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UNISEXUAL *AMBYSTOMA* FROM THE BASS ISLANDS
OF LAKE ERIE

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The *Ambystoma jeffersonianum* complex as currently recognized consists of two diploid ($2n = 28$), biparental and two triploid ($3n = 42$), gynogenetic species (Clanton, 1934; Minton, 1954; Uzzell, 1964). The triploid *A. tremblayi* is associated with certain populations of the northern *A. laterale*, whereas the second triploid, *A. platineum*, occurs with those of the more southerly distributed *A. jeffersonianum*. The triploid eggs of the gynogenetic females result from the reductional division of germ cells that enter meiosis in a temporarily hexaploid condition (MacGregor and Uzzell, 1964; Uzzell, 1970; Cuellar, 1976). On the basis of morphological and geographic intermediacy, and the fact that males of both diploid species can successfully court either of the triploids, Uzzell (1964) considered the unisexual populations to have originated through hybridization of the two diploids. An analysis of electrophoretic patterns of serum proteins strongly supported this theory of hybrid origin (Uzzell and Goldblatt, 1967).

The triploid populations occur where the geographic ranges of the diploid species approach one another or interdigitate. Although Uzzell and Goldblatt (1967) recorded both *A. tremblayi* and *A. platineum* in northwestern Ohio, other unisexual populations from that region are not referable to either form and suggest that the genomes of unisexual

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Ambystoma are more varied than previously supposed. The present paper evaluates the status of such populations from North Bass and Middle Bass Islands in the western basin of Lake Erie. These populations are significant because (1) the unisexuals, although including triploids, are predominately diploid; (2) biparental species are apparently absent from one of the islands that supports a unisexual population; (3) the origin of the unisexuals appears to have involved hybridization between a member of a *A. jeffersonianum* complex (*A. laterale*) and a species (*A. texanum*) that is apparently only distantly related to that complex.

MATERIALS AND METHODS

Uzzell (1964) pointed out the difficulty, and often impossibility, of distinguishing between preserved diploid and triploid females of the *A. jeffersonianum* complex. Fortunately, the ploidy of living salamanders can usually be ascertained from differences in the sizes of the erythrocytes (Davison, 1959; Uzzell, 1964). While investigating populations of *Ambystoma* in the Great Lakes region, I have recorded data from some 700 preserved specimens, including many of unknown ploidy. The conclusions presented here, however, are largely based on about 400 specimens collected since 1966, and for which ploidy was known or could be surmised from the mean erythrocyte area. I have also relied on data recorded from preserved material, of known ploidy, identified by Uzzell during his earlier study.

The recognition of the triploid species, and of their surprisingly extensive geographic distribution (see Uzzell, 1967a and 1967b; Creusere, 1971; Briggs, 1974; Gilhen, 1974; Morris, 1974; Weller and Sprules, 1976) has cast doubt on the composition of many populations of the complex sampled prior to these discoveries. This uncertainty is increased by the discovery reported herein that a third bisexual has contributed to the genomes of certain unisexual populations. I have therefore compared the island populations most closely with selected mainland populations of known composition. These include *A. laterale* and *A. tremblayi* from Washtenaw and Livingston counties, Michigan; *A. jeffersonianum* and *A. plattineum* from Lorain Co., Ohio; and *A. jeffersonianum* and *A. texanum* from Wayne Co., Ohio. Since *A. jeffersonianum* and its

triploid associate *A. platineum* have apparently not influenced the island populations, data for these species are largely omitted from this report. Comparable data, as well as photographs of the four members of the *A. jeffersonianum* complex, are provided by Uzzell (1964).

Salamanders were maintained in the laboratory for various lengths of time. Coloration was recorded for many of these, and cell size determined for all. Blood taken from toe webbing was suspended in amphibian Ringer's solution and the long and short axes of 20 erythrocytes measured with a calibrated ocular micrometer. Areas were calculated and averaged to give a mean erythrocyte area. This method differs from the planimetric technique employed by Uzzell (1964), but provides comparable values. The relationship between cell size and ploidy was determined by preparing karyotypes of some individuals and by noting nucleolar numbers in others.

Chromosomal preparation followed, with modifications, the procedure outlined by Cuellar and Kluge (1972), using velban as an arrestor and intestinal mucosa as a tissue source. For salamanders, the incubation period was modified to between 10 and 12 hours at room temperature, and the pieces of excised intestine were bathed in the sodium citrate solution for up to three hours prior to staining with aceto-orcein. Sealed temporary mounts were searched and photographed through phase optics. Testes, when available, were similarly treated, but remained in the citrate solution for only 15 minutes. Healthy animals produced adequate numbers of countable spreads, but ambystomatid chromosomes are large and spreads without overlapping chromosomes were rarely observed. Nucleolar counts were recorded from small pieces of tail tip, minced and squashed in 45 percent glacial acetic acid, stained with Giemsa, and destained in 3 percent methanol.

The electrophoretic analysis of serum proteins was based primarily on the results of a vertical slab procedure carried out in the laboratory of Stephen G. Tilley at Smith College. The gels were 7.5 percent acrylamide, catalyzed by ammonium persulfate and TMED. Tris HCl (pH 8.9) served as the gel buffer, and tris glycine (pH 8.3) as the bath buffer. The initial voltage of 350 v was increased to 450 v after the first 20 minutes. The proteins were separated during a period of two hours, and stained with amido black.

Total length and standard length (snout to posterior angle of vent) were measured on preserved specimens with a millimeter rule. The number of costal grooves, including one each in axilla and groin, and costal folds between adpressed limbs were recorded. The lower jaw was ranked as projecting forward beyond the upper, even with the tip of the upper, or ending short of it. Snout length, canthal length, and internarial distance were measured directly to the nearest 0.1 mm with an ocular micrometer.

Teeth on the premaxillary-maxillary arch were counted, and their arrangement in one or in multiple rows was noted. Similar information was recorded for the medial and, if present, lateral rows of prevomerine teeth. When present, the lateral patches of teeth were scored as spatially separated from, or contiguous with, the medial patch. In some specimens the shape of the teeth was noted. The plicae of the tongue were scored as radiating from the posterior margin of the lingual pad or diverging from a medial furrow.

Marked differences between certain skeletal elements characterize the subgenera *Ambystoma*, which includes the *A. jeffersonianum* complex, and *Linguelapsus*, which includes *A. texanum* (Tihen, 1958). A few specimens were therefore macerated to provide material for a comparison of premaxillaries, prevomers, and parasphenoids.

Breeding experiments were carried out in natural ponds in Scio Tp., Washtenaw Co., Michigan in the spring of 1971, and in Chester Tp., Wayne Co., Ohio during 1973, 1974, and 1975. Island females captured during the spring breeding season have not been available. Attempts were made to bring salamanders into breeding condition by overwintering them in outdoor cages in Wayne Co., but these were unsuccessful and resulted in the loss of many specimens. As a result, only females maintained in the laboratory through the winter have been available for breeding studies. Such females were confined alone or with one or more males in cylindrical breeding cages fashioned like those used by Uzzell (1964). Cages were checked daily for spermatophores or eggs until the experiment was terminated. In 1971, males of *A. laterale* and *A. texanum* were trapped at drift fences, or in minnow traps within the water, in Washtenaw Co. In subsequent years, males of *A. texanum* from two sites in Wayne Co., Ohio were utilized. Partial fencing of the ponds was ineffective at these sites, and therefore most of the males were

trapped in the water. Such males may have courted females prior to entering a trap, and usually had the opportunity to court within the trap itself. Since the prior reproductive history of a given male was often unknown, males failing to court in the breeding cages within a few days of introduction were usually replaced by a second male. Similarly, a female failing to elicit courtship from a male known to be unmated was replaced by another female.

RESULTS AND CONCLUSIONS

SEX RATIOS AND PLOIDY

Available evidence indicates that males outnumber females in breeding aggregations of biparental species of *Ambystoma* (Husting, 1965; Uzzell, 1969). In the *A. jeffersonianum* complex, however, mixed populations of biparental and uniparental species may exhibit extraordinary percentages of females due to the abundance of the uniparental triploids (Uzzell, 1964; Wilbur, 1971). Although the island specimens were not collected during the breeding season, evidence for a preponderance of females is overwhelming. Indeed, all of the 113 sexed individuals from North Bass Island are females, as are ninety (93 percent) of the 97 from Middle Bass Island.

