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Reproductive Ecology and Hybridization of the Endangered Houston Toad (*Bufo houstonensis*)

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ABSTRACT.—**Initiation of breeding activity of *Bufo houstonensis* followed a rise in minimum air temperature in January or February to above approximately 14°C. Rain was not a direct necessary stimulus to breeding in this species; additional site-specific stimuli apparently are necessary; rapid algal growth may be such a stimulus.**

Amplectant male *B. houstonensis* were significantly larger than non-amplectant males. Based on growth data from recaptured males, it is likely that older male *B. houstonensis* are more likely to achieve amplexus than are first-season males. Data on breeding behavior and movements to and from breeding sites are presented.

Natural hybrids between *B. houstonensis* and *B. woodhousei* and between *B. houstonensis* and *B. valliceps* were identified morphologically and electrophoretically. Only one suspected backcross product was found. Hybridization among these species was minimal in the study areas—in all cases less than 1% of the parental populations. The primary isolating mechanisms are temporally offset breeding seasons (of *B. houstonensis* and *B. valliceps*) and habitat isolation (*B. houstonensis* and *B. woodhousei*).

The population sizes of *B. houstonensis* in Bastrop Co., Texas, are larger than previously reported; present population sizes in this area appear not to be critically low. The restricted range of the species coupled with habitat destruction seem to be the primary factors in the endangerment of *B. houstonensis*.

The *Bufo americanus* species group of North American toads has received considerable attention with respect to interspecific hybridization, isolating mechanisms, and population ecology (A. P. Blair, 1941, 1942, 1955; W. F. Blair, 1956*a*, *b*, 1957, 1959, 1961, 1972*c*; Bragg, 1940; Cory and Manion, 1955; Guttman, 1969; Henrich, 1968; Oldham, 1966; Sanders, 1961; Thornton, 1955; Volpe, 1959; Zweifel, 1968, and references cited therein). These studies have contributed considerably to our present understanding of speciation and species interactions of anurans (W. F. Blair, 1962, 1964, 1972*a*, 1973). However, several restricted and isolated taxa of *Bufo* (Pleistocene relicts according to W. F. Blair, 1972*b*) that are closely related to more widely distributed members of the *B. americanus* species group have not been studied to a comparable degree.

An example of a Pleistocene isolate that is particularly suited for a comparative study is *Bufo houstonensis*, a species closely related to *B. americanus* (see A. P. Blair, 1957; W. F. Blair, 1965, 1972*b*). In addition to the evolutionarily interesting aspects of *B. houstonensis*, it is currently listed on national (Gottschalk, 1970) and international (Honegger, 1970) lists of endangered species. Despite the public attention and publicity given *B. houstonensis* in recent years because of its endangered status, little basic life history information is known for this species. Possible reasons for the decline in populations of *B. houstonensis* usually are listed as natural climatic changes, habitat destruction by humans, and hybridization with sympatric congeners (Brown, 1971), but supporting data are scarce. Since the

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description of *B. houstonensis* by Sanders (1953), only two papers have presented original data on aspects of the population ecology of this species, and in both cases these data were in the form of descriptive notes ancillary to the main thrust of the papers (Kennedy, 1962; Brown, 1971).

The purposes of this study were threefold: (1) to document basic aspects of the population ecology of *B. houstonensis* over several years (with detailed, quantified data from one complete breeding season), and to compare these data with past studies on the ecology of *B. americanus*; (2) to study the present extent, causes, and possible effects of hybridization among *B. houstonensis* and two partly sympatric congeners (*Bufo woodhousei* and *Bufo valliceps*) for comparison with similar data reported a decade earlier by Brown (1971); and (3) to determine the current status of populations of *B. houstonensis* in the only area of their range in which they are known to be relatively abundant—the isolated loblolly pine regions of Bastrop Co., Texas.

Methods

Twelve study sites in Bastrop Co., Texas, were selected prior to the start of the 1981 breeding season of *B. houstonensis* (Fig. 1). Site selection was based on observations made in previous seasons: sites 1 and 12 were known to have supported populations of *B. woodhousei* and *B. valliceps*, but not of *B. houstonensis*; sites 2 and 11 were suspected sites of hybridization of *B. woodhousei* and *B. houstonensis*, that also supported populations of *B. valliceps*; sites 3, 4, 5, 6, 8, and 10 were known to have supported breeding populations of *B. houstonensis* (as well as *B. valliceps*); and sites 7 and 9 were selected on the basis of negative evidence—no *B. houstonensis* had been found at these sites during previous years despite their similarity to the sites occupied by it.

Each of the study sites was visited nightly from 1 January through 4 June 1981. Most of the sites also were visited during periods of weather believed to be favorable for reproduction of *B. houstonensis* during 1979 (at least twice per week) and sporadically in 1980. During each site visit, data on water temperature, air temperature, relative humidity (1981 only), precipitation, sky conditions, and activity of *Bufo* (number of males present and/or calling, number of females present, number of pairs in amplexus, number of egg masses, relative number and stage of development of tadpoles) were recorded. During 1981, air temperature was recorded continuously at a location near site 1 (Fig. 1), as were daily precipitation data. The locality at which these data were collected was less than 10 km (straight-line distance) from each of the study sites. Daily water temperature minima and maxima (at 10 cm depth) were measured at site 5. Turbidity (in ppm SiO_2) was measured at each of the sites at the start of the 1981 breeding season of *B. houstonensis*, and pH, dissolved nitrate (ppm), and dissolved phosphate (ppm) were measured one week before and one week after the first choruses of *B. houstonensis* in 1981 at sites 4, 5, 6, 7, 9, and 10. Individuals in the first 1981 chorus of *B. houstonensis* at site 5 were marked by toe-clipping; our system differentiated between amplectant and non-amplectant males and females.

