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EXPERIMENTAL F_1 HYBRIDS BETWEEN *BUFO VALLICEPS* AND *BUFO FOWLERI*[1]

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Investigators familiar with the bufonid fauna of Louisiana have long suspected the occurrence of hybridization between *Bufo valliceps* Wiegmann and *Bufo fowleri* Hinckley. The distributional ranges of the two species overlap in southern Louisiana, and both species may be found breeding in the same body of water. Orton (1951) and Liner (1954) have observed interspecific matings at different localities in Louisiana. Sixteen mis-mated pairs have been noted by the author during the spring and summer months of 1954 and 1955 at a local breeding site (Audubon Park, New Orleans) occupied by both species. Seven of the cross-mated pairs involved females of *B. valliceps* clasped by males of *B. fowleri;* the other nine comprised reciprocal mis-mated pairs.

Laboratory crosses between the two species have revealed the extent to which interspecific gene exchange is possible. F_1 hybrids from the experimental cross *B. valliceps* ♀ X *B. fowleri* ♂ died in an early stage of development. In contradistinction, the majority of F_1 hybrids from the reciprocal cross *B. fowleri* ♀ X *B. valliceps* ♂ were viable. The metamorphosed F_1 hybrids more closely resembled the *B. valliceps* than the *B. fowleri* parent. The effects of the *B. fowleri* parent were more pronounced in the larval period of the F_1 hybrid than in the transformed stage.

The purpose of this paper is to describe and illustrate the appearance of laboratory-raised F_1 hybrid tadpoles and metamorphosed young. This information is an essential prerequisite to analysing the constituent individuals of a natural hybrid population.

Materials and Methods

Breeding adults of *B. valliceps* and *B. fowleri* were collected on several occasions during the spring and summer months of 1954 and 1955 from a pond at Audubon Park, New Orleans. In the laboratory hybridizations performed in 1954 the method of artificial fertilization was employed. Fertilized eggs were obtained by the routine procedure (Rugh, 1934) of inducing ovulation by pituitary injection and stripping the eggs into a sperm suspension. The latter was prepared by crushing a pair of testes in 20 cc of 0.10 Ringer's solution. In order to eliminate any possible variability caused by individual genetic dif-

[1] Research supported by a grant from the National Science Foundation (NSF-G1319). This study is part of a long-term project aimed at reconstructing experimentally the sequence of events that has led to the establishment and maintenance of hybrid individuals between *Bufo valliceps* and *Bufo fowleri* at Audubon Park, New Orleans. The illustrations were prepared by Mrs. Carolyn Thorne Volpe.

ferences, the eggs of a single female were fertilized in two batches; the first with sperm of the same species ("normal" cross), and the second with sperm of the other species ("hybrid" cross). Eggs of *B. valliceps* were fertilized with sperm of *B. fowleri* on six different occasions (each accompanied by a normal cross); the reciprocal cross *B. fowleri* ♀ X *B. valliceps* ♂ was performed nine times (each accompanied by a normal cross).

In the experiments carried out in 1955, the eggs were fertilized by the natural process. The eggs were inseminated by the male (during amplexus) as they emerged from the cloaca of the female. No difficulty was encountered in inducing males of one species to clasp females of the other species. Six interspecific crosses were made, three of which involved females of *B. valliceps* mated with males of *B. fowleri.* The other three were reciprocal crosses.