A predominance of triploids in the island populations, anticipated on the basis of the sex ratios, is not indicated by the data on erythrocyte sizes. Mean erythrocyte areas from island individuals, although including some that clearly indicate a triploid genome, fall largely in a size range intermediate between those characterizing the diploid *A. laterale* and its triploid associate, *A. tremblayi* (Fig. 1). The correlation between cell size and ploidy was determined by chromosomal and nucleolar counts. Karyotypes are available for 22 individuals; of these, the ploidy of sixteen had been previously determined, without contradiction, by nucleolar counts. The ploidy of an additional 57 salamanders was ascertained by the nucleolar technique. The mean erythrocyte areas of 62 diploids range from 750 to $1040\mu^2$, and those of 17 triploids from 1030 to $1400\mu^2$ (areas rounded to nearest $10\mu^2$). For diploids, the mean of means was 873.1 and the standard error 8.11; for triploids, 1170.0 and 26.78. Although a narrow zone of overlap, and uncertain ploidy,

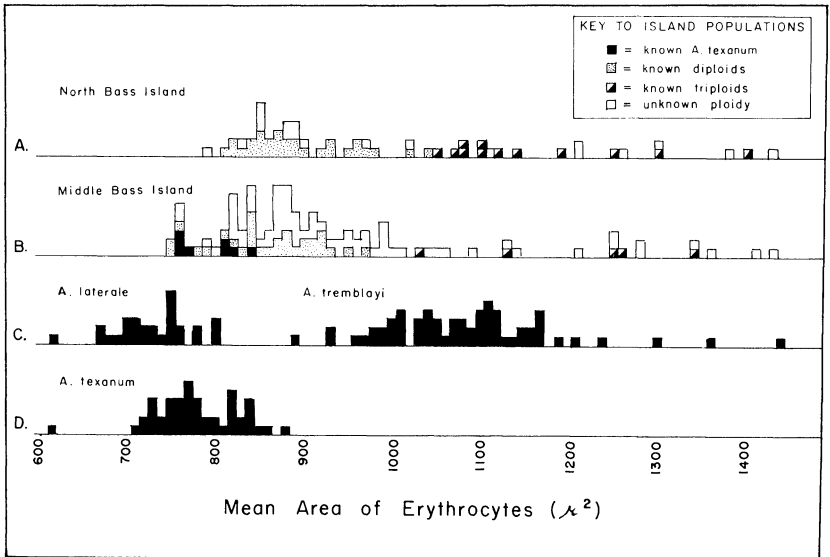


FIG. 1. Individual mean erythrocyte areas (μ^2) of salamanders of the genus *Ambystoma* from selected localities. A. North Bass Island, Ottawa Co., Ohio; B. Middle Bass Island, Ottawa Co., Ohio; C. Washtenaw Co., Michigan (*A. laterale* and *A. tremblayi*); D. Wayne Co., Ohio (*A. texanum*).

exists, the distribution of mean erythrocyte areas clearly indicates that most of the island individuals are diploid. Cell sizes (760 to $810\mu^2$) are available for five of the seven males known from Middle Bass Island, and are comparable to those of diploid males of *A. laterale*, *A. jeffersonianum*, and *A. texanum*.

IDENTITY OF THE DIPLOID BISEXUALS

On the basis of their respective geographic ranges, both *A. laterale* and *A. texanum* might be expected to inhabit the islands. The seven males known from Middle Bass Island are all, however, assignable to *A. texanum*. They agree with males of that species, and contrast to those of *A. laterale*, in having a short and narrow snout (Table 1; Fig. 2), the lower jaw projecting forward beyond the upper, 14 or 15 costal grooves (Table 2), teeth arranged in more than a single row, no prevomerine tooth rows lateral to the internal nares, and in the presence of a lingual furrow. The largest island males are considerably larger than males of *A. laterale*, but comparable in

TABLE 1
RELATION OF STANDARD LENGTH TO SNOUT LENGTH AND INTERNARIAL DISTANCE IN SELECTED POPULATIONS
OF *AMBYSTOMA*.

Population	Sex	N ¹	Standard length (mm)		Snout length (mm)		Internarial distance (mm)	
			O.R. ¹	\bar{x} ¹	O.R.	\bar{x}	O.R.	\bar{x}
Diploid Bisexuals:								
<i>A. laterale</i> Washtenaw Co.	♂♂	22	47-60	(52.91)	0.61-0.80	(0.71)	0.50-0.59	(0.54)
	♀♀	18	46-69	(56.61)	0.61-0.76	(0.67)	0.45-0.57	(0.52)
<i>A. texanum</i> Wayne Co.	♂♂	58	63-85	(72.85)	0.42-0.60	(0.49)	0.24-0.35	(0.31)
	♀♀	36	62-88	(77.78)	0.41-0.56	(0.48)	0.26-0.32	(0.29)
Middle Bass Island	♂♂	7	57-87	(69.71)	0.45-0.58	(0.49)	0.26-0.33	(0.31)
	♀♀	3	63-80	(73.00)	0.43-0.51	(0.48)	0.29-0.32	(0.30)
Diploid Unisexuales:								
Middle Bass Island	♀♀	50	58-77	(66.84)	0.50-0.70	(0.59)	0.38-0.47	(0.42)
North Bass Island	♀♀	27	55-77	(68.82)	0.49-0.73	(0.55)	0.37-0.45	(0.43)
Triploid Unisexuales:								
Middle Bass Island	♀♀	10	63-89	(70.90)	0.50-0.66	(0.56)	0.32-0.42	(0.38)
North Bass Island	♀♀	7	63-82	(72.43)	0.48-0.64	(0.56)	0.33-0.40	(0.36)
<i>A. tremblayi</i> Washtenaw Co.	♀♀	55	57-82	(68.96)	0.58-0.86	(0.70)	0.47-0.61	(0.53)

¹N = sample size; O.R. = observed range; \bar{x} = sample mean.

size to those of *A. texanum* (Table 1). The color pattern features the grayish-white frosting and lichen-like lateral blotches of *A. texanum* rather than the blue-spotted pattern of *A. laterale* (Fig. 6).

Three females from Middle Bass Island share this suite of characters with the males, and can be confidently referred to *A. texanum*. The mean cell sizes of these females (760 to 835 μ^2) are within the range of variation shown by *A. texanum* on the mainland. All have 15 costal grooves, narrow snouts (Fig. 2), and lack lateral patches of prevomerine teeth.

The failure to discover a single male among 113 specimens from North Bass Island is strong evidence that bisexual species are absent. The 95% confidence limits for this percentage of males are 0.0 and 3.2 percent (Rohlf and Sokal, 1969). The specific absence of *A. texanum* is further indicated by the absence of specimens with 15 costal grooves, the modal number for that species on Middle Bass Island. I have seen no living individuals from North Bass Island that immediately impressed me as belonging to one of the bisexual species. In contrast, the

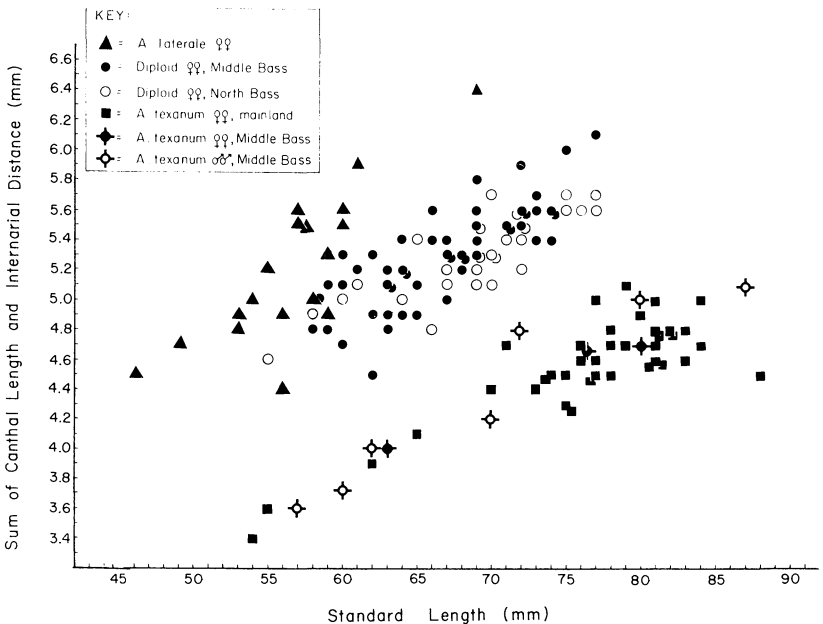


FIG. 2. The relation of the sum of the canthal length and internarial distance to standard length in diploid bisexual and unisexual *Ambystoma*.

TABLE 2
ABSOLUTE AND RELATIVE TAIL LENGTHS IN SOME
POPULATIONS OF *AMBYSTOMA*.

Population	Sex	N ¹	Tail length (mm)		Tail length (mm)	
			O.R. ¹	\bar{x} ¹	Standard length (mm)	\bar{x}
Diploid bisexuals:						
<i>A. laterale</i>	♂♂	20	33-59	(43.75)	0.62-0.98	(0.83)
Washtenaw Co.	♀♀	16	36-55	(44.50)	0.64-0.85	(0.79)
<i>A. texanum</i>	♂♂	50	42-70	(55.76)	0.64-0.94	(0.77)
Wayne Co.	♀♀	32	48-71	(58.22)	0.65-0.84	(0.75)
Diploid unisexuals:						
Middle Bass Island	♀♀	45	39-62	(49.73)	0.59-0.90	(0.74)
North Bass Island	♀♀	23	43-63	(54.65)	0.71-0.89	(0.80)

¹N = sample size; O.R. = observed range; \bar{x} = mean.