Hybrids between *B. houstonensis* and *B. valliceps* can be distinguished from the parental species by several morphological and auditory characters; these hybrids are reportedly infertile, so backcrossing to the parental species probably cannot occur (Brown, 1971). However, Brown (1971) found *B. woodhousei* × *B. valliceps* hybrids difficult to distinguish from *B. valliceps* × *B. houstonensis* hybrids; he also reported that "as *B. houstonensis* and *B. woodhousei* are quite similar morphologically, it is difficult to distinguish their hybrids from either parental species . . . . The hybrids are intermediate in size and for other morphological characters they fall within

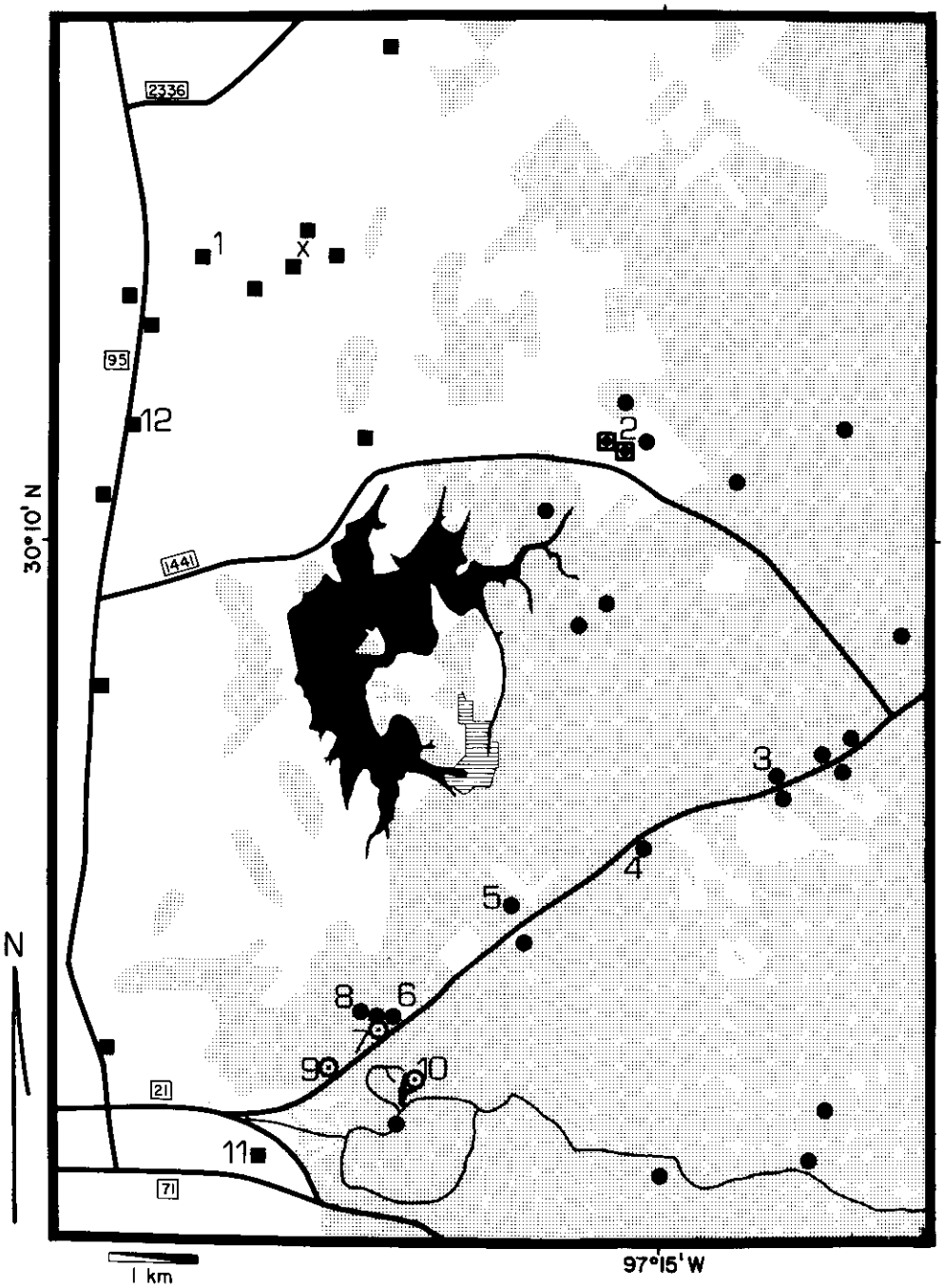

FIG. 1. Map of the study sites in Bastrop County, Texas. Numbers in boxes are highway route numbers. The shaded area represents the primary distribution of loblolly pine (*Pinus taeda*) in the study area in March 1981 as determined by infrared aerial photography and field inspection. The black squares represent known breeding localities of *Bufo woodhousei* during 1979 and 1981, and the black dots represent known breeding localities of *B. houstonensis* during this time period. The circles surrounded by squares represent sites of hybridization between *B. woodhousei* and *B. houstonensis*. The

TABLE 1. Means ± standard deviations and ranges of seven morphometric characters for samples (N = 10) of *B. houstonensis, B. woodhousei,* and *B. valliceps.* The starred (*) characters were utilized in a discriminant function analysis that was used to identify hybrid combinations of these three species (Fig. 2). Measurements that could be made on the left and right sides were averaged for each individual.

Character (mm)	*B. houstonensis*	*B. woodhousei*	*B. valliceps*
Head width	20.06 ± 1.99 (17.1–23.0)	30.67 ± 2.82 (25.3–34.0)	26.91 ± 2.82 (23.8–33.6)
*Distance between interocular crests	4.65 ± 0.54 (3.9–5.5)	6.24 ± 0.66 (4.9–7.1)	10.53 ± 1.05 (9.2–12.6)
*Length of paratoid gland	11.58 ± 1.03 (10.2–12.85)	15.24 ± 1.19 (13.25–17.65)	8.87 ± 0.87 (7.4–10.0)
Width of paratoid gland	5.40 ± 0.39 (4.75–5.95)	6.67 ± 0.58 (6.0–7.9)	5.58 ± 0.47 (4.5–6.1)
*Length of tibiofibula	19.74 ± 2.37 (17.0–24.0)	31.81 ± 2.74 (26.3–35.8)	27.35 ± 3.16 (23.4–33.8)
Snout–urostyle length	56.61 ± 5.03 (51.4–64.9)	79.48 ± 6.28 (67.5–87.7)	71.64 ± 8.54 (62.8–92.3)
*Distance between paratoid gland and transverse axis of postorbital crest	1.90 ± 0.49 (1.35–2.65)	0.12 ± 0.15 (0.0–0.4)	2.94 ± 0.43 (2.4–3.7)

the ranges of variation of the parental species." In addition, backcrossing of *B. woodhousei* × *B. houstonensis* hybrids is possible because at least the males of this combination (no hybrid females have been reported) are fertile (Brown, 1971; W. F. Blair, 1972*c*).

To identify hybrids of any combination of *B. houstonensis, B. woodhousei,* and *B. valliceps* by external morphology, a stepwise discriminant function analysis was performed on seven morphometric measurements (Table 1) with the three parental species as a priori groups (program BMDP7M of the statistical package described by Dixon and Brown, 1977). The *B. valliceps* and *B. woodhousei* in the a priori groups each were collected at sites within the study area at which only that species bred; the *B. houstonensis* were from site 5, where *B. woodhousei* does not occur and *B. valliceps* is uncommon (less than 0.2% of the *Bufo* at the site). Two canonical axes (CA I and CA II) were derived that taken together discriminated among all three species (Fig. 2); suspected hybrids were identified by intermediate scores along these two axes or by call parameters (Brown, 1971).