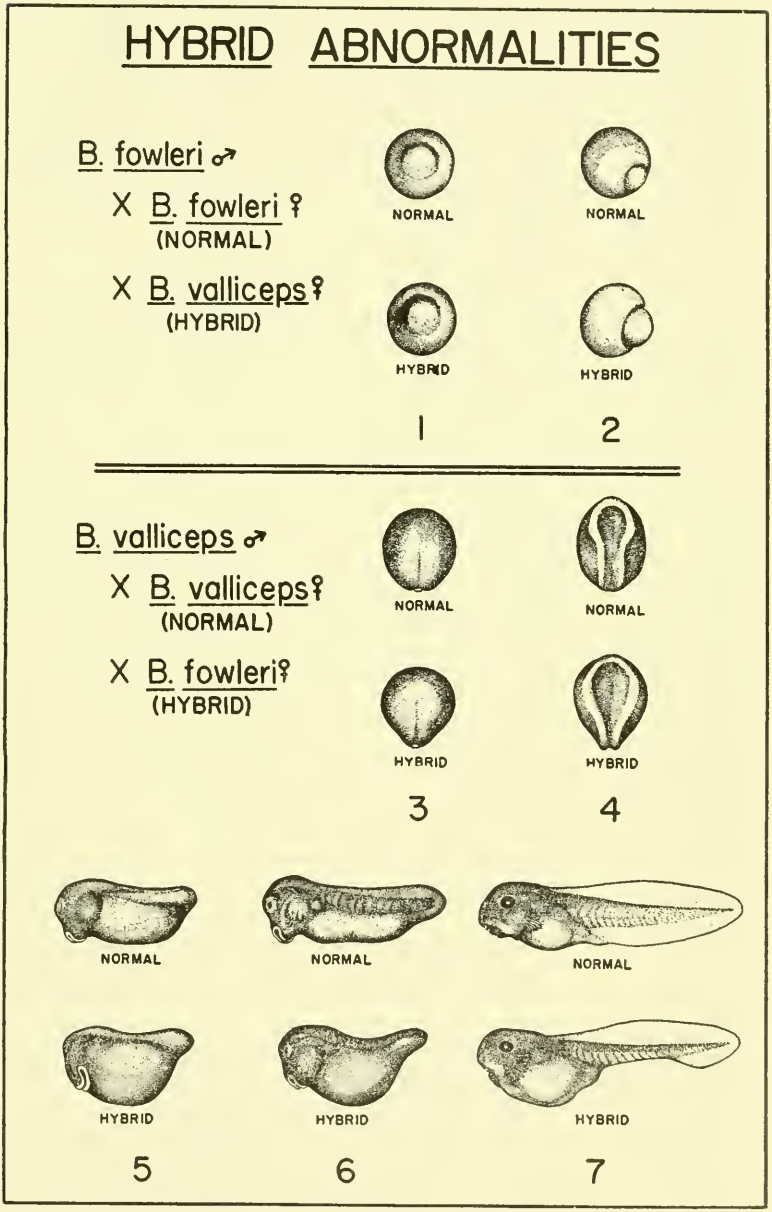
The procedures used in rearing the embryos were essentially similar to those employed in other work of this type (Volpe, 1952, 1954, 1955a, b). The egg strings from each fertilization were cut into small groups. Approximately seventy-five eggs were placed in a finger bowl containing 200 cc of pond water. Four finger bowls were used for each cross. The embryos in the pre-feeding stages (embryonic stages 1-25; defined in Shumway, 1940) were maintained in a 25° C. "Precision Scientific" constant temperature unit. At the onset of feeding activity (larval stage 1; defined in Taylor and Kollros, 1946), at least one hundred embryos from each of the successful crosses were transferred to enamel pans (12″ X 8″ X 2″) containing pond water at a depth of one inch. Each enamel pan, containing approximately twenty embryos, was kept at room temperature. Tadpoles were fed slightly boiled spinach. As the tadpoles approached metamorphosis, the water level in the pans was reduced and pebbles were added to enable the young toads to emerge from the water. Juvenile toads were fed vestigial-winged fruit flies, meal-worm larvae, and pieces of liver dipped in bone meal.

The embryos were examined at regular intervals during their development. At each observation, records were made of the external features of the embryos, types and proportions of abnormalities, and mortality. Embryos derived from the artificial crosses (1954 experiments) developed in an identical manner as those obtained from the natural crosses (1955 experiments). The data from the artificial and natural crosses are treated collectively in the discussion of the results.

Results

A. The Crosses: *B. valliceps* ♀ X *B. fowleri* ♂ (Hybrid)
B. valliceps ♀ X *B. valliceps* ♂ (Normal)

Embryos from the hybrid crosses (nine experiments) died in the late gastrula stages. The cleavage furrows in the early stages of development (stages 3-9; Shumway, *op. cit.*) were normal. The first indication of hybrid abnormality occurred during early gastrulation.



Figures 1-7. Abnormalities in the embryonic stages of F_1 hybrids from reciprocal crosses between *Bufo valliceps* and *Bufo fowleri*.

