1	4.6
<i>B. kelloggi</i> male × <i>B. kelloggi</i> female						
a	63	100	100.00	11.1	1.6	..
b	59	100	64.4	64.4	45.7	28.8

TABLE 12.—Results of crosses made during the summer of 1963 using *Bufo kelloggi* females

Cross	No. eggs	No. to reach stage 20	% stage 20	No. feeding larvae	% feeding larvae
<i>B. kelloggi</i> × <i>B. kelloggi</i>	100	80	80	26	26
<i>B. punctatus</i> × <i>B. kelloggi</i> No. 1	109	9	8.25	6	5.5
<i>B. punctatus</i> × <i>B. kelloggi</i> No. 2	79	4	5.06	4	5.06
<i>B. retiformis</i> × <i>B. kelloggi</i> No. 1	203	53	26.1	29	14.3
<i>B. retiformis</i> × <i>B. kelloggi</i> No. 2	59	9	15.4	9	15.4
<i>B. debilis</i> × <i>B. kelloggi</i> No. 1	153	40	26.1	35	22.9
<i>B. debilis</i> × <i>B. kelloggi</i> No. 2	101	1	.99	0	..

TABLE 13.—Results of crosses made during summer of 1962 using *Bufo punctatus* females. Letters and numbers at left indicate series and cross numbers within series (series A = more than 40 embryos; series B = less than 40 embryos)

Cross	No. embryos	No. met.	% met.	Mean and range of metamorphosis time in days
A <sub>0</sub> <i>B. punctatus</i> × <i>B. punctatus</i>	71	53	74.5	99.1±5.3 (29-180)
A <sub>1</sub> <i>B. retiformis</i> × <i>B. punctatus</i>	67	25	37.4	169.6±13.4 (83-306)
A <sub>3</sub> <i>B. retiformis</i> × <i>B. punctatus</i>	70	18	26.7	133.9±8.6 (101-193)
A <sub>4</sub> <i>B. retiformis</i> × <i>B. punctatus</i>	66	20	30.3	136.8±9.8 (42-181)
A <sub>7</sub> <i>B. retiformis</i> × <i>B. punctatus</i>	67	22	32.8	132.1±4.1 (74-216)
A <sub>9</sub> <i>B. retiformis</i> × <i>B. punctatus</i>	60	23	38.4	153.5±8.3 (87-223)
A <sub>2</sub> <i>B. kelloggi</i> × <i>B. punctatus</i>	66	58	88.0	67.4±4.3 (27-142)
A <sub>5</sub> <i>B. kelloggi</i> × <i>B. punctatus</i>	66	43	65.0	80.4±6.0 (25-167)
A <sub>8</sub> <i>B. kelloggi</i> × <i>B. punctatus</i>	70	49	70.0	52.8±3.2 (24-128)
A <sub>6</sub> <i>B. debilis</i> × <i>B. punctatus</i>	55	22	40.0	129.5±7.9 (74-216)
B <sub>1</sub> <i>B. retiformis</i> × <i>B. punctatus</i>	40	29	72.5	91.6±8.9 (29-175)
B <sub>2</sub> <i>B. punctatus</i> × <i>B. punctatus</i>	32	27	84.5	32.1±4.8 (22-50)
B <sub>3</sub> <i>B. kelloggi</i> × <i>B. punctatus</i>	23	18	78.3	25.8±0.9 (21-31)
B <sub>4</sub> <i>B. debilis</i> × <i>B. punctatus</i>	36	33	91.5	74.5±6.1 (27-140)
B <sub>5</sub> <i>B. kelloggi</i> × <i>B. punctatus</i>	33	6	18.4	53.2±11.8 (25-84)
B <sub>6</sub> <i>B. retiformis</i> × <i>B. punctatus</i>	29	16	55.2	121.0±9.1 (86-175)
B <sub>7</sub> <i>B. retiformis</i> × <i>B. punctatus</i>	27	7	26.9	109.4±15.0 (41-163)

would not be complemented in the ancestral stock. Thus, the most recent member of a species group may likely show a greater proportion of newer genetic material that is incompatible with the genetic assemblage of the ancestral stock. Dobzhansky (1951) has stated this proposition as follows: "In the course of evolution, the function of a gene in the development may undergo such changes that a gene may subtend developmental processes other than those with which it was previously concerned." Thus, a greater number of genes in a derivative species that are present in the near-ancestral species should permit greater development of the hybrid offspring.