A. texanum from Middle Bass Island were immediately recognized.

THE DIPLOID UNISEXUALS

The majority of the salamanders from the island populations are diploid females for which there are no corresponding males. The characteristics of these diploid unisexuals are generally intermediate between those of *A. laterale* and *A. texanum*. Standard lengths of mature unisexuals overlap, but fall generally between, those of mature females of the two bisexual species (Table 1). The mean tail lengths of the island populations are also between those of the bisexual forms. In relative tail length, the sample mean of the population from North Bass Island is greater than that from Middle Bass Island, and comparable to females of *A. laterale*. The sample from Middle Bass Island is closer to *A. texanum* in relative tail length (Table 2).

The relative length of the limbs decreases with increasing body size in all forms, and since *A. laterale* is a smaller species than *A. texanum* it seemingly has longer limbs. Comparably-sized individuals of the two species, however, have equivalent leg lengths. The adpressed limbs of *A. laterale* may overlap by as

many as two costal folds in small individuals, but be separated by three folds in large ones. In *A. texanum*, the addressed limbs are separated by from 1.5 to 5.5 folds. Reflecting the intermediate size of the individuals, the addressed limbs of the diploid unisexuals are neither as relatively long as in small *A. laterale* nor as short as in the largest individuals of *A. texanum* (Fig. 3).

The modal number of costal grooves is 13 in *A. laterale* and 14 in *A. texanum* from the mainland. In the small sample

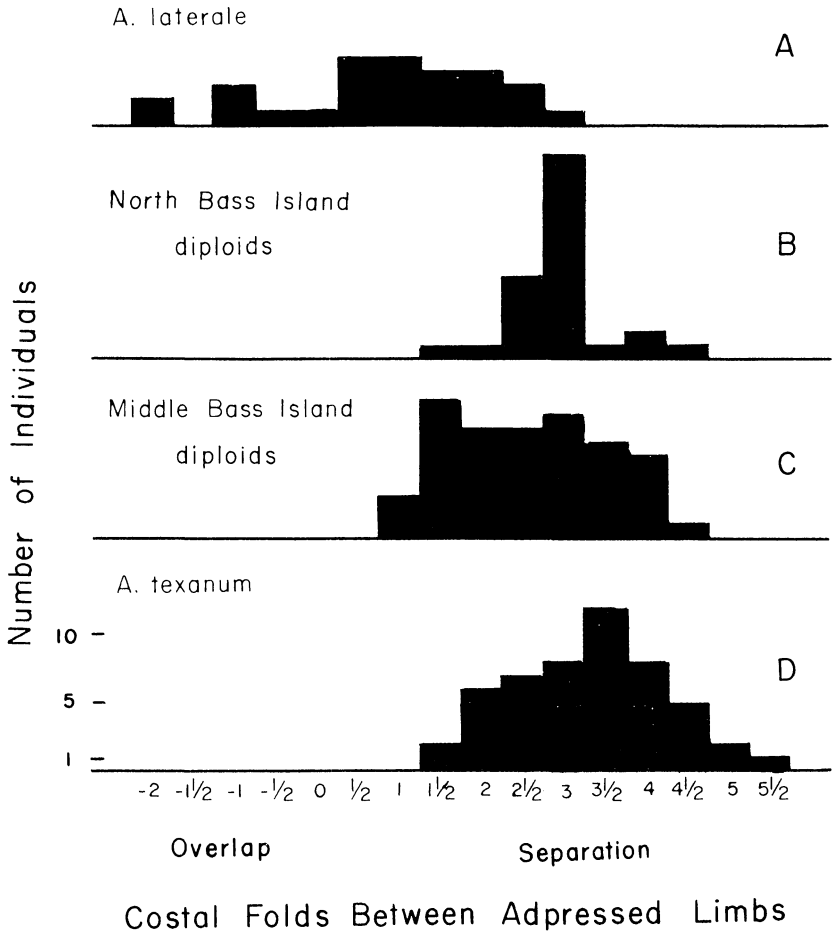


FIG. 3. The number of costal folds between the addressed limbs of diploid females of unisexual and bisexual *Ambystoma*. A. *A. laterale*; B. Diploid unisexuals, North Bass Island; C. Diploid unisexuals, Middle Bass Island; D. *A. texanum*.

of the latter species from Middle Bass Island, the modal number is 15. Most diploid unisexuals from the Bass Islands have 14 costal grooves, but those that do not have 13 (Table 3).

Marked differences in snout and canthal length, and in snout width, distinguish *A. laterale* and *A. texanum*. When these dimensions are related to standard length, the values for the island females are intermediate between those of the two bisexual species (Table 1). This intermediacy is clearly shown when measures of the length and width of the snout are summed and graphed against standard length (Fig. 2). The relative length of the lower jaw also differs in the bisexual forms. In the members of the *A. jeffersonianum* complex, including *A. laterale*, the upper jaw protrudes forward and overhangs the tip of the lower, whereas in *A. texanum* the lower projects beyond the upper. Among diploid unisexuals from Middle Bass Island, the lower jaw projected in nine individuals, the jaws were of equal length in 24, and the upper jaw exceeded the lower in 17. The number of specimens from North Bass Island in these categories were 10, 15, and 3 respectively. A similar ambiguity of character expression characterizes the tongue of the island females. Some 74 percent of them were scored as similar to *A. laterale* in lacking a medial furrow and having the plicae radiating from the posterior part of the lingual pad. Six percent had the *A. texanum* condition of plicae diverging from a lingual furrow. The remaining 20 percent, perhaps due to

TABLE 3

NUMBER OF COSTAL GROOVES IN CERTAIN POPULATIONS OF *AMBYSTOMA*

Population	Number of Costal Grooves			
	12	13	14	15
Diploid bisexuals:				
<i>A. laterale</i> , Washtenaw Co.	1	41	19	
<i>A. texanum</i> , Wayne Co.			71	29
<i>A. texanum</i> , Middle Bass Island			3	7
Diploid unisexuals:				
Middle Bass Island		9	41	
North Bass Island		3	24	
Triploid unisexuals:				
Middle Bass Island			10	
North Bass Island			7	
<i>A. tremblayi</i> , Washtenaw Co.		47	7	

artifacts of preservation, were unassignable either because the plicae appeared divergent despite the absence of a groove or because a lingual furrow was present but the plicae were not divergent.

Premaxillary and maxillary teeth are characteristically arranged in a single row in *A. laterale*, but in at least two rows in *A. texanum*. In 22 diploid unisexuals from the Bass Islands, the teeth are in a single row, and therefore comparable to *A. laterale*. The majority of individuals (47), however, were ranked in an intermediate position. This intermediate condition ranged from having at least some teeth, usually in the premaxillary region, crowded into a staggered row to having a well defined second row along part of, but not more than one-half of, the dentiferous arch. None of the island unisexuals exhibit the full expression of the condition found in *A. texanum*. The shape of individual teeth also differs in the bisexual species. In labial aspect, the tooth crown in *A. laterale* is bluntly rounded and about as high as broad. The dominant cusp in *A. texanum* is sharply pointed and often thrice as long as broad. Island unisexuals have pointed cusps of intermediate length.

Despite the multiple-rowed tooth arrangement in *A. texanum*, the combined number of premaxillary and maxillary teeth is not significantly different in the female samples of the bisexual species (Table 4). The shorter dentiferous arch imposed by the short and narrow snout of *A. texanum* apparently offsets any effect of the additional tooth row in that species. The mean number of teeth in the sample of diploid unisexuals from Middle Bass Island is comparable to that of the sample from North Bass Island, but significantly higher than in female *A. laterale* ($t = 3.2987$, $p < 0.01$) and *A. texanum* ($t = 5.7352$, $p < 0.001$). Unisexuals from North Bass Island also have significantly higher tooth counts than either *A. laterale* ($t = 2.2538$, $p < 0.05$) or *A. texanum* ($t = 3.9666$, $p < 0.001$).

In dorsal view the nasal process of the premaxilla in *A. laterale* is a broad, flat structure (Fig. 4). In *A. texanum*, the process is laterally compressed and vertically thickened just behind the dentiferous arm. In six macerated skulls of *A. texanum*, the narrowest width of this constricted neck averages 5.8 percent (range, 4 to 7) of the total length of the premaxilla. The corresponding point in three skulls of *A. laterale* averages 14.0 percent (range, 10 to 17) of the total length, and for five island unisexuals 9.2 percent (range, 8 to 11). A similar

TABLE 4
NUMBER OF PREMAXILLARY-MAXILLARY AND PREVOMERINE TEETH
IN FEMALES OF SELECTED POPULATIONS OF *AMBYSTOMA*.