The identifications of hybrids as described above were verified by horizontal starch gel electrophoresis. Liver and muscle tissue were separately homogenized in 0.01 M tris-HCl, 0.001 M EDTA, and 0.001 M 2-mercaptoethanol, pH 7.5. Homogenates were frozen at −80°C and then centrifuged for 15 minutes at 14,000 rpm. Two buffer systems were employed: tris-citrate pH 7.0 (0.13 M tris-0.043 M citrate electrode buffer, 0.009 M tris-0.003 M citrate gel buffer);

←

numbered localities are sites that were visited nightly from late January to early June 1981 and are numbered in order of nightly visitation. At three of these sites (open circles) no *Bufo* bred during 1981. *B. valliceps* was found throughout this area at almost all sites; hybrids of this species with *B. houstonensis* were found only at sites 4 and 6. The × denotes the location at which the 24-hour meteorological data were recorded. The large black area represents Lake Bastrop, and the adjoining barred area represents the location of the facilities of the Sam Gideon power plant.

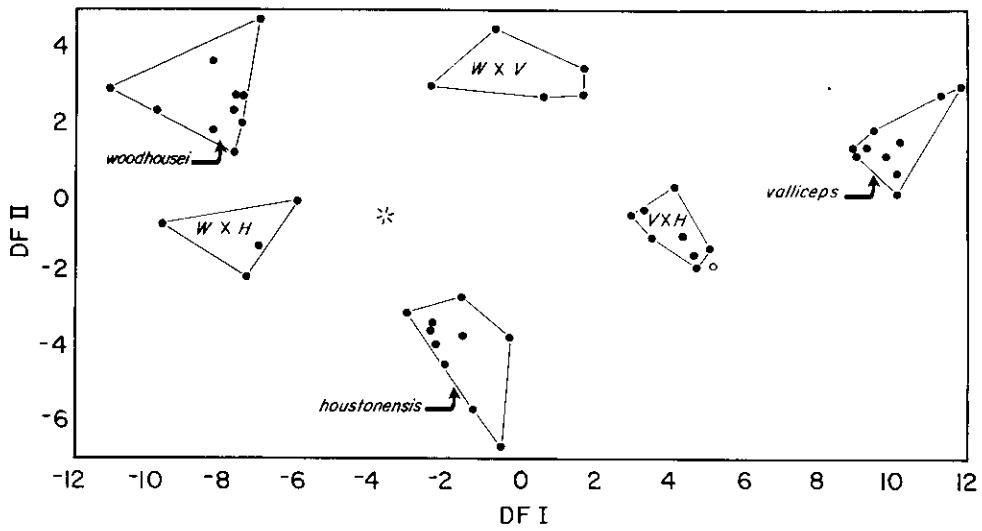

FIG. 2. Plot of scores along two canonical axes showing the morphological intermediacy of hybrids of *B. houstonensis*, *B. woodhousei*, and *B. valliceps*. Identifications of hybrids were confirmed by subsequent electrophoretic examination. The open circle designates the score along the two axes of a *B. valliceps* × *B. houstonensis* hybrid from site 5 that was identified by call characteristics and measured by Nancy Jacobson in 1982 (not verified electrophoretically). The asterisk designates the multivariate location of a probable backcross of a *B. woodhousei* × *B. houstonensis* hybrid to *B. houstonensis* as determined electrophoretically.

and tris-versene-borate pH 8.0 (0.5 M tris-0.016 M EDTA-0.65 M borate electrode buffer, 0.05 M tris-0.0016 M EDTA-0.065 M borate gel buffer). Two drops of 2-mercaptoethanol were added to the gel buffer mixture after boiling and degassing. The staining procedures are described in Siciliano and Shaw (1976) and Harris and Hopkinson (1976).

Enzyme systems examined included superoxide dismutase (SOD; two loci, one of which was diagnostic), esterase (EST; several loci, two of which were diagnostic), lactate dehydrogenase (LDH; two loci, one of which was diagnostic), malic enzyme (ME; two loci, one of which could be used to differentiate between *B. woodhousei* and the other two species), and malate dehydrogenase (MDH; two loci, one of which could be used to differentiate between *B. valliceps* and the other two species). The common electromorph in the *B. houstonensis* sample was scored as 100; all other electromorphs were scored according to their mobilities relative to electromorph 100. The MDH-2 electromorph of *B. houstonensis* and *B. woodhousei*, scored as 100 in both cases, was actually slightly slower in *B. woodhousei* than in *B. houstonensis*, but the two electromorphs could not be distinguished consistently and therefore were lumped.

RESULTS AND DISCUSSION

Breeding Stimuli.—Kennedy (1962) stated that in *B. houstonensis* "calling and spawning activity is ephemeral and is apparently initiated by heavy rains with warm temperature. The earliest known spawning occurred on February 22 and the latest on June 26" (observations in Houston). At Bastrop sites we have never observed gravid female *B. houstonensis* after 2 May, but breeding usually is initiated in the Bastrop populations before the earliest date reported by Kennedy (earliest date of chorusing: 22 Jan-

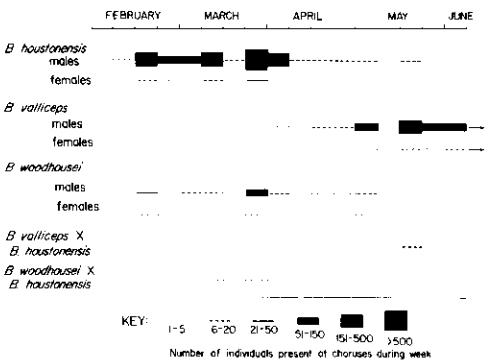

FIG. 3. Numbers of *B. houstonensis*, *B. woodhousei*, *B. valliceps* and hybrids present at the study sites during week-long intervals in 1981.