The dorsal blastopore lip was more heavily pigmented and undercut the embryo more deeply than in the normal gastrula (fig. 1). As the lateral and ventral blastopore lips formed in the hybrid embryo, a large mass of yolk was extruded through the open blastopore. Figure 2 illustrates the exogastrular condition of the hybrid embryo. Shortly thereafter all hybrid embryos cytolysed. No defects were noted in embryos from the normal crosses. The external appearance of a tadpole of *B. valliceps* derived from a normal cross is shown in Figure 8. Its features will be discussed later.

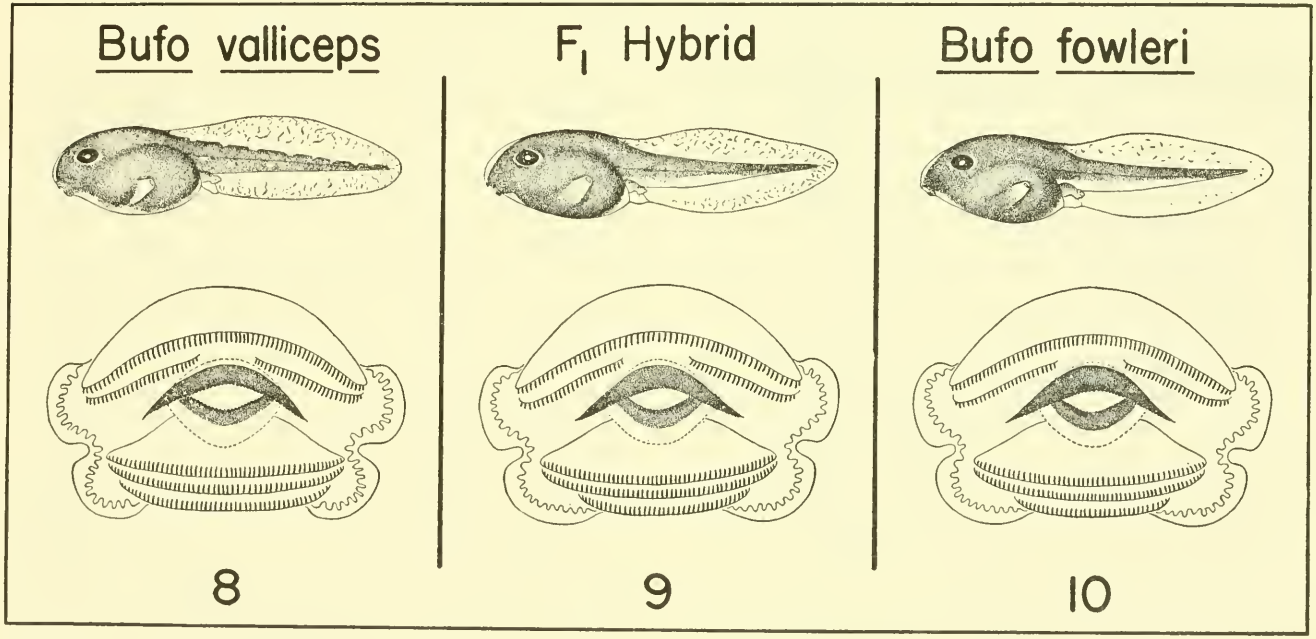
B. The Crosses: *B. fowleri* ♀ X *B. valliceps* ♂ (Hybrid)
B. fowleri ♀ X *B. fowleri* ♂ (Normal)

Early embryonic development (pre-feeding stages).—The cleavage and gastrulation processes (stages 3-12) in all hybrid embryos were normal. In later development, twenty-six to thirty-eight percent (minimum and maximum percents in twelve experiments) of the hybrid embryos died at various stages of development.