Population	NUMBER OF TEETH			
	Premaxillary-maxillary		Prevomerine	
	N ¹	$\bar{x} \pm 95\%$ conf. lim.	N	$\bar{x} \pm 95\%$ conf. lim.
<i>A. laterale</i> Washtenaw Co.	17	95.41 \pm 3.65 (85-113)	18	34.11 \pm 1.64 (29-39)
<i>A. texanum</i> Wayne Co.	28	89.50 \pm 4.71 (68-110)	30	28.30 \pm 1.62 (21-37)
Diploid Unisexuales:				
Middle Bass Island	50	101.86 \pm 1.97 (88-115)	50	39.92 \pm 1.27 (24-50)
North Bass Island	27	99.48 \pm 1.94 (84-105)	27	37.82 \pm 1.38 (32-45)

¹The prevomerine count includes the teeth of the medial row and, when present, those in isolated lateral rows. The observed range is given parenthetically below the mean and 95 percent confidence limits of the mean. N = sample size.

proportional intermediacy characterizes the shape of the parasphenoid in the island females. In *A. texanum*, the lateral expansion of the posterior third of the parasphenoid is particularly pronounced, its greatest width ranging from 56 to 60 percent (mean, 57.2) of the length of the bone in six skulls. In three *A. laterale* this ratio ranges from 45 to 49 percent (mean, 46.7), and in five unisexuales from the island populations from 50 to 57 percent (mean, 51.8).

The prevomers of *A. laterale* and *A. texanum* are highly distinctive (Fig. 5). The sutural margins are relatively smooth in the former species, but jagged in the latter. In *A. texanum*, a bony shelf projects posteriorly beyond the row of teeth. A comparable structure is but poorly developed in *A. laterale*. Most impressively, the prevomer of *A. laterale* has a postero-lateral extension that forms the entire posterior margin of the internal nares; it bears, lateral to the nares, an isolated short row of teeth. In *A. texanum*, this extension is shorter, forms only the postero-medial margin of the nares, and lacks teeth. Diploid unisexuales from the Bass Islands are strikingly diverse in the

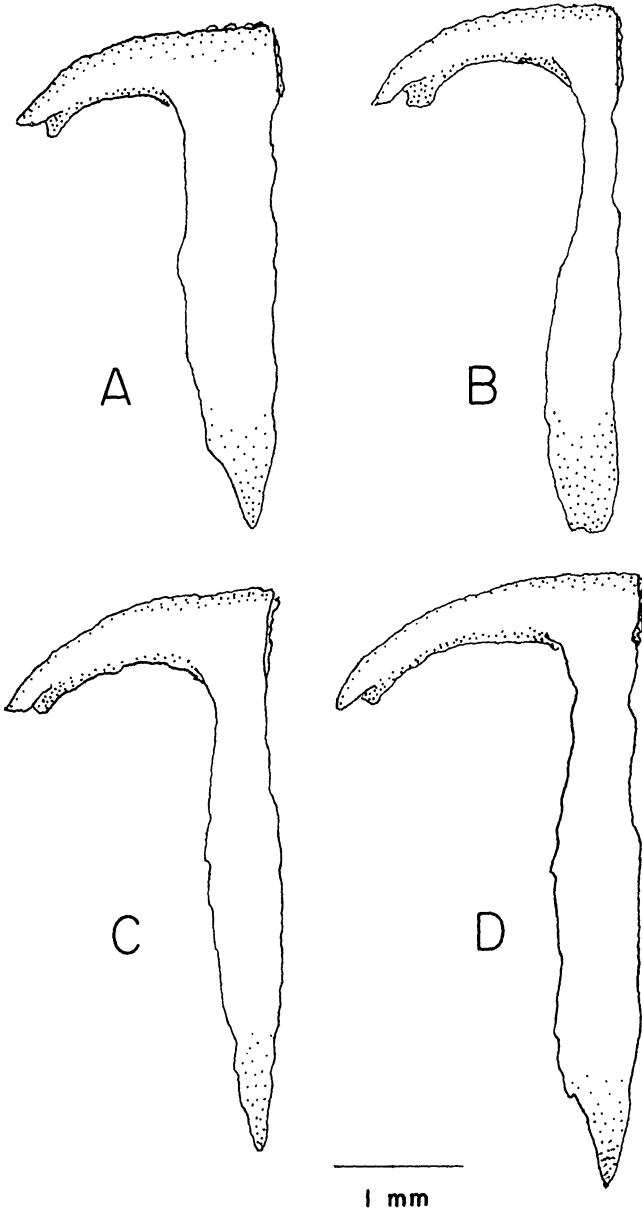


FIG. 4. Dorsal view of premaxillaries of bisexual and unisexual *Ambystoma*. A. *A. laterale*, Washtenaw Co., Michigan; B. *A. texanum*, Wayne Co., Ohio; C. Diploid unisexual, Middle Bass Island, Ottawa Co., Ohio; D. Triploid unisexual, Middle Bass Island, Ottawa Co., Ohio.

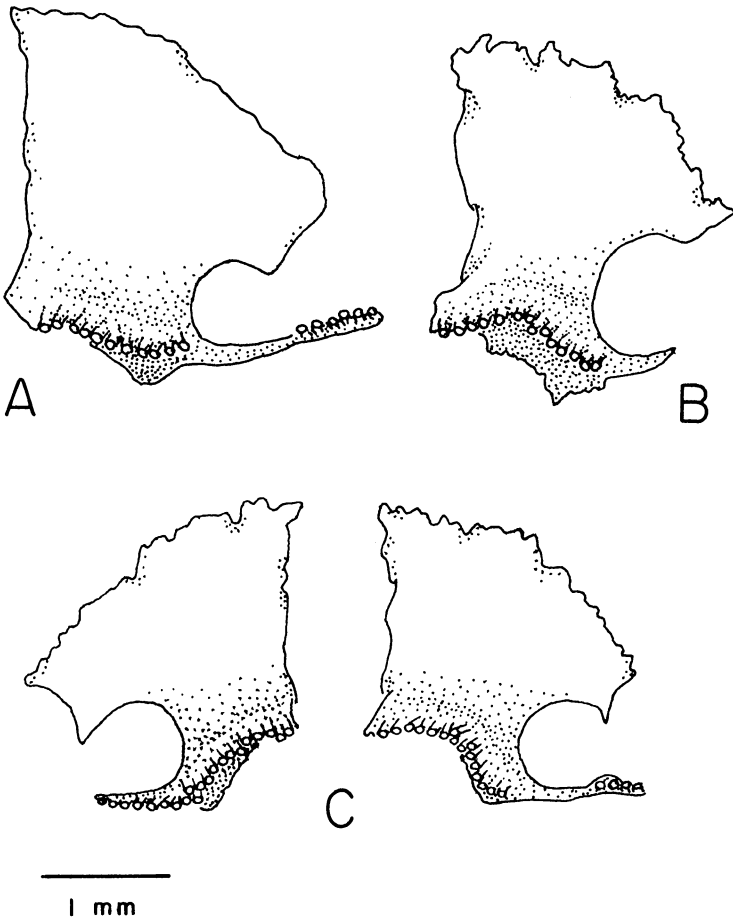


FIG. 5. Ventral view of prevomers of bisexual and unisexual *Ambystoma*. A. Left prevomer of *A. laterale*, Washtenaw Co., Michigan; B. Left prevomer of *A. texanum*, Wayne Co., Ohio; C. Asymmetrical right and left prevomers of a diploid unisexual from Middle Bass Island, Ottawa Co., Ohio.

structure of the prevomer. Although the postero-lateral extension may be somewhat shorter and bear fewer teeth, the prevomers of 42 individuals agree with those of *A. laterale* in possessing an isolated lateral row of teeth on each side of the palate. Two individuals have only the medial row of prevomerine teeth, and therefore agree with *A. texanum*. Eleven specimens have an intermediate condition in which the lateral teeth are present, but are borne on a short extension and are thus contiguous with

those of the medial row. The prevomers of the remaining 23 individuals are asymmetrical, with an isolated lateral row of teeth on one side of the head and the more medial, contiguous row on the other (Fig. 5). The sculpturing of the sutural margins, although difficult to quantify, also appears, in the unisexuales, intermediate between the conditions found in the two bisexual species. Differences in the average number of prevomerine teeth parallel those for the jaw teeth in that the highest values are found in the island populations (Table 4). The sample mean for diploid unisexuales from Middle Bass Island is slightly, but significantly ($p < 0.05$), greater than that for females from North Bass Island. The differences between *A. laterale* and *A. texanum*, and between each and the two island populations, are significant at a 99 percent level of confidence.

Living individuals of *A. laterale* and *A. texanum* are readily distinguished on the basis of coloration. The former is a blackish salamander with generally discrete blue spots along the sides of the body. In *A. texanum*, the light pigment is pale gray and distributed in lichen-like patches that often coalesce into a more or less continuous suffusion of the light pigment. The diffuse blotches often extend to the mid-dorsal region, but are darker and less conspicuous there. The pale gray flanks contrast sharply with a blackish venter that bears only a few patches of grayish-white pigment. Small diploid females from the island populations often bear discrete bluish lateral spots, and are thus not unlike *A. laterale* in appearance. In larger individuals, however, the distribution of bluish pigment is more extensive and diffuse (Fig. 6). Only the ventral surface and the tail retain the appearances of discrete bluish patches against a black background. The sides of the body are covered with diffuse, often coalesced, blotches of pale bluish-gray. The dorsum remains darker, often with a brownish cast, but it too has a suffusion of the light pigment. The coloration of the island diploids thus combines the blue pigment of *A. laterale* with the pattern of *A. texanum*.