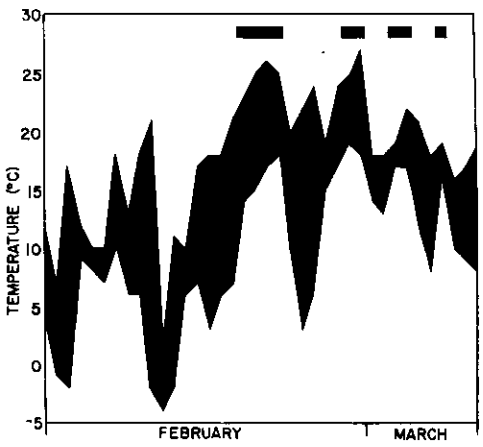

FIG. 4. Air temperature minima and maxima during 24-hour periods previous to 1800 hours (approximate time of chorus assembly) in February and early March 1981. Bars across the top indicate nights on which breeding of *B. houstonensis* took place. Note presence of breeding each time that the minimum air temperature was above 14°C.

uary 1982—N. Jacobson, pers. comm.; initiation of spawning: 18 February 1981; latest date for initiation of chorusing and spawning: 23 February 1979). In each of the years for which we have made observations, initiation of chorusing was not directly associated with rain; in 1981 (the only year for which continuous on-site data are available), no rain had fallen in the area for 8 days when breeding choruses of *B. houstonensis* first assembled on 18 February. Breeding choruses continued to assemble through the beginning of April without any detectable association with rain. From late April to early June (Fig. 3), choruses of *B. valliceps* began to assemble on rainy nights. These choruses often included a few male *B. houstonensis* as well, although we found only one gravid female *B. houstonensis* after 2 April (on 2 May 1981). Therefore, rains apparently do not necessarily serve (at least directly) as a stimulus to breeding in *B. houstonensis*. Instead, at our study area in 1981, breeding choruses of *B. houstonensis* assembled each time that the minimum air temperature for the preceding 24 hours did not fall below 14°C (Fig. 4). While we did not collect continuous on-site temperature data during 1979, 1982, or 1983, chorusing also began in these years when the 24-hour air temperature minimum rose above 14°C at Austin, Texas (approximately 45 km west of the study area). The earliest date that such a rise in overnight air temperature minima would stimulate breeding in *B. houstonensis* is unknown, but in 1982 this rise occurred in January and chorusing did take place (N. Jacobson, pers. comm.). On the last night of several breeding periods during 1981, air temperature fell below 14°C during chorusing; a causal relationship may exist here in which the subsequent night's calling was inhibited. Brown (1967), earlier, often observed calling in *B. houstonensis* below this temperature.

This explanation of breeding stimulation holds true for all of the study sites considered together, but it does not explain the intersite variation in breeding dates that was observed during this study. For instance, whereas breeding choruses assembled first during 1981 at sites 3, 4, and 5 from 18 to 21 February, no *B. houstonensis* choruses assembled at site 6 until 31 March. Choruses were never fully synchronized among the study sites, even when they occurred during approximately the same time

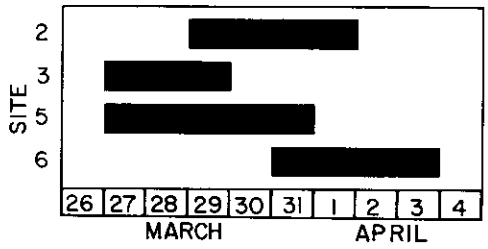

FIG. 5. Temporal occurrence of breeding choruses of *B. houstonensis* at four sites during a representative period in late March and early April 1981. Note the non-correspondence of these intervals in time.

TABLE 2. Dissolved nitrate (N) and phosphate (P) concentrations (in ppm) at six study sites on two dates in 1981. Notice the drop in these concentrations between the two dates (typical of algal blooms) at the three sites where breeding of *B. houstonensis* took place. u = undetectable; t = trace (<0.1 ppm).

Site	10 February		25 February		Date of first breeding
4	0.1	N	0.1	N	18 February
	0.5	P	0.1	P	
5	0.5	N	0.4	N	18 February
	0.6	P	0.1	P	
6	0.3	N	u	N	30 March
	1.2	P	0.1	P	
7	u	N	u	N	None
	t	P	t	P	
9	u	N	u	N	None
	t	P	t	P	
10	0.1	N	0.1	N	None
	t	P	t	P	

period (Fig. 5). It is likely that some additional on-site stimuli are necessary to initiate chorusing in *B. houstonensis*, even when temperature conditions are favorable.

A factor that possibly accounts for intersite variation in breeding dates is rapid algal growth. Savage (1961 and papers cited therein) considered spring algal blooms to be the primary stimulus to breeding in *Rana temporaria*. We became interested in this hypothesis in 1979 when we noticed that the first breeding choruses of *B. houstonensis* appeared just before an algal bloom (of unidentified species) appeared at the site of chorusing.

Table 2 presents dissolved nitrate and phosphate levels present in the water at six of the study sites in 1981. The measurements were first made on 10 February, eight days before the first breeding choruses of *B. houstonensis* appeared at two of these sites. The second set of data was taken seven days after the first breeding choruses. During this time, phosphate levels fell between five-fold and twelve-fold at three of the sites; phosphate levels were barely detectable or not detectable at all (our apparatus had a lower sensitivity limit of about 0.1 ppm) on either date at the other three sites. There was also a drop in nitrate level at most of the sites during this time, although it was not as large as the drop in phosphate level (Table 2). Rapid drops in nitrate and (especially) phosphate levels are characteristic of algal blooms (Smith, 1950). It is of interest that the three sites at which phosphate levels were barely detectable on both dates did not support breeding of *B. houstonensis* in 1981. All three sites occur within the range of *B. houstonensis* (Fig. 1), and the immediate area around each of the ponds appears to be suitable (sandy soil and pine forest) for *B. houstonensis*. In fact, we observed a few *B. houstonensis* calling at site 10 in 1979. Savage (1961) noted that year-to-year variations in water chemistry (thus affecting algal growth) may have caused *Rana temporaria* to use a pond in one year but not in another. Our data suggest that either *B. houstonensis* are not stimulated to breed in ponds with low phosphate levels (and presumably little algal growth), or ponds with low productivity cannot support populations of *B. houstonensis*. However, the data do not explain the delay of breeding initiation at site 6 until 30 March, because there was also a drop in phosphate and nitrate levels at this pond between 10 and 25 February (Table 2).

Studies that have been conducted on *B. americanus* generally have concluded that rising spring temperatures stimulate breeding in this species (Christein and Taylor, 1978; Oldham, 1966). However, no critical high or low temperatures have been reported.

Mate Selection.—A number of studies have been conducted recently that have dealt with mate selection among various anurans. Although four of these studies (Gatz, 1981; Kruse, 1981; Licht, 1976; and Wilbur et al., 1978) dealt at least in part with *B. americanus*, they are divided on their conclusions. Licht (1976) and Gatz (1981) reported significant size-based selection of males by female *B. americanus*, whereas Wilbur et al. (1978) and Kruse (1981) reported random mating of males and females with respect to body size in this species. Licht (1976) hypothesized that female *B. americanus* select males of optimum size relative to their own body size in order to maximize fertilization (as has been experimentally demonstrated for *Bufo bufo* by Davies and Halliday, 1977), but Kruse (1981) and Gatz (1981) reported nonsignificant correlation values of male body size to female body size in several populations of this species (from $r = -0.299$ to $r = 0.261$), and Kruse (1981) reported no fertilization advantage for any particular male-to-female ratio in experimental crosses of *B. americanus*. Wilbur et al. (1978), however, suggested that female toads choose the largest males in a chorus—an hypothesis supported by Gatz (1981) but refuted by Kruse (1981).