The defects observed in the hybrid embryos are illustrated in Figures 3-7. When the neural plate formed dorsally (stage 13), the abnormal hybrid embryos were tapered in the region of the blastopore (fig. 3). During the formation of the neural folds (stage 14), the lateral posterior folds in the defective hybrid embryos were more convergent than in the normal embryos (fig. 4). This condition foreshadowed the narrow tail that appeared in later development. At the tail bud stage (stage 17), the abnormal hybrid embryos exhibited a slight dorsal bending, an upturned tail, an enlarged abdomen, and divergent suckers (fig. 5). The gill plate was compressed against the sensory structures of the head region. The ectodermal surface of the abdominal region was thin and an excessive amount of fluid filled the abdominal cavity. Most of the defective hybrid embryos died at this stage. A few survived to stage 18 (fig. 6). The head structures appeared more normal, apparently due to regulative processes, but the dorsal bending was more pronounced, the tail bud was more irregularly curved, and the abdominal region remained enlarged. Some of these embryos continued to develop as far as stage 23 (fig. 7). Development was arrested at this stage, and the embryos invariably cytolysed. The most conspicuous defects were the dorsal flexure, the edematous abdomen, and the absence of a large portion of the ventral tail fin.

Sixty-two to seventy-four percent of the hybrid embryos were normal, or exhibited the above-mentioned defects to such a small degree as not to have affected their later development.

Late embryonic development (feeding stages).—In terms of survival, the most critical period in hybrid development was the embryonic pre-feeding phase. No abnormalities were observed in the hybrid embryos (tadpoles) during the feeding stages. Samples of hybrid tadpoles were preserved at various stages of development and compared with a similarly preserved series of tadpoles of each parental



Figures 8-10. External features and mouthparts of tadpoles of *Bufo valliceps* (fig. 8), *Bufo fowleri* (fig. 10), and the F_1 hybrid derived from the cross *Bufo valliceps* ♂ X *Bufo fowleri* ♀. The distribution of black chromatophores (melanophores) is shown in the tadpoles; the yellow and white chromatophores are omitted.

species.[2]

During the differentiation of the hind limb buds (stages I-XVII; Taylor and Kollros, *op. cit.*), the tadpoles acquired a characteristic pattern of body pigmentation and a specific arrangement of mouthparts. The most reliable method of distinguishing the tadpoles of the F_1 hybrids from those of each parental species was that based on utilizing both the distributional pattern of pigment and the structural arrangement of mouthparts. In Figures 8-10, the tadpole of an F_1 hybrid is compared with that of *Bufo valliceps* and *Bufo fowleri.*

The dorsal and ventral tail fins of tadpoles of *B. valliceps* (fig. 8) are heavily mottled with reticulate melanopores. The dorsal edge of the tail musculature is characterized by an alternating series of light and dark areas, an effect created by a group of yellow pigment cells interposed among the melanophores at regular intervals along the dorsal edge. The remainder of the tail musculature is black except for a few light areas or blotches (of yellow pigment cells) in the ventral half. Wright (1929) characterized the pigmentation of the tail of tadpoles of *B. valliceps* as follows: ". . . upper edge of tail musculature with 8-10 black bars with intervening pale olive-buff areas; irregular black or brown band on tail with cartridge buff or tilleul buff below it; . . .". I surmise that the 8-10 black bars refer to the dark areas interrupted by light areas on the dorsal edge of the musculature. I am unable to interpret the latter part of Wright's description.

The tail musculature of tadpoles of *B. fowleri* (fig. 10) is covered uniformly with melanophores in the dorsal half; most of the ventral half is devoid of melanophores. The tail fins contain stellate melanophores, more abundant in the dorsal half than in the ventral half. This description compares favorably with that found in Orton (1952).

The pigmentation in the tail fins of F_1 hybrid tadpoles (fig. 9) resembles the pattern in tadpoles of *B. valliceps.* However, the reticulate melanophores are less numerous, and a few stellate melanophores, characteristic of tadpoles of *B. fowleri,* are present. Compared with those in tadpoles of *B. fowleri,* the melanophores covering the tail musculature extend farther, and more irregularly, into the ventral half of the tail musculature. The alternating series of light and dark areas on the dorsal edge of the tail musculature, characteristic of tadpoles of *B. valliceps,* is not present in the F_1 hybrid tadpoles. Although the pigmentation pattern varies at different stages of development, the F_1 hybrid tadpoles always possess a *fowleri*-like pigmentation of the tail musculature and a *valliceps*-like pigmentation of the tail fins.

The mouthparts of tadpoles of *B. valliceps* (fig. 8) and *B. fowleri* (fig. 10) are almost identical. The length of the third lower tooth row constitutes the only difference between the two species. In *B.*

[2] Approximately 200 tadpoles of each group (F_1 hybrid, *B. valliceps,* and *B. fowleri*) have been deposited in the amphibian collections of Tulane University.