Morphologically, many features of the diploid unisexuales from the Bass Islands are directly intermediate between those of *A. laterale* and *A. texanum*, combine elements of the two species, or are more like those of *A. laterale* in some individuals and like those of *A. texanum* in others. Although some minor differences in the expression of certain characters occur, there is no compelling evidence that the diploid unisexuales from Middle

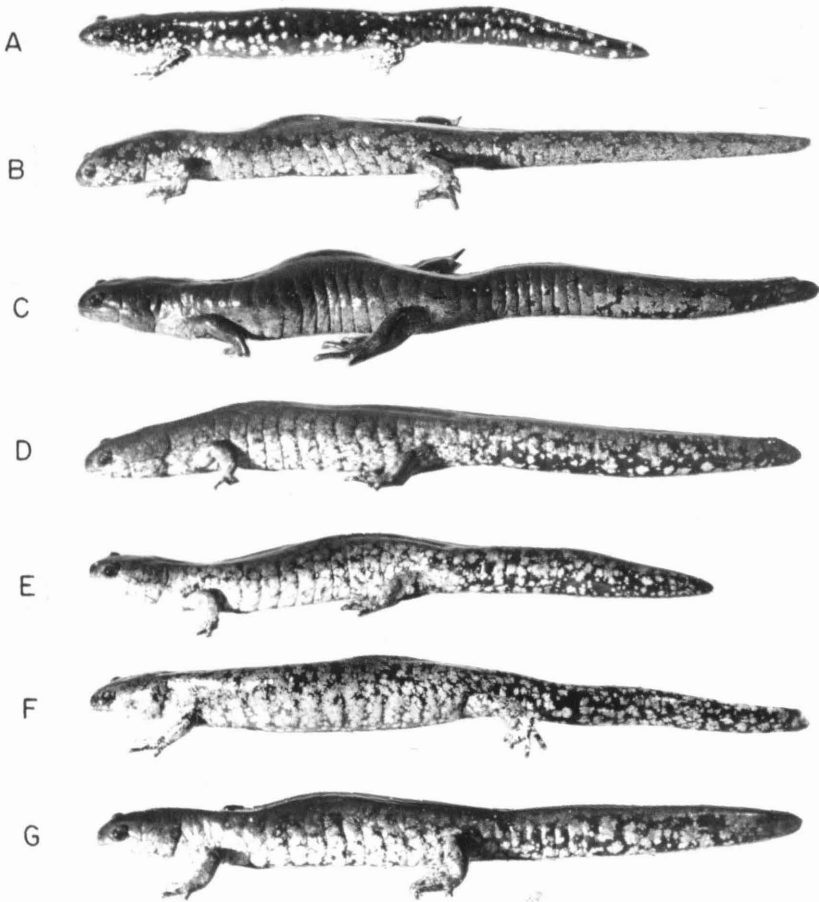


FIG. 6. Representatives of bisexual and unisexual *Ambystoma*. A. *A. laterale*, female, Washtenaw Co., Michigan; B. *A. texanum*, female, Wayne Co., Ohio; C. *A. texanum*, male, Middle Bass Island, Ottawa Co., Ohio; D. Diploid unisexual, Middle Bass Island; E. Diploid unisexual, North Bass Island; F. Triploid unisexual, Middle Bass Island; G. Triploid unisexual, North Bass Island.

Bass Island are fundamentally different than those from North Bass Island. The conclusion that the diploid unisexual populations on the two islands consist of hybrid individuals seems inescapable. Those few features, such as the size of the erythrocytes and the number of teeth, in which the values of the unisexuals exceed those of the diploid bisexuals are presumably influenced by heterosis.

THE TRIPLOID FEMALES

On both Middle and North Bass Islands, triploid females are less numerous than the diploid unisexuals. Although the small number of available specimens precludes a definitive judgement, it is likely that the triploids are unisexual. The homogeneity of the triploids from Middle Bass Island, in which the sum of the premaxillary and maxillary teeth ranges from 81 to 145, is in some doubt. Those individuals with high tooth counts were, with one exception, scored as having two rows of teeth. Those with lower counts have from one to one and one-half rows. Since these rankings are essentially arbitrary subdivisions of a continuum, and since the two groups possess no correlated suites of characters that otherwise distinguish them, the triploids are for the present considered a single sample. The tooth counts (range, 86-103) and other features of the triploids from North Bass Island raise no suspicion of heterogeneity.

The available samples of triploids from the two islands do not differ significantly in size or snout shape (Table 1), nor can they be distinguished on the basis of coloration (Fig. 6). There are, however, certain differences between the two groups that indicate a more homogeneous population on North Bass Island. None of the individuals from that island approach the high tooth counts found in some individuals from Middle Bass Island. In other respects, however, the specimens from North Bass Island are no more similar to those specimens from Middle Bass Island possessing comparably low tooth counts than to those with the highest counts. Lateral patches of prevomerine teeth are lacking in five of the 8 specimens from North Bass Island, and from one side of the head in two others. In contrast, one-half of the ten individuals from Middle Bass Island have tooth patches on both sides, two have the patch on one side, and in only three salamanders are the patches absent. Specimens from North Bass Island consistently have a protruding lower jaw and at least some development of a medial furrow on the tongue. The sample from Middle Bass Island is more variable in both features, having the lower jaw protrude or not and having a tongue with a well-developed central groove, a weakly-developed groove, or no groove at all. Despite the greater variability of the sample from Middle Bass Island, the populations of the two islands appear to be fundamentally similar.

The island triploids are clearly different than the other known triploids in the genus, *A. tremblayi* and *A. platineum*. Of the latter, only *A. tremblayi* is sufficiently similar to warrant discussion. The most striking difference between the island forms and *A. tremblayi* is in head shape; the latter species has a much broader and longer snout (Table 1). The sum of the internarial distance and the canthal length, when plotted against standard length, clearly distinguishes *A. tremblayi* from the island triploids (Fig. 7). The snout extends beyond the lower jaw in *A. tremblayi*, but the lower jaw usually protrudes in the island triploids. All of the triploids from the islands have 14 costal grooves, whereas the modal number in *A. tremblayi* is 13 (Table 3). Dentitionally, *A. tremblayi* resembles its diploid associate, *A. laterale*, in having the teeth in a single row and in possessing isolated lateral rows of prevomerine teeth. One island triploid had a single row of teeth, but that individual lacked

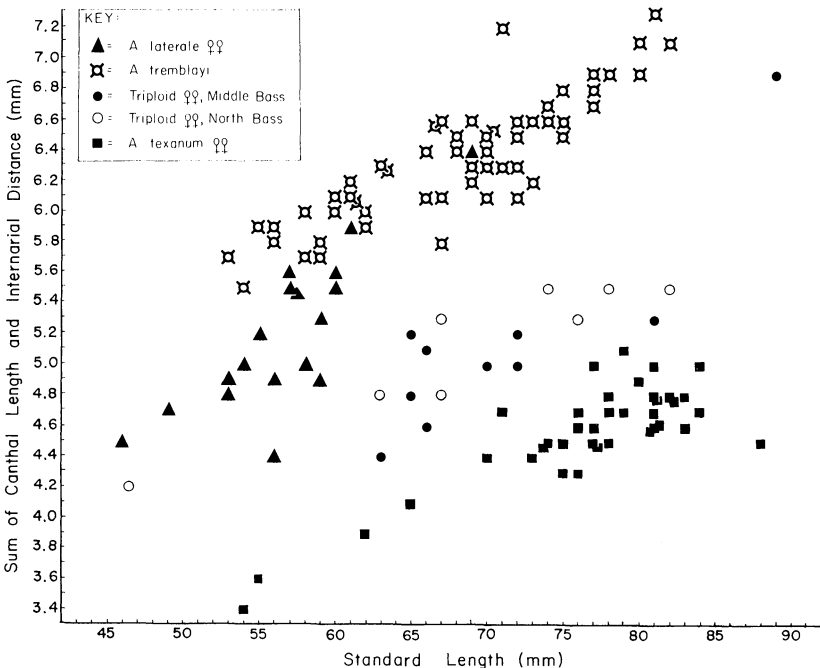


FIG. 7. The relation of the sum of the canthal length and internarial distance to standard length in females of certain diploid, bisexual and triploid, unisexual *Ambystoma*. *A. laterale* and *A. tremblayi* are from Washtenaw Co., Michigan and *A. texanum* from Wayne Co., Ohio.

lateral prevomerine teeth. The teeth are crowded into more than a single row for at least part of the dentiferous arch in other island specimens. Although the lateral patches of prevomerine teeth may be present or absent, the majority of the island triploids lack the patch on at least one side. Finally, the bluish pigment of *A. tremblayi* is usually in the form of discrete spots and flecks, that of the island triploids in more diffuse blotches.