The sexual size difference within populations of *B. houstonensis* is not as great as that within the populations of *B. americanus* in which mate selection has been studied. In three populations of *B. americanus* (Canada—Licht, 1976; North Carolina—Wilbur et al., 1978; and Illinois—Kruse, 1981), reported average male body length ranged from 82.1% to 86.7% of average female body length. In contrast, we found the mean body length of male *B. houstonensis* (57.11 mm, N = 309, SD = 4.20) to be 90.6% of the mean body length of females (63.02 mm, N = 60, SD = 5.09). In the population of *B. americanus* studied by Kruse (1981), only the lower 38% of the distribution of female body length overlapped the range of male body length, whereas 96% of the female *B. houstonensis* that we measured fell within the size range of the males (Fig. 6). This difference between the two species in relative sexual size is reflected in the size ratios of amplectant pairs; Kruse (1981) reported only one out of 63 amplectant pairs in which the male *B. americanus* was larger

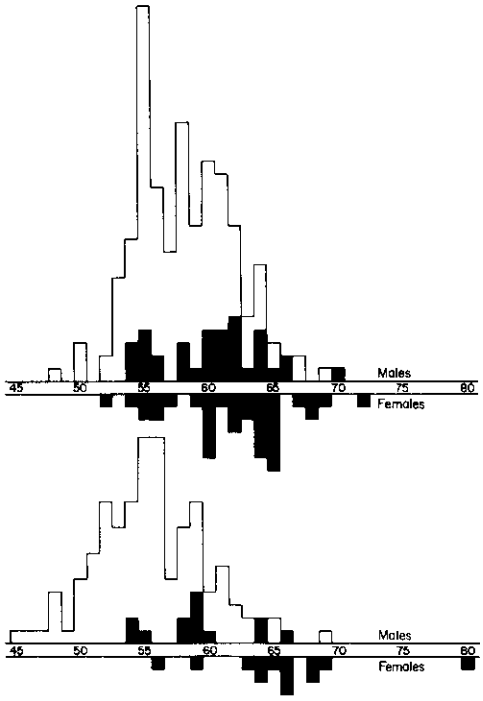

FIG. 6. Size-based mate selection in two choruses of *B. houstonensis*. The upper figure indicates the size distribution of individuals at site 5, and the lower figure indicates the distribution of individuals at site 3. In both cases, males are figured above the line and females below the line. All females were in amplexus; males in amplexus are represented in black. In both cases, the mean size of the males in amplexus was significantly larger than that of males not in amplexus ($P < 0.001$).

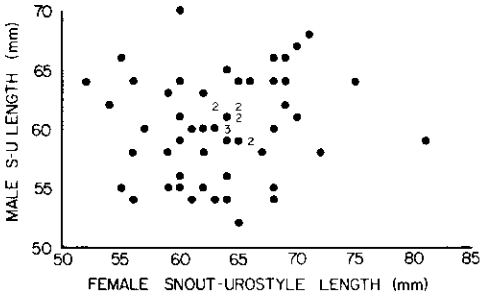

FIG. 7. Plot of male versus female snout-urostyle lengths of amplectant pairs of *B. houstonensis* ($r = 0.128$, $0.2 < P < 0.4$). Overlap of two or three points is indicated by the appropriate numeral.

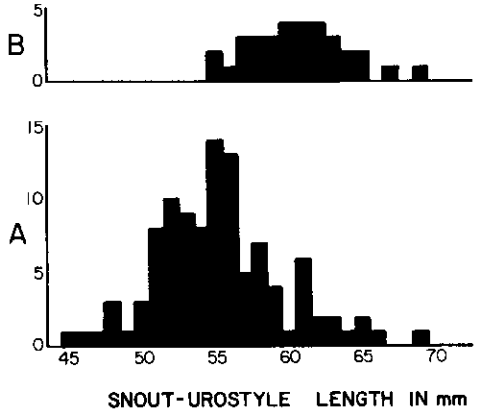

FIG. 8. A. Size distribution of 104 male *B. houstonensis* marked on 18 February 1981. B. Size distribution of 33 recaptured males one year later (17-22 February 1982).

than the female, whereas we found 12 out of 60 amplectant pairs of *B. houstonensis* in which the male was larger (Fig. 7).

In the two populations of *B. houstonensis* in which we studied mate selection (Fig. 6), the males in amplexus were significantly larger than the non-amplectant males ($t = 3.457$, $P < 0.001$ for site 5; $t = 3.627$, $P < 0.001$ for site 3). In contrast, the correlation of male body length to female body length (Fig. 7; $r = 0.128$, $0.2 < P < 0.4$) is not significant. If female *B. houstonensis* select males (instead of vice versa), then they select the largest available males, not males that result in an optimum size ratio. However, Kruse (1981) suggested that male-male interactions could be more important than mate selection by females in determining pairings of male and female *B. americanus*. Unpaired male *B. americanus* have been observed attempting to dislodge males in amplexus (Licht, 1976); presumably, large males are more successful in such attempts than are small males. Data on the relationship of size to age of male *B. houstonensis* (Fig. 8) support this possibility. The size distribution of marked males recaptured after one year at site 5 (Fig. 8) corresponds closely to the size distribution of amplectant males of the 1981 season at this site (Fig. 6). The mean snout-urostyle (SU) length of the marked males that survived to 1982 was 60.7 mm (SD = 3.31), whereas the mean SU length of amplectant 1981 males was 60.2 mm (SD = 4.01). These two groups are not significantly different ($t = 0.559$; $0.5 < P < 0.6$). Therefore, it is likely that the majority of the amplectant males in 1981 were in at least their second breeding season. If this is correct, then male experience or dominance could be more important than female choice in determining successful breeding.

Breeding Dynamics.—About 20 minutes before sunset on nights that choruses of the Houston toad occurred, male *B. houstonensis* began to call underground from the burrows to which they had retreated the previous evening (similar underground calling behavior has been reported in *B. punctatus* by Brown and Pierce, 1965). These burrows ranged from within a meter of the pond to at least 40 m from the shore. The majority of the burrows of *B. houstonensis* observed were along gulleys that led to the ponds; as choruses ended, individuals often left the ponds by traveling up these gulleys. We never observed *B. houstonensis* creating new burrows; instead, the toads utilized existing holes (commonly mammal bur-

rows in root entanglements of undercut banks) or spaces beneath logs. Several individuals were uncovered from beneath thick piles of pine needles in gulleys. One male with a uniquely deformed limb mark was observed for three consecutive nights at the pond, and after each night was found in the same burrow. During these three nights, this toad was found calling at various sides of the pond; thus it was not merely retreating to the nearest available burrow. After this period, the toad was no longer present at the breeding chorus nor in the burrow.