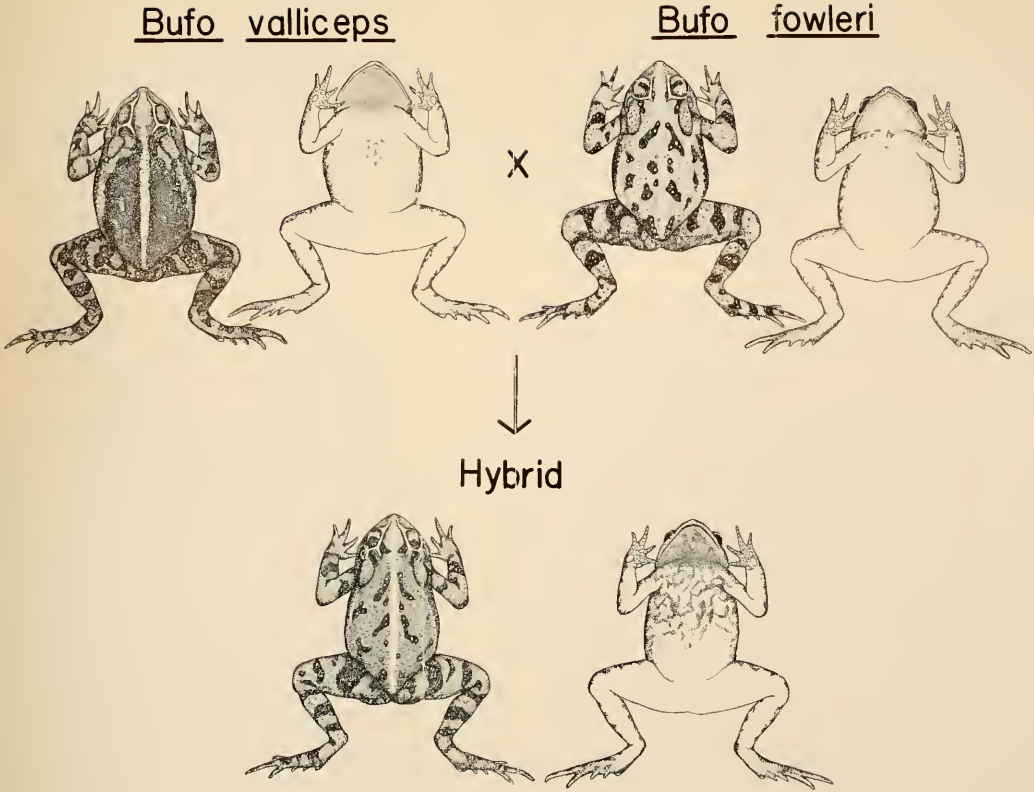


Figure 11. External features of the parents used in one of the interspecific crosses performed in 1954 and of the F_1 hybrid derived from this cross.

fowleri, the third lower tooth row is considerably shorter than the first and second lower tooth rows, as has also been noted by Nichols (1937) and Orton (1952). In *B. valliceps,* the lower tooth rows are essentially equal in length. In other respects, the mouthparts are identical in the two species. The labial tooth formula is 2/3. In the upper labium, the first upper tooth row is continuous and the second is divided medially. Each lateral segment of the second tooth row is approximately twice the length of the median space. The labial papillae are confined to the sides and extend to slightly below the edge of the third lower row. Each lateral fringe bearing the papillae is folded inward between the upper and lower tooth rows.

The mouthparts of tadpoles of the F_1 hybrid (fig. 9) resemble those of *B. fowleri.* Compared with that of *B. fowleri,* the third lower tooth row is as short, or slightly longer. The third lower tooth row is never as long as the corresponding row in *B. valliceps.*

Thus, the F_1 hybrid tadpoles can be readily identified by the *fowleri*-like pigment pattern on the tail musculature, the *valliceps*-like pigment distribution on the tail fins, and the *fowleri*-like arrangement of the mouthparts.