Although most individuals can be identified by cell size, individual triploids have no combination of external features that allow them to be distinguished from the diploid unisexuals with which they occur (Fig. 6). The variation among triploids parallels that of the diploids in presenting different degrees of *laterale*-like and *texanum*-like character states. As groups, however, the triploid and diploid unisexuals differ in the expression of several characters. The triploids reach a greater maximal and average size than the diploids (Table 1). Although the relative length of the snout is comparable in the two groups, the triploids have relatively narrower snouts than the diploids (Table 1). The number of costal grooves is 14 in the triploids and in most diploids, but about 15 percent of the latter have 13 grooves, a number not known to occur among triploids. Diploid unisexuals almost always have lateral rows of prevomerine teeth that are often contiguous with the medial row, whereas the majority of the triploids lack these lateral teeth on one or both sides. The tongue of most diploids shows no evidence of a medial groove, but the groove is at least partially developed in most triploids. In each of these features, the triploids show a greater resemblance to *A. texanum* than do the diploid unisexuals. The morphological evidence therefore suggests that the triploid genome combines two sets of chromosomes from *A. texanum* and a single set from *A. laterale*.

SERUM PROTEINS

Serum proteins of the members of the *A. jeffersonianum* complex were studied electrophoretically by Uzzell and Goldblatt (1967). They found a single densely staining band of high mobility in each of the diploid bisexual species of the complex, with the protein of *A. laterale* showing greater mobility than that of *A. jeffersonianum*. Artificially produced diploid hybrids showed two bands, one corresponding to each of the parental types. Both bands were also present in the triploid unisexuals, *A.*

tremblayi and *A. platineum*, indicating a hybrid origin. Furthermore, the relative staining properties of the two bands in the triploids suggested that *A. tremblayi* possesses two alleles from *A. laterale* and one from *A. jeffersonianum*, and *A. platineum* two from *A. jeffersonianum* and one from *A. laterale*. When that interpretation is extrapolated to the entire triploid genome it provides a logical basis for the close morphological similarity between *A. tremblayi* and *A. laterale*, and between *A. platineum* and *A. jeffersonianum*.

Uzzell and Goldblatt (1967) analyzed sera from 18 *A. laterale* and 8 *A. jeffersonianum* and, despite drawing their samples from different parts of the respective geographic ranges, found no evidence of heterozygosity for the structural gene specifying the densely-staining, species-specific protein. The 10 *A. laterale* analyzed in my study, all from Washtenaw Co., Michigan, are also homozygous (Fig. 8B). Among 7 *A.*

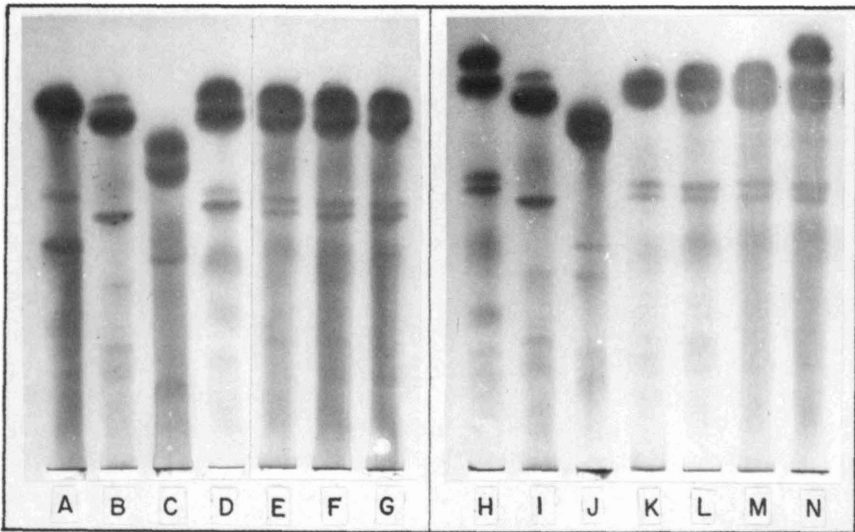


FIG. 8. Electrophoretic patterns of serum proteins in selected *Ambystoma*. Gels A through G and H through N are from two simultaneous runs. A. Homozygous *A. texanum*, Washtenaw Co., Mich.; B. Homozygous *A. laterale*, Washtenaw Co., Mich.; C. Heterozygous *A. jeffersonianum*, Wayne Co., Ohio; D. Male *A. texanum*, Middle Bass Island, Ottawa Co., Ohio; E. Diploid unisexual, Middle Bass Island; F. Diploid unisexual, North Bass Island; G. Diploid unisexual, North Bass Island; H. Heterozygous *A. texanum*, Wayne Co., Ohio; I. Homozygous *A. laterale*, Washtenaw Co., Mich.; J. Homozygous *A. jeffersonianum*, Portage Co., Ohio; K. Diploid unisexual, Middle Bass Island; L. Triploid unisexual, Middle Bass Island; M. Diploid unisexual, North Bass Island; N. Triploid unisexual, North Bass Island.

jeffersonianum from Wayne and Portage Cos., Ohio, four show the homozygous condition (Fig. 8J) but three, representing both counties, are heterozygous. The second band in the heterozygotes has a lower mobility than the previously known band (Fig. 8C).

The mobile, densely-staining proteins of *A. laterale* and *A. jeffersonianum* have counterparts, presumably homologous, in other species of *Ambystoma* (Uzzell and Goldblatt, 1967). The two *A. texanum* from Washtenaw Co., Michigan tested by me were both homozygous, showing a single band of greater mobility than those of the other two bisexual species (Fig. 8A). Among nine *A. texanum* from Wayne Co., Ohio, four were heterozygous, showing a second band of even greater mobility (Fig. 8H). The known serum protein patterns of the three bisexual species are thus distinctive and identifiable.

The electrophoretic patterns of the unisexuals from the Bass Islands support the morphological evidence that the island populations are of hybrid origin. Diploid unisexuals from both Middle Bass Island (Figs. 8E, 8K) and North Bass Island (Figs. 8F, 8G, 8M) show a heterozygous pattern of two equally dense bands, one corresponding in mobility to that of *A. laterale* and the other to the slower of the two bands known for *A. texanum*. Two triploids from Middle Bass Island exhibit these same two bands, but the faster protein, representing the *A. texanum* component, stains more densely than the slower one, a situation that presumably reflects a 2:1 allelic ratio in the triploid genome (Fig. 8L). Despite the electrophoretic similarity, these two triploids differ in their expression of *texanum*-like and *laterale*-like characters. One agrees with *A. texanum* in lacking lateral patches of prevomerine teeth and in having a well-defined medial groove on the tongue. The second has lateral prevomerine patches and no lingual groove. Triploids from Middle Bass Island exhibit considerable variation in the number of premaxillary and maxillary teeth, and I have questioned their homogeneity on that basis. The two individuals for which serum protein patterns are available have modest tooth counts, and the pattern of those with high tooth counts remains unknown. Modest tooth counts also characterize triploid unisexuals from North Bass Island, but the electrophoretic patterns of the two individuals analyzed differ from those of the triploids from Middle Bass Island. The triploids from North Bass Island possess three major bands, which appear to correspond to the single

band of *A. laterale* and the two bands of a heterozygous *A. texanum* (Fig. 8N). One of the two specimens has lateral prevomerine tooth patches, the second does not.

The serum protein pattern of a male from Middle Bass Island is heterozygous (Fig. 8D), and the slower of the two bands is disturbingly similar to, although slightly in advance of, the band of *A. laterale*. The morphological features (Fig. 6C) and erythrocyte size ($811\mu^2$) of the specimen agree with those of *A. texanum*, and since the mobilities of its two protein bands are only slightly less than those of a heterozygous *A. texanum* from the mainland it seems appropriate to assume that the protein pattern of the island specimen falls within the range of variation for that species. The serum of a second male from Middle Bass Island was investigated in a disc gel apparatus. Inconsistent migration rates among the various tubes prevented a direct comparison with other sera, but the male exhibited the single band of a homozygote.

The island populations show no morphological resemblance to *A. jeffersonianum* or its triploid associate, *A. platineum*. The electrophoretic patterns of the island individuals support the conclusion that *A. jeffersonianum* has not influenced the genetic constitution of the unisexuals from the Bass Islands (Figs. 8C, 8J).

BREEDING EXPERIMENTS

The ability of both triploid forms to elicit courtship from males of either *A. laterale* or *A. jeffersonianum* contributed to Uzzell's (1964) initial conclusion, later supported with electrophoretic data, that the unisexual species of the *A. jeffersonianum*-complex originated through hybridization of the two diploid, bisexual species. Similar evidence was sought in this study, with *A. laterale* and *A. texanum* the presumed parental stocks. The courtship of *A. laterale* has been described by Kumpf and Yeaton (1932), Uzzell (1964), and Storez (1969), and includes a dorsal capture by the male and subsequent leading of the female. Two conflicting descriptions of the courtship of *A. texanum* have recently appeared. Wyman (1971) reported a pattern similar to that of *A. laterale*, but involving a longer nudging phase, in *A. texanum* from northern Illinois. Observing animals from southern Illinois, Garton (1972) found no capture stage, and no leading of the female by the male.

Rather, after actively nudging the female, the male moved away, deposited one or more spermatophores, and returned to repeat the sequence. When several males courted the same female, a large concentration of spermatophores within a small surface area resulted. Such concentrations were found in the breeding ponds of the *A. texanum* used in my study, including a site at which no other species of *Ambystoma* occur, suggesting that courtship in these populations follows the pattern described by Garton.