Approximately 10 minutes before sunset, male *B. houstonensis* emerged from burrows and began to move toward the breeding pond, calling between movements. Males began to arrive at the pond shortly after sunset and continued to arrive at the pond until shortly after midnight. Some males began to leave the pond several hours before midnight. Females rarely arrived at the pond until several hours after sunset; partly because of the skewed sex ratio (Fig. 6), females remained unpaired for a very short time. The last females arrived by 0200 hours, and chorus sizes usually began to decrease markedly at about this time (Fig. 9).

Guttman and Wilson (1973) observed heterozygote deficiencies in some populations of *B. americanus* and suggested that toads might orient to a particular shore of a breeding pond at which they would breed with other individuals that had the same shore preference. Such shore-specific breeding did not occur in *B. houstonensis* populations; males did not remain calling from a single location, but usually moved several times during a given night—often to an opposite shore.

Male *B. houstonensis* vocalized from along the shoreline, in shallow water, or from the top of an object within several meters in either direction from the shore. Height appeared to be important in call-site selection, as all of the highest projections along a shoreline usually were occupied by calling males. Logs that projected above the surface of the pond often served as calling sites for several males.

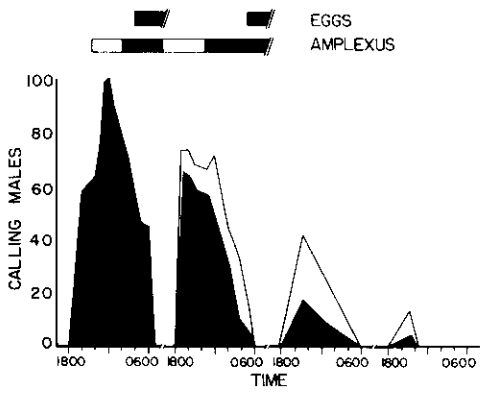

FIG. 9. Representative breeding period of *B. houstonensis* (18–21 February 1981; site 5). The number of calling males represented in black are those that were calling on the first night of the period (all were marked); those represented in white joined the chorus after the first night. Bars marked "eggs" and "amplexus" indicate the periods during which individuals were ovipositing or in amplexus, respectively; at the point each night when the last female arrived and entered amplexus, the amplexus bar changes from white to black.

Pairs of *B. houstonensis* remained in amplexus for a minimum observed time of six hours before oviposition began. Many females had not begun to lay eggs by dawn, and at least some amplectant pairs remained together without oviposition until the following night (Fig. 8). During daylight hours, we observed these non-laying amplectant pairs resting upon or partly concealed beneath detritus at the bottom of the ponds. However, once individual females began to oviposit, they continued until finished; because oviposition usually did not begin until a few hours before dawn (Fig. 9), many of the eggs were laid during the early daylight hours.

Nightly choruses usually were present at a given pond for three to five day periods (Fig. 5), unless cold weather shortened this period. Even with favorable weather conditions (as evi-

denced by choruses present at other ponds), choruses never were observed at a pond for more than six consecutive nights. These three- to five-day periods never were completely synchronized at our study sites.

At one site (#6), there was only one period during which *B. houstonensis* bred (and at this site, several male *B. houstonensis* called at early choruses of *B. valliceps*). At other sites at which *B. houstonensis* bred, there were two or three primary periods of breeding activity, separated by two to six week intervals during which no (or occasionally a few) males called. We found only one gravid female at a breeding site during these intervals. Some males that were marked on the first night of the first breeding period (18 February) at site 5 also called during the second breeding period (27–31 March) at this site. Males marked during the first period constituted 33.5% of the pooled males present during the second period, and ranged from 22.4% to 41.6% of the males present at the breeding site on any one night. Some of the males that were in amplexus during the first period also called during the second period; these males made up a slightly larger percentage (10.4%) of the marked males that were present during the second period than they had in the first period (8.4%). This indicates that the successful males also were among the most persistent attendants at the choruses.

Survivorship and Growth.—Of the 104 males and 8 females marked on 18 February 1981 at site 5, 35 males and no females were recovered during an intensive study at this site during 1982 by Jacobson (1983). Fig. 8 shows the size distribution of the 104 males in 1981 and 33 of the 35 recovered males in 1982, one year to the week later. The remaining two males were recovered much later in the 1982 season after a longer opportunity for growth. The average size (SU length) of these males in 1981 was 55.3 mm (SD = 4.40); by 1982 the surviving males averaged 60.7 mm (SD = 3.31; 1982 males significantly larger than 1981 males: $t = 7.49$, $P < 0.001$). The upper limits of the size distribution of these toads remained the same from 1981 to 1982. This suggests that most of the toads 56 mm and less in SU length are first year breeders. If this is correct, then during the 1981 breeding season approximately twice as many toads were in their first breeding season as toads that were returning for at least a second year. Arbitrarily assuming annual breeding and fidelity to one site, we speculate that a high proportion of males in a breeding chorus are first and second year attendants and that proportionately few males breed for more than two seasons.

Tadpoles and Metamorphosis.—The first egg-strings of *B. houstonensis* laid in 1981 (18 February) hatched in seven days in water that ranged from 8°C to 17°C during this time period. These tadpoles metamorphosed 60–61 days after the eggs were deposited (19–20 April). Between hatching and metamorphosis, the water temperature ranged from 11°C to 31°C. The period from oviposition to metamorphosis was relatively constant during our study; the longest period we observed for eggs laid in February was 64–65 days (23 February to 28–29 April 1979), while the 60–61 day interval above was the shortest. Tadpoles ranged from 6.1 mm to 6.7 mm total length at hatching and reached a maximum total length of 20–22 mm. Just after metamorphosis, the young *B. houstonensis* were 7–9 mm in length.

In both 1979 and 1981, metamorphosis of most of the tadpoles from various clutches laid within several days of each other occurred over a period of a few hours at each of the sites. This resulted in postmetamorphic aggregations of *B. houstonensis* similar to those described for several other species of *Bufo* (Arnold and Wassersug, 1978). As the postmetamorphic *B. houstonensis* left the ponds, they moved in large numbers along the same gulleys that were used by the adults during the breeding season for

nightly trips to and from the ponds (see above). Movement of the postmetamorphic toads occurred during both day and night. During daylight, young *B. houstonensis* were found moving away from the ponds in gulleys as far as 100 m from the ponds.