Metamorphosis.—Two hundred and sixty-three F_1 hybrid tadpoles transformed from the twelve experiments.[3] At metamorphosis, the young toad exhibits the dorsal pattern of warts and the median dorsal stripe, but lacks the cranial crests, the tympana, and the parotoid glands. The metamorphosed toads were raised in an air-conditioned laboratory, in which the temperature was approximately 20° C. Rearing the toads at this low temperature has the disadvantage of retarding differentiation, but has the advantage of permitting a closer examination of the structural changes occurring during growth. The parotoid glands were not fully developed until four months after metamorphosis. The cranial crests and tympana made their appearance at five months after metamorphosis. Thus, under laboratory conditions, the young toad approached the adult in external features after five months or more of growth after metamorphosis.

Mortality is considerable in young toads maintained under laboratory conditions. Thirty-two toads have been reared for a period of five months or more.

The young hybrid toads were consistent in their external features. Figure 11 illustrates the appearance of each parental species and an F_1 hybrid. Their characteristics are listed in Table 1. The strong influence of *B. valliceps* is evident in the nature of the cranial crests, the character of the dorsal median stripe, the nature of the metatarsal tubercles, the type of lateral stripe, and the shape of the snout. The F_1 hybrids are modified in the direction of *B. fowleri* mainly with respect to the pattern of spots on the dorsal surface. The dorsal spots

[3] One hundred and eighty-six experimental F_1 hybrids, exhibiting varied degrees of structural differentiation, have been deposited in the amphibian collections of Tulane University.

TABLE 1

SEGREGATION OF CHARACTERS IN AN F_1 HYBRID BETWEEN A MALE *Bufo valliceps* AND A FEMALE *Bufo fowleri*

Character	*B. valliceps*	*B. fowleri*	F_1 Hybrid	Remarks
I. Cranial Crests				
a. Interorbitals (between orbits)	Divergent from nostrils; high and trenchant; widely separated.	Parallel to each other; low; not widely separated.	Divergent from nostrils; high and trenchant; widely separated.	Like *valliceps*.
b. Postorbitals (below orbits)	Meet interorbitals at an angle greater than 90° (approximately 120°).	Meet interorbitals at approximately right angles.	Meet interorbitals at an angle greater than 90° (approximately120°).	Like *valliceps*.
c. Preorbitals (above orbits)	Distinct.	Weakly developed.	Distinct.	Like *valliceps*.
d. Preparotoids (between parotoid gland and postorbital crest)	Conspicuous connecting ridge.	Absent (parotoid gland generally in direct contact with postorbital).	Conspicuous connecting ridge.	Like *valliceps*.
e. Parietals (junction of interorbitals and postorbitals)	Prominent; curve inward toward mid-line.	Short, spur-like.	Prominent; curve inward toward mid-line.	Like *valliceps*.
f. Supratympanics (between tympanum and orbit)	Well-defined.	Weakly developed.	Well-defined.	Like *valliceps*.
g. Canthals (extension of interorbitals cephalad)	Prominent; extend to nostrils.	Absent.	Prominent; extend to nostrils.	Like *valliceps*.

h. Infraorbitals (ventral to orbit)	Well-defined.	Absent.	Well-defined.	Like *valliceps*.
II. Parotoid gland	Small; triangular; dark brown tubercles on pitted surface.	Long and narrow; pitted surface not conspicuously warted.	Subtriangular (apex of triangle more rounded); dark brown tubercles on pitted surface.	Shape approaching *fowleri;* size and warts like *valliceps*.
III. Dorsal spots and warts	Body covered with closely-set, spiny-tipped warts; no spots on dorsum; irregular pattern of bands on legs.	Spots with rounded warts; skin between spots finely warted; regular pattern of bands on legs.	Two prominent pairs of spots along mid-dorsal line; several smaller spots on dorsum; skin between spots contain spiny-tipped warts; regular pattern of bands on legs.	More like *fowleri*.
IV. Ventral spotting	Yellow-green throat; skin over subgular vocal sacs colored dark green; venter sprinkled with light brown spots.	Immaculate to moderately spotted venter (black throat and gular vocal sacs in male).	Yellow-green throat mottled with black; subgular vocal sacs; heavily reticulated venter.	Throat pigmentation combination of both species; subgular vocal sacs like *valliceps;* heavy, unique mottling of venter.
V. Dorsal median stripe	Light, broad streak.	Light, narrow streak.	Light, broad streak.	Like *valliceps*.
VI. Lateral stripe	Broad, light lateral area extending diagonally backward to the groin.	Irregular, light lateral area.	Narrow, light lateral area.	More like *valliceps*.
VII. Metacarpal tubercles	Prominent inner palmar; prominent outer thenar.	Prominent palmar; weakly developed thenar.	Prominent palmar; prominent thenar.	Like *valliceps*.
VIII. Snout shape (lateral profile)	Longer and more pointed.	Shorter and blunter.	Longer and more pointed.	Like *valliceps*.