The presence of spermatophores in the breeding cages was assumed to mean that courtship had occurred. Sexual activity by the male depends on the presence of a female in an appropriate physiological state (Uzzell, 1969; Storez, 1969; Garton, 1972). In ten of the 12 instances of spermatophore deposition in the breeding cages, the deposition occurred on the first or second night of confinement, suggesting a clear response to the female. In the other two cages, spermatophores were not discovered until the twelfth and thirteenth mornings. This delay, however, was associated with severe weather that hampered the checking process and apparently inactivated the salamanders. A comparable, simultaneous delay in spermatophore production occurred in three other cages not involving island females, and fertile eggs subsequently appeared in two of these. The delay, therefore, does not alter the assumption that courtship is a necessary prerequisite of the deposition of spermatophores.

In four years of trials, 61 combinations of males and females, involving 40 different females, were confined in breeding cages. Courtship occurred in twelve of these, and two of the courted females spawned fertile eggs. No significance can be attached to the number of combinations that failed to court, nor to those females that were courted but failed to deposit eggs. Because the attempts to maintain selected island females in outdoor cages through the winter months failed, other individuals had to be substituted. There was little expectation that these substitute females, maintained in the laboratory, would be in a physiological state appropriate for reproductive activity. Upon introduction to the water, many made vigorous attempts to escape it, a response characteristic of these terrestrial salamanders when they are not in breeding condition.

Most of the trials utilized females from the Middle Bass Island population. Thirteen such females (12 diploid, one triploid) were tested with males of *A. laterale*. One male

produced 8 spermatophores during his first night of confinement with a diploid unisexual, but the latter failed to deposit eggs.

Males of *A. texanum* were tested with diploid unisexuals from Middle Bass Island in 33 trials, resulting in 8 courtships. Five spermatophores were deposited in a cage housing two males and a single female. In courtships involving single males, the minimal numbers of spermatophores were 4, 4, 8, 14, 15, 18 (6 one night, 12 the following), and 19 (mean, 11.7). The females confined with the two males that produced the largest number of spermatophores in a single night (19 and 15, respectively) deposited fertile eggs. The resulting larvae did not survive through metamorphosis in the laboratory, and their sex and ploidy are unknown.

Nine combinations involving triploid females from Middle Bass Island and males of *A. texanum* were tested. In two of these, the female was confined with two males and courtship took place (10 and 12 spermatophores, respectively). Neither female deposited eggs.

Females from North Bass Island and males of *A. laterale* have not been simultaneously available for testing. Five females (4 diploid, one triploid) were tested in six combinations with males of *A. texanum*. A diploid unisexual confined with two males elicited 10 spermatophores, but no eggs resulted.

Some females from each island were placed in breeding cages without males for a minimum of two nights. One of the ten diploids from Middle Bass Island deposited a small mass of about 20 eggs, but these did not develop. The other nine, plus two triploids from Middle Bass Island and two diploids and one triploid from North Bass Island, failed to lay eggs spontaneously. None of these females elicited courtship from the males that were subsequently introduced to the cages, suggesting that the females were not in a reproductive condition. These results do not, therefore, negate the possibility that the island females are capable of parthenogenetic reproduction.

DISCUSSION

The close genetic relationship between *A. laterale* and *A. jeffersonianum* has not been seriously doubted. Indeed, their distinctness as separate species was not generally recognized until 1954, when Minton convincingly distinguished the two forms.

The markedly unbalanced sex ratios in certain populations of the complex, first noted by Clanton (1934), subsequently led to the recognition (Uzzell, 1964) of the associated triploid forms, *A. tremblayi* and *A. platineum*, and of the hybrid nature of the triploid genomes (Uzzell and Goldblatt, 1967). Although bizarre, this hybrid origin of the triploid unisexuals at least involves the failure, perhaps only upon initial contact, of the anti-hybridization mechanisms of two closely related parental species.

In contrast, the hybridization necessary to account for the populations on the Bass Islands involves parental species that have always been considered only distantly related. Cope (1889), and most recently Freytag (1959), placed *A. laterale* and *A. texanum* in separate genera. Most recent herpetologists have followed Tihen (1958) in placing the two in separate subgenera within *Ambystoma*. Even Tihen, however, acknowledged that the most specialized members of the subgenus *Linguelapsus*, such as *A. texanum*, might warrant generic recognition were it not for the presence of less specialized, intermediate forms. The occurrence of natural hybrids between *A. laterale* and *A. texanum* is therefore not a little surprising; it argues strongly that the two are congeneric.

Uzzell and Goldblatt (1967) concluded that the origin of the triploid unisexual *Ambystoma* could be most plausibly explained by assuming an initial hybridization that produced diploid offspring possessing one set of chromosomes from each parental species. Individuals with such a hybrid genome apparently produced unreduced gametes, and by backcrossing to the two parental species produced the triploid genomes of *A. tremblayi* and *A. platineum*. The number of unisexual vertebrates is surprisingly large (see Cuellar and Kluge, 1972, for a recent listing), and an initial hybridization has been repeatedly invoked in the explanations of their origins. Indeed, Maslin (1968) stated that parthenogenesis developing after hybridization is the only type known in vertebrates. Cuellar (1974) has recently challenged the universal application of the hybridization theory, pointing out that little evidence supports the assumption that hybridization leads to parthenogenesis. He argued that diploid parthenogens are more likely to arise spontaneously. Allotriploids would originate when such autodiploid unisexuals hybridized with a second species. Although certain vertebrate parthenogens may have arisen spontaneously, the hybrid origin for unisexual *Ambystoma* proposed by Uzzell and Goldblatt

gains considerable support from the situation on the Bass Islands, where allodiploids and allotriploids coexist. It seems reasonable to assume that the diploid hybrids developed prior to the triploids, and produced the latter by backcrossing to *A. texanum*.

The reproductive mechanism of the hybrid females remains unknown. Critical data on the ploidy of the eggs, and on the role of males, are lacking. Unisexual vertebrates are known to reproduce gynogenetically, hybridogenetically, or parthenogenetically. Within the genus *Ambystoma*, the uniparental *A. tremblayi* and *A. platineum* are courted by males of their respective associated biparental species, but produce triploid eggs and reproduce clonally by gynogenesis. The limited information available demonstrates that males of *A. texanum* and *A. laterale* will court the hybrid females from the Bass Islands, and that courted females sometimes deposit fertile eggs. Although consistent with a gynogenetic mode of reproduction, this information does not preclude the other possibilities, and the temptation to equate the method of procreation of the island females with those of *A. tremblayi* and *A. platineum* ignores an important difference in the structure of the respective populations. The latter species are consistently triploid, whereas diploids comprise the majority of the island populations. Although it is conceivable that both diploids and triploids are gynogenetic, the origin of triploids from such diploids is not easily explained, since in gynogenetic reproduction the sperm does not contribute its genome to the offspring.

The mixed diploid-triploid populations of *Ambystoma* are paralleled by those of a ranid frog in Europe. *Rana esculenta*, a hybrid species, usually occurs sympatrically with one or both of its parental forms, *R. lessonae* and *R. ridibunda* (Uzzell and Berger, 1975, and references therein). Most *R. esculenta* are diploid, but triploids are also present in certain populations (Günther, 1970; 1975). Although both male and female *R. esculenta* exist, the perpetuation of the esculenta phenotype results from hybridogenetic backcrossing to the parental forms (Berger, 1973; Tunner, 1973; Uzzell and Berger, 1975). Uzzell *et al.* (1975) discovered that diploid females of *R. esculenta* may simultaneously produce both haploid and diploid eggs, and therefore both diploid and triploid offspring. This discovery provides a clear explanation of the origin of triploid individuals in this hybridogenetic species, and perhaps a model that can be

applied to the hybrid *Ambystoma*. The backcrossing characteristic of hybridogenesis, and specifically its implicit genetic contribution by the male, might explain the variable expression shown by several features of the hybrids. Such variability is less easily explained if one presumes a strictly clonal method of reproduction. There remains the possibility, supported by the apparent absence of males on North Bass Island, that the island *Ambystoma* are capable of thelytokous parthenogenesis.

The existence of parthenogenesis in certain populations of clonally-reproducing *Ambystoma* has been previously suggested. Uzzell (1964) noted several localities at which he found triploid, but not diploid, members of the *A. jeffersonianum* complex. More recently, presumably on the basis of more intensive sampling, the opinion that diploid members of the complex are absent from certain populations in Boone and Delaware Cos., Indiana, has been more confidently voiced (Uzzell and Goldblatt, 1967; Uzzell, 1969). Uzzell (1970) and Cuellar (1974) have suggested that these populations are parthenogenic, and the apparent absence of biparental species on North Bass Island is further evidence that spontaneous egg development has emancipated certain uniparental populations from their reliance on even the gynogenetic role of males. The hybrid populations described herein, however, permit the serious consideration of an alternative explanation suggested by Uzzell (1970). That explanation concerns the potential involvement of males of other species of *Ambystoma*.