Accordingly, the sperm of *B. punctatus* is able to efficiently complement the genetic material of the eggs of *B. debilis*, *B. retiformis*, and *B. kelloggi*. In experimental crosses which did not involve *B. punctatus*, it was found that the sperm of *B. kelloggi* had greater affinity for the eggs of *B. debilis* than for those of *B. retiformis* (Tables 9, 10). In 1961, the reciprocal crosses were made using the sperm of *B. debilis* and *B. retiformis* (Table 11). Slightly better results were obtained in the crosses involving males of *B. retiformis*. When these crosses were attempted again in 1963, better results were obtained with the sperm of *B. debilis* than with that of *B. retiformis* (Table 12).

The time for metamorphosis varies between crosses and should represent a parameter indicating genetic affinity. The range of the time for metamorphosis in days is quite short when *Bufo punctatus* and *B. kelloggi* males are crossed with *Bufo punctatus* females, while, in comparison, the crosses between *B. debilis* females and *B. retiformis* males demonstrate both a greater range of metamorphosis time and a significantly longer time between fertilization and first metamorphosis (Table 13).

With respect to mean time for metamorphosis and per cent metamorphosis, the *B. kelloggi* crosses are very similar to the *B. punctatus* control (Fig 15). When viewed in this manner, the *Bufo retiformis*

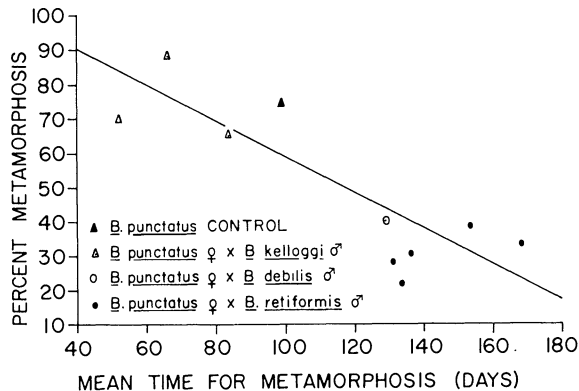


Fig. 15.—Regression of percent metamorphosis on mean time for metamorphosis.  $Y = 106.53 - 0.478 X$ ,  $r = 0.944$ ,  $p = 0.01$  and  $N = 10$ .

and *B. debilis* crosses in this series are considerably different from the control.

The colors of the feeding larvae did not differ markedly. Blair (1963) reports hybrid abnormalities that he refers to as a "hybrid syndrome." Individuals with this syndrome are colorless due to a failure of the melanophores to dilate. Larvae of this type resulted from a cross in 1961 between a *B. debilis* male and a *B. retiformis* female. In general, the tadpoles from the pure species matings were dark (almost black) with interspersed subcutaneous gold stellate markings. Tadpoles of *Bufo debilis* females  $\times$  *B. punctatus* males were fairly light in color, approaching amber in some cases. Tadpoles resulting from *Bufo debilis* females  $\times$  *B. retiformis* males (1962) were pale with reddish brown pigmentation medially and with black pigmentation around the eyes.

*Metamorphosed Hybrids.*—At metamorphosis all individuals from all crosses were amber to dark brown. This coloration did not differ significantly among hybrids or controls. Within a few days after metamorphosis, those crosses involving a *Bufo punctatus* parent began to exhibit red and black punctations against a brown background, which is characteristic of juvenile *B. punctatus*. By the time the juveniles had attained a snout-vent length of 15 mm, those resulting from crosses between *B. kelloggi* males and *B. punctatus* females (Fig. 16) were dark brown with some of the characteristic brown reticulate pattern of *Bufo kelloggi*, and all had small pigment spots characteristic of *Bufo punctatus*.

Individuals resulting from *B. debilis* males  $\times$  *B. punctatus* females (Fig. 17) become olive with some amber coloration laterally and on the legs, the red punctations of *B. punctatus* were also present, being