At sites where two or more periods of breeding occurred, several size classes of *B. houstonensis* tadpoles overlapped temporally. At one site (#3) we observed *B. houstonensis* tadpoles from the first breeding period consuming the jelly envelopes of recently hatched *B. houstonensis* tadpoles from a second breeding period. None were observed eating eggs before hatching occurred.

Tadpoles of *B. houstonensis* were observed to form aggregations at several sites during the day, but they dispersed at night. During the day, *B. houstonensis* tadpoles (whether single or in aggregations) remained on the bottom of the ponds, whereas at night they fed on material attached to vegetation, on the surface of the water, and along the shore. During both 1979 and 1981 large numbers of tadpoles were found along a shore at which wind had accumulated 1–3-cm thick layers of pine pollen (*Pinus taeda*) from the surface of the pond. In both years, the intestines of the tadpoles were filled with pine pollen.

Hybridization and Isolating Mechanisms.—Fig. 2 shows the bivariate plot for scores of *B. houstonensis, B. woodhousei,* and *B. valliceps* (the a priori groups) as well as the three hybrid combinations for two canonical axes. The first canonical axis is defined by CA I = 2.05A − 0.57B − 0.43C + 1.40D + 1.08, where A = distance between the interorbital crests, B = length of the paratoid gland, C = length of the tibiofibula, and D = distance between the paratoid gland and the transverse axis of the postorbital crest (all to the nearest 0.1 mm). Likewise, the second canonical axis is defined by CA II = 0.44A − 0.64B + 0.42C − 1.86D − 3.50. This method of discrimination was found to be a reliable means of identifying the hybrid toads (Fig. 2) and was confirmed electrophoretically: all of the suspected hybrids had hybrid genotypes at six genetic loci, five of which were diagnostic for each comparison with *B. houstonensis* (see Tables 3 and 4). Mating calls are also useful for identifying calling hybrids (Brown, 1971). An additional character that can be used to identify some hybrid combinations is vocal sac coloration. Vocal sacs of *B. valliceps* are yellow, whereas those of *B. houstonensis* and *B. woodhousei* are black. The latter two species can also be distinguished on the basis of vocal sac condition: the skin that covers the vocal sac of *B. houstonensis* is translucent and the underlying muscle can be seen through it, whereas in *B. woodhousei* it is thick and

TABLE 3. Allelic frequencies of *B. woodhousei* (N = 10), *B. houstonensis* (N = 20), and *B. valliceps* (N = 10) at six presumptive genetic loci.

		Species		
Locus	Allele	*woodhousei*	*houston-ensis*	*valliceps*
SOD-1	55	—	—	1.000
	60	—	0.150	—
	65	0.350	—	—
	80	—	0.225	—
	100	—	0.575	—
	110	0.650	0.050	—
EST-2	90	—	0.125	—
	94	0.550	—	—
	100	—	0.450	—
	102	0.350	—	—
	105	—	0.425	—
	108	0.100	—	—
	110	—	—	0.250
	112	—	—	0.750
EST-3	100	—	1.000	—
	110	—	—	1.000
	120	1.000	—	—
LDH-1	100	—	0.700	—
	106	—	0.275	—
	108	—	0.025	—
	112	1.000	—	—
	125	—	—	1.000
ME-2	100	—	0.875	1.000
	110	—	0.125	—
	120	1.000	—	—
MDH-2	70	—	—	1.000
	100	1.000	1.000	—

TABLE 4. Observed genotypes of *B. woodhousei* × *B. houstonensis* hybrids (N = 4), *B. valliceps* × *B. houstonensis* hybrids (N = 8), and one presumed backcross of *B. woodhousei* × *B. houstonensis* to *B. houstonensis*. Parental origin of each of the alleles is indicated by the appropriate initial. SOD-1^{110}, indicated as an allele of *B. woodhousei* (marked with a star), was also present in low frequency (0.05) in the sample of *B. houstonensis*. ME-2^{100} is present in both *B. houstonensis* and *B. valliceps*, and MDH-2^{100} is present in both *B. woodhousei* and *B. houstonensis*.

	Hybrid combination		
Locus	*woodhousei* × *houstonensis*	(*woodhousei* × *houstonensis*) × *houstonensis*	*valliceps* × *houstonensis*
SOD-1	80-H/110-W* (2) 60-H/65-W (1) 65-W/100-H (1)	60-H/110-W* (1)	55-V/60-H (1) 55-V/80-H (2) 55-V/100-H (5)
EST-2	90-H/102-W (1) 94-W/100-H (1) 94-W/105-H (2)	100-H/105-H (1)	100-H/110-V (2) 100-H/112-V (2) 105-H/110-V (1) 105-H/112-V (3)
EST-3	100-H/120-W (4)	100-H/100-H (1)	100-H/110-V (8)
LDH-1	100-H/112-W (3) 106-H/112-W (1)	100-H/112-W (1)	100-H/125-V (5) 106-H/125-V (3)
ME-2	100-H/120-W (4)	100-H/120-W (1)	100-H/100-V (8)
MDH-2	100-H/100-W (4)	100- ?/100- ? (1)	70-V/100-H (8)

opaque. *B. valliceps* × *B. houstonensis* hybrids have a small amount of yellow pigment on their vocal sacs, and are readily distinguishable from either parental species. *B. houstonensis* × *B. woodhousei* hybrids are also intermediate with respect to the parental species in vocal sac morphology, but approach the *B. woodhousei* condition more closely.

Hybridization among the three species is low within the study area. No *B. woodhousei* × *B. valliceps* hybrids were found at any of the study sites (the individuals represented in Fig. 2 are from Austin, in adjacent Travis Co.). *B. woodhousei* × *B. houstonensis* hybrids were found at only one of the study sites (#2), and only four F_1-hybrids (as well as one probable backcross to *B. houstonensis*) were found there. *B. valliceps* × *B. houstonensis* hybrids were found at two of the sites—two at site 4 and six at site 6. In each of these cases, hybrids represent less than 1% of the parental populations. Although no *B. valliceps* × *B. houstonensis* hybrids were found during 1981 at site 5, Nancy Jacobson (pers. comm.) found one such hybrid out of 387 marked or individually recognizable *B. houstonensis* at this locality in 1982 (Fig. 2).

The low levels of hybridization and the restriction of hybrids to only a few localities suggest that premating isolating mechanisms are well developed among *B. houstonensis*, *B. woodhousei*, and *B. valliceps*. Brown (1971) considered the premating isolating mechanisms among these three species and concluded that ". . . all premating isolating mechanisms except mating call and size seem to have partially broken down." He noted that breeding seasons of the three species overlapped widely, considered habitat isolation to be poorly developed, and reported that differences in species densities were a "possible contributing cause of natural hybridization."