in individuals of *B. fowleri* tend to be arranged in pairs along the mid-dorsal line. At least two pairs of spots are present in all hybrids, each spot containing several warts. The pigmentation of the ventral surface of the hybrids is unique. The clear, yellow-green throat (characteristic of males of *B. valliceps*) is mottled with black pigment (males of *B. fowleri* possess a black throat). The abdominal surface is heavily reticulated. Individuals of each species may be found with pigment markings in the abdominal region, but the pigmentation is not as strongly expressed as in the F_1 hybrids. The extent of ventral spotting in *B. fowleri* and *B. valliceps* varies from unspotted (omitting the pigmentation on the throat of males) to moderately mottled.[4] The hybrid condition is apparently independent of the extent of ventral markings of either parental species. Relatively unspotted parents (as illustrated in fig. 11) give rise to hybrids which possess the same reticulated ventral condition as offspring resulting from two mottled parents.

The sex of the experimental hybrids presents a challenging problem. The throat region of the hybrids is pigmented as in males, but the gonads are composed of half ovarian and half testicular tissue. This condition apparently does not represent a bipotential gonad in the process of differentiation, since all natural hybrids (discussed below) exhibit the same structural arrangement of ovarian and testicular tissue.

Discussion

As mentioned in the introduction, seven mis-mated pairs involving a female *B. valliceps* and a male *B. fowleri* have been observed in nature. Reproductive energy has been wasted, since no viable hybrids could result from these mis-matings. With respect to the nine reciprocal cross-mated pairs (*B. fowleri* ♀ X *B. valliceps* ♂) that have been noted in the field, approximately seventy percent of the embryos could develop normally. The survival of the hybrid embryos in nature would depend upon many factors, one of which would be their ability to compete successfully with embryos of each parental species. It is of interest that an F_1 hybrid embryo can be identified on a morphological basis. A study of samples of tadpoles taken at regular intervals from a hybrid population would provide information as to the relative survival of hybrid and non-hybrid tadpoles.

At least some of the F_1 hybrids in a natural population have reached

[4] Confirming Blair's (1943) observations, *B. fowleri* individuals from the lower Mississippi valley region are characterized by more extensive ventral markings than representatives of this species from the eastern seaboard. Bragg (1955; also Bragg and Sanders, 1951) does not deny the possibility that the ventral spotting in Louisiana *B. fowleri* (=*B. woodhousei fowleri*) may have been derived from past hybridization with *B. terrestris* or a *terrestris*-like stock, but favors the interpretation that Fowler's toad of southeastern Louisiana is either the new subspecies *B. woodhousei velatus* Bragg and Sanders or an intergrade between this subspecies and *B. woodhousei fowleri*.

maturity, as evidenced by fifteen adult F_1 hybrids that have been collected to date from Audubon Park, New Orleans. The natural hybrids are identical to the experimental hybrids described in the preceding section. All natural hybrids exhibit male secondary sexual characters, *i.e.,* a darkened thumb and a pigmented throat. Their calls consist of feeble, discontinuous trills. Only two of the fifteen hybrids have been found in amplexus; in each case with a female of *B. fowleri.* As in the experimental hybrids, the gonads are atypical. The testis portion of each gonad is much reduced. The anterior portion is composed of a large mass of ovarian tissue, which may represent an overdeveloped Bidder's organ. The ovary-like Bidder's organ is small in relation to the size of the testes in both *B. fowleri* and *B. valliceps.* A histological study of the gonads of F_1 hybrids is in progress. The fertility of the F_1 hybrids is also being tested.