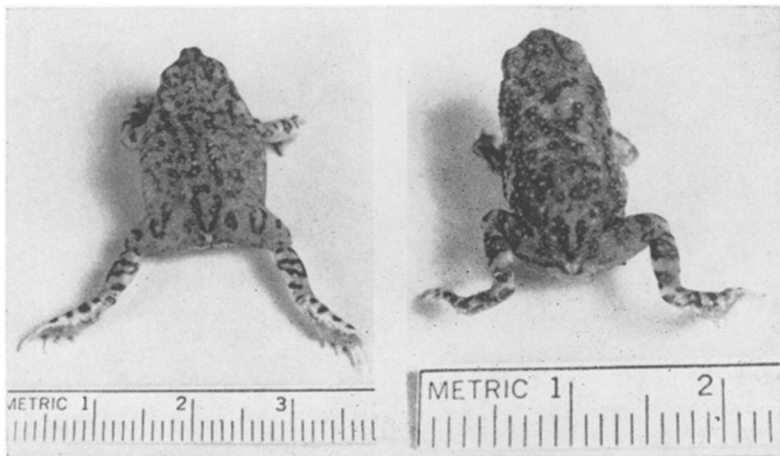


Fig. 16.—Hybrids produced in the laboratory during the summer of 1962. Left, *B. punctatus* female  $\times$  *B. retiformis* male (UAZ 11941). Right, *B. punctatus* female  $\times$  *B. kelloggi* male (UAZ 11942).

particularly abundant on the dorsal surface of the head and on the ventral surface of the pectoral region.

Hybrids from the cross between *B. punctatus* males and *B. debilis* females (Fig. 17) were similar to their reciprocals, all being green with small black and red punctations. As in the *B. retiformis* hybrids, no black reticulate pattern was formed.

All individuals resulting from crosses between *B. retiformis* males and *B. punctatus* females (Fig. 16) were identical in coloration. They were olive green with red pigmentation and some black spots that were arranged in a very similar fashion to those of the individuals resulting from *B. punctatus* females  $\times$  *B. debilis* males and *B. debilis* females  $\times$  male *B. punctatus*. This dark pigmentation did not join in a reticulate pattern as in *B. retiformis*.

In summary, it is evident from the results of hybridization in this laboratory that *B. punctatus* belongs to the same species group as *Bufo debilis*, *B. retiformis*, and *B. kelloggi* and that the genetic affinities of this group fall in the following order: *B. punctatus* — *B. kelloggi* — *B. debilis* — *B. retiformis*. As noted above, from the fossil evidence it is probable that a *Bufo punctatus*-like species existed during the Miocene (Tihen, 1962b). This species certainly could have eventually given rise to both *B. punctatus* of the Recent, and a divergent branch which resulted in a *B. kelloggi*-like species also in the early Madro-Tertiary Geoflora of Western North America. From this *B. kelloggi*-like species, a progenitor (of *B. debilis* and *B. retiformis*) probably developed in a grassland habitat during Pliocene or Mio-Pliocene time and gave rise to *B. debilis*, which is found in

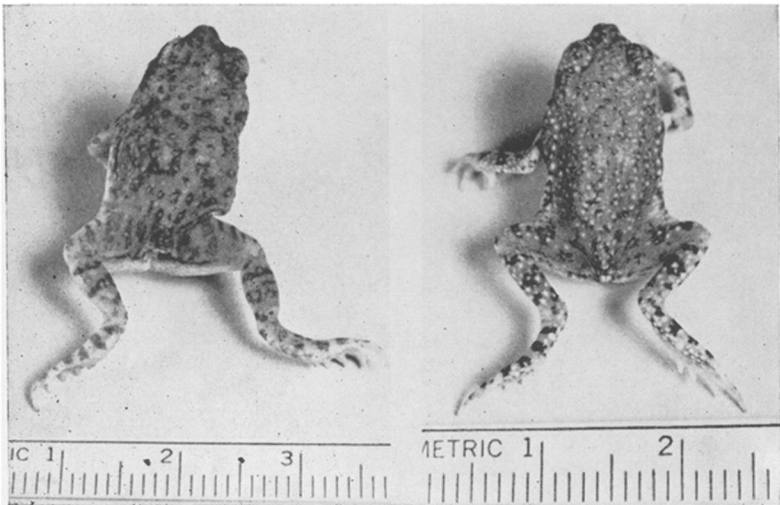


Fig. 17.—Hybrids produced in the laboratory during the summer of 1962. Left, *B. punctatus* female  $\times$  *B. debilis* male (UAZ 11943). Right, *B. debilis* female  $\times$  *B. punctatus* male (UAZ 11944).

the grassland today, and to *B. retiformis*, which is now almost exclusively restricted to adjacent disjunctive environments within the Sonoran Desert that receive ample summer rains; it appears beyond reasonable doubt that speciation occurred as a result of events during the formation of the North American deserts during Plio-Pleistocene time.

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