At present *B. houstonensis* and *B. valliceps* seem to be isolated primarily by differences in their respective breeding seasons (Fig. 3). No actual breeding of *B. valliceps* occurred before May in 1981, although a few males of this species were present at some of the sites as ear-

ly as 3 April. In contrast, only one female *B. houstonensis* was found after 2 April in 1981 (on 2 May). W. F. Blair (1963) reported that laboratory crosses of females of the *B. valliceps* species group to males of the *B. americanus* species group result in death of the offspring before metamorphosis; therefore, the only hybridization between *B. houstonensis* and *B. valliceps* expected to result in adults is the reciprocal combination (Brown, 1971). Kennedy (1962) reported offspring raised to maturity from such a cross. In 1981, no hybridization between these two species was observed at any of our study sites, and the only female *B. houstonensis* that we found after the seasonal onset of chorusing of *B. valliceps* arrived at site 3 on a night when no *B. valliceps* were present. This female was found in amplexus with a conspecific male.

In some years, temporal isolation of *B. valliceps* and *B. houstonensis* is not so complete as it was in 1981, as evidenced by the eight hybrids between these two species present at choruses in 1981. In 1979 we found a female *B. houstonensis* in amplexus with a conspecific male on 17 April in a chorus of 25 *B. houstonensis* and five *B. valliceps*. Brown (1971) found two male *B. valliceps* in amplexus with two female *B. houstonensis*, with one male *B. houstonensis* calling at the site (date not reported).

All of the hybrids between *B. houstonensis* and *B. valliceps* were present at choruses that were composed primarily of the former species, but with a few males of the latter species calling as well (Fig. 3). These hybrids are reportedly sterile (Brown, 1971), so presumably backcrossing cannot occur (no backcrossed individuals were found in our electrophoretic analysis). All of the females present at these choruses were *B. valliceps*, each of which was in amplexus with a conspecific male.

The breeding seasons of *B. houstonensis* and *B. woodhousei* are similar in the Bastrop area (Fig. 3), so temporal isolation is not in effect between these two species. However, *B. houstonensis* and *B. woodhousei* are essentially parapatric in the Bastrop area with virtually no overlap in their ranges (Fig. 1; Brown, 1971). *B. houstonensis* occurs throughout most of the approximately 20,000-hectare disjunct island of *Pinus taeda* in Bastrop Co., whereas *B. woodhousei* populations surround this island of pine forest. This co-occurrence of *B. houstonensis* and *Pinus taeda* in Bastrop Co. (there are no pines in some other parts of the range of *B. houstonensis*) is probably due to the fact that pines grow only in sandy soil in the region (Youngman, 1965), and *B. houstonensis* also displays a strong preference for this type substrate.

Breakdown of the habitat isolation between *B. woodhousei* and *B. houstonensis* probably can occur whenever clearing activities by humans around the periphery of the pine forest cause erosion and other disturbance (pers. obs.; Brown, 1971). In 1981, we found *B. houstonensis* × *B. woodhousei* hybrids at only one locality (site 2)—a pair of lakes that were constructed in the early 1970's in an area that was cleared on the edge of the pine forest (Fig. 1). Much of the sand in the area has been lost to erosion, and presumably *B. woodhousei* has invaded what had been *B. houstonensis* habitat, with limited hybridization the result. In 1981, the *B. houstonensis* population at this site was much larger than the population of *B. woodhousei*; however, with continuing change of habitat through exposure, construction, and erosion, this area may become more favorable to *B. woodhousei* and less favorable to *B. houstonensis*. Evidence for this possibility rests with the condition in 1981 of a previously reported (Brown, 1971) site of hybridization (during the mid-1960's) near site 11. In 1979–1981, *B. woodhousei* was present at this locality but *B. houstonensis* was absent (Fig. 1). The hybridization was reported shortly after construction had altered the area (Brown, 1971); by 1979, the area apparently had become unsuitable for supporting populations of *B. houstonensis*.

Besides the two primary premating isolating mechanisms considered above, several other mechanisms are probably effective in further reducing hybridization. As reported by Brown (1971), male *B. houstonensis* are significantly smaller than either male *B. woodhousei* or male *B. valliceps* (Table 1). Female *B. houstonensis* may be able to discriminate between the males of these species by size, or male *B. houstonensis* may be too small to effectively clasp some females of the other species. However, the size range of female *B. woodhousei* widely overlaps the size range of female *B. houstonensis*. In addition, the actual limitations of amplexus size-differential are not reached by the difference in size of male *B. houstonensis* and female *B. woodhousei*; a small male *B. houstonensis* (55 mm) experimentally placed with a large female *B. woodhousei* (90 mm) grasped the female and remained in amplexus for eight days (the female *B. woodhousei* was not in breeding condition). The other reported possible premating isolating mechanism is the difference among the three species in mating calls (Brown, 1971). All three species can be recognized by their mating calls (as can the hybrid combinations); presumably, the females of each species are also able to distinguish the calls of conspecific males from those of other species.

Status of the Extant Populations.—*B. houstonensis* has been reported from seven counties in southeastern Texas (W. F. Blair, 1956*a*; Brown, 1971; and Sanders, 1953). Since 1977, *B. houstonensis* has been found only in Bastrop and Burleson counties, and only five males have been found at the Burleson Co. locality from 1981 to 1983 (J. R. Dixon, pers. comm.). Older Harris Co. and Fort Bend Co. localities for *B. houstonensis* have been considerably altered by the expansion of Houston. We visited the Austin Co., Liberty Co., and Colorado Co. localities in 1981, and found areas where seemingly favorable habitat remained, especially at the Liberty Co. locality. However, no *B. houstonensis* have been reported from any of these localities since the 1950's. At present, the only known substantial populations of *B. houstonensis* are in Bastrop Co.

In Bastrop Co., populations of *B. houstonensis* are large and seem to occur throughout the areas that are forested in pine. Brown (1975) estimated that in 1967 "there were probably no more than 300 *B. houstonensis* (possibly less than 100) in Bastrop Co.," but also noted that it was difficult to estimate population sizes for the species. Our study indicates the present existence of much higher population levels. An accurate population estimate remains a difficult objective, especially considering that only a small portion of the pine forest in Bastrop Co. has been surveyed. However, present population sizes of *B. houstonensis* in Bastrop Co. appear not to be critically low; the restricted range of the species coupled with habitat destruction within this area seem to be the primary factors in the endangerment of *B. houstonensis*.

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