The data presented here compare favorably with Thornton's (1955) results of experimental hybridization between *B. valliceps* and *B. woodhousei.* The latter species is closely related to *B. fowleri.* Embryos derived from the cross-mating of a *B. valliceps* female with a *B. woodhousei* male died during the early stages of development. Persistent yolk plugs were noted in the hybrid embryos. The same abnormality (fig. 2) was observed in hybrid embryos from the cross *B. valliceps* ♀ X *B. fowleri* ♂. Viable hybrids resulted from the reciprocal cross *B. woodhousei* ♀ X *B. valliceps* ♂. In one experiment, Thornton estimated that seventy to eighty percent of the fertilized eggs developed to the tadpole stage. I suspect that the other twenty to thirty percent died of the same defects as illustrated in Figures 3-7. The metamorphosed F_1 hybrids more closely resembled the *B. valliceps* parent than the *B. woodhousei* parent. Thornton also described two unique features of the hybrids; the first, the heavy spotting on the ventral surface, and the second, the peculiar appearance of the testes.

Apparently the sperm of *B. valliceps* interacts with the eggs of *B. fowleri* and *B. woodhousei* in a similar manner. This finding is striking confirmation of the prevalent view that *B. fowleri* and *B. woodhousei* are closely related (in fact, the two are often referred to as subspecies). The limited amount of gene exchange possible between *B. valliceps* and *B. fowleri* or *B. woodhousei* provides additional support for the thesis that *B. valliceps* belongs to an entirely different phylogenetic series. Hybridization studies not only yield information as to the degree to which interspecific gene exchange is possible, but serve also to substantiate or clarify assumed systematic relationships.

Summary

1. Experimental F_1 hybrids from the cross *Bufo valliceps* ♀ X *Bufo fowleri* ♂ are inviable. The embryos die in the late gastrula stage.

2. Sixty-two to seventy-four percent (minimum and maximum percents in twelve experiments) of the hybrid embryos from the reciprocal cross *Bufo fowleri* ♀ X *Bufo valliceps* ♂ develop normally. The

external defects observed in the remaining percent of the embryos have been described and illustrated.

3. The F_1 hybrid tadpoles are distinguishable from those of the parental species. They are characterized by a *fowleri*-like pigment pattern on the tail musculature, a *valliceps*-like pigment pattern on the tail fins, and a *fowleri*-like arrangement of the mouthparts.

4. The F_1 hybrid young toads possess more features of *B. valliceps* than of *B. fowleri.* Their characteristics have been described in detail.

5. The F_1 hybrid tadpoles and metamorphosed young have been illustrated so as to facilitate the identification of the tadpoles and adults in nature. A natural population in which hybridization is suspected can not be properly analyzed without precise knowledge of the embryonic and adult features of its constituent individuals.

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Abstract

The distributional ranges of *Bufo valliceps* and *Bufo fowleri* overlap in southern Louisiana. Both species of toads may be found breeding simultaneously in the same pond, and males of each species will readily clasp females of either species both in nature and in the laboratory.

Laboratory crosses have revealed the extent to which interspecific gene exchange is possible. Experimental F_1 hybrids from the cross *B. valliceps* ♀ X *B. fowleri* ♂ are inviable. All embryos die in the late gastrula stage. Approximately seventy percent of the F_1 hybrid embryos from the reciprocal cross *B. fowleri* ♀ X *B. valliceps* ♂ develop beyond metamorphosis. The F_1 hybrid tadpoles can be distinguished from those of the parental species. They are characterized by a *fowleri*-like pigment pattern on the tail musculature, a *valliceps*-like pigment distribution on the tail fins, and a *fowleri*-like arrangement of the mouthparts. The transformed F_1 hybrid toads possess cranial crests characteristic of the *valliceps* parent, a modified form of dorsal spotting inherited from the *fowleri* parent, and a unique reticulated pattern of pigment on the ventral surface which is not found in either parental species. Consequently, adult F_1 hybrids may be identified easily in the field.

All experimental and natural F_1 hybrids are apparently males. They exhibit male secondary sexual characteristics, but their testes are atypical. The anterior portion is composed of an unusually large mass of ovarian tissue, which may represent an overdeveloped Bidder's organ. The testicular portion is much reduced. A histological study of the gonads of experimental and natural F_1 hybrids is in progress.