Small samples from several localities (e.g., in Ottawa and Wood Cos.) in northwestern Ohio convince me that hybrids combining features of *A. texanum* and *A. laterale* exist on the mainland as well as on the islands. Furthermore, a population of triploids under study in Wayne Co., Ohio may combine genetic contributions from *A. texanum* and *A. jeffersonianum*. The latter triploids are as large as *A. jeffersonianum* or *A. platineum*, but have relatively narrower snouts than any member of the *jeffersonianum*-complex, and a blotchy, *texanum*-like color pattern. Whether or not they are genetically related, these triploids and *A. texanum* occur together and males of the latter, when confined with triploids in breeding cages, successfully court them. The offspring are triploid, indicating a gynogenetic relationship.

It is therefore perhaps significant that *A. texanum* is abundant at the sites in Boone and Delaware Co., Indiana where

triploids occur in the absence of *A. laterale* and *A. jeffersonianum* (Uzzell, 1969). Indeed, the latter two species are not known to occur within the broader area of central Indiana that includes Boone and Delaware Counties (Minton, 1972). *A. texanum* is also present at the localities in Hancock Co., Ohio from which Uzzell (1964) identified two kinds of triploids but no diploid members of the complex. Narrow-snouted triploids from Hancock Co. were assigned to *A. tremblayi* by Uzzell, but two features suggest that these may be related to *A. texanum*: They have narrower snouts than any other population assigned to *A. tremblayi*, and the color pattern is described as "heavily frosted with gray, blue-gray, and light gray on the sides and dorsum" (Uzzell, 1964). It thus seems likely that at least one of the two kinds of triploids in Hancock Co. can utilize males of *A. texanum* in its reproduction. The possibility that other populations of triploids, including those at the sites in Indiana, are courted by males of *A. texanum* is an intriguing one. Certainly we are only beginning to appreciate the complexity of relationships that exist among unisexual and bisexual members of the genus *Ambystoma*.

Maslin (1968) outlined the taxonomic problems posed by unisexual vertebrates. Many parthenogenetic lizards, and the gynogenetic triploids of the *A. jeffersonianum* complex, have been accorded binomial recognition. Schultz (1969), dealing with unisexual poeciliid fishes, proposed hyphenated names denoting the genomic contributions of the two parental species. If the hybrid genomes of unisexual *Ambystoma* are as varied as suggested herein, the taxonomic problems will be correspondingly complex. At present, and particularly until their reproductive biology and geographic ranges are more completely understood, it seems imprudent to grant formal taxonomic recognition to the unisexual populations on the Bass Islands.

The islands in the western basin of Lake Erie are of considerable herpetological interest. In addition to the hybrid ambystomatids, an endemic subspecies of watersnake and a relict population of timber rattlesnakes are notable elements of the fauna (Langlois, 1964). Many herpetofaunal populations have declined in recent years, and the remaining habitat for ambystomatid salamanders seems particularly precarious. A few museum specimens attest to the former presence of the hybrid *Ambystoma* on South Bass Island, but at present that island apparently lacks adequate breeding sites, and ambystomatids

have not been recorded there in recent years. A similar fate may await the population on Middle Bass Island, where the sole remaining breeding site appears to be Haunck's Pond. The pond was used as a dump for many years, and is badly polluted. Although its use as a dump has been discontinued, the pond is also threatened by the high water levels maintained in Lake Erie in recent years. Such levels have submerged or carried off many of the rotting logs and other objects that serve as terrestrial retreats for the salamanders and, in combination with violent storms, have resulted in at least one breaching of the narrow gravel bar that normally separates the pond from the Lake. Until recently, the salamanders also utilized Fischer's Pond, a relatively pristine pond at the northwestern edge of the island. Unfortunately, that pond has been converted to a marina and is in open communication with the lake. The situation on North Bass Island, where the salamander population is centered around Fox's Pond, is more encouraging. Although the problems posed by the high water levels are applicable to the pond, human disturbance is minimal, and the wooded area around the pond is more extensive than that on Middle Bass Island.

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SPECIMENS EXAMINED

In addition to personally collected specimens, I have examined comparative material in the Museum of Zoology, University of Michigan (UMMZ) and the Franz Theodore Stone Laboratory of Ohio State University (OSU-SL). Many of the specimens collected, and thus observed alive, by me are deposited in the collections of the Museum of Zoology at the University of Michigan. In the listing below, the samples from the individual islands are given after those of the five mainland species to which they were compared.

A. jeffersonianum: OHIO: Delaware Co.: 1.6 m E Norton: UMMZ 122718 (3); Marlboro Tp., 0.5 m S Mayfield Corner: UMMZ 122584 (4), 123057 (4), 123058-60, 123062; Hocking Co.: nr Logan: UMMZ 122785 (6); Lawrence Co.: Rome Tp., Little Paddy Creek: UMMZ 122148-49; Lorain Co.: Amherst Tp., 1.5 m WNW North Amherst: UMMZ 122839-47; 125918, FLD (21); Portage Co.: Suffield Tp., Mogadore Reservoir: FLD (28); Wayne Co.: Franklin Tp., Sec. 22, Munson's Knob: FLD (19).

A. laterale: INDIANA: Fulton Co.: nr Delong: UMMZ 103440; Jasper Co.: 2 m E Parr: UMMZ 100942; Jasper-Pulaski St. Pk.: UMMZ 103441 (5), 124012 (6); Porter Co.: Indiana Dunes St. Pk.: UMMZ 124015; Pulaski Co.: 4.5 m W Winamac: UMMZ 129036; Starke Co.: 4 m S Knox: UMMZ 106594 (2); Steuben Co.: Clear Lake: UMMZ 122715 (2). MICHIGAN; Livingston Co.: E.S. George Reserve: UMMZ 123397, 123402 (2); Washtenaw Co.: UMMZ 123398 (3); Northfield Tp., Sec. 20: UMMZ 123400 (4), 123403 (6); Scio Tp., Sec. 28: UMMZ 123399 (4); Silver Lake: UMMZ 123396, 123401 (2), FLD (16).

A. platineum: INDIANA: Boone Co.: UMMZ 126602 (5); 4 m E Whitestone: UMMZ 126354 (3); Delaware Co.: nr Gaston: UMMZ 128369 (2); St. Joseph Co.: Rum Village, South Bend: UMMZ 123292 (5). OHIO: Hamilton Co.: Columbia Tp., Indian Hill Village: UMMZ 126603; Anderson Tp., "Woodmar Farms": UMMZ 123291; Hancock Co.: Van Buren Tp., Sec. 5: UMMZ 123562 (2); Lorain Co.: Amherst Tp., 1.5 m WNW North Amherst: UMMZ 122835-38, FLD (29); Wayne Co.: Franklin Tp., Sec. 22, Munson's Knob: FLD (7).

A. texanum: MICHIGAN: Hillsdale Co.: Amboy Tp., Sec. 12: UMMZ 99589 (6); Sec. 4: UMMZ 99592 (2); Sec. 6: UMMZ 101112; Washtenaw Co.: Scio Tp., Sec. 28: UMMZ 101117, 101118 (2), 101161, 122623, 122624 (4), 122580, 125996 (2), 122689 (14), FLD (2). OHIO: Auglaize Co.: German Tp., Sec. 5: UMMZ 122593 (4); Moulton Tp., Sec. 36: UMMZ 122595 (9); Clermont Co.: Glen Este: UMMZ 122569; Goshen: OSU-SL 715; Darke Co.: 5.5 m WNW Greenville: UMMZ 111551 (2); Franklin Co.: Blendon Tp., 1 m E Hoover Dam: UMMZ 122478; Hamilton Co.: Amberley Village: UMMZ 122570; Hancock Co.: Eagle Tp., Sec. 19: UMMZ 122388 (7), 122389 (7), 123571, 123575; Van Buren Tp., Sec. 5: UMMZ 124035 (13); Lucas Co.: Toledo: UMMZ 69292 (2), 75876 (2); Montgomery Co.: nr Centerville: UMMZ 111545-46; Dayton: UMMZ 111547 (8); nr Northridge: UMMZ 111552; 3/4 m NW Centerville: UMMZ 111548 (5); Wayne Co.: Chester Tp., Sec. 25: UMMZ 123209 (7), 123213 (3), FLD (32); Chippewa Tp., Sec. 18: UMMZ 123210 (6), 123214 (6); Franklin Tp., Sec. 6: UMMZ 123211 (5); Sec. 18: UMMZ 123212 (8), 123215 (4), 123216 (2), FLD (2); Sec. 20: UMMZ 123217 (6); Wooster Tp., Sec. 32: FLD (23).

A. tremblayi: INDIANA: Delaware Co.: 11 m N and 3 m W Muncie: UMMZ 122783 (6); Marshall Co.: 1 m E Culver: UMMZ 122864 (4); St. Joseph Co.: Liberty Tp., NE corner, Sec. 16: UMMZ 122714 (4). MICHIGAN: Washtenaw Co.: UMMZ 123361 (3), 123353; Northfield Tp., Sec. 20: UMMZ 123356 (2), 123358 (3), 123360, 123365 (3); Scio Tp., Sec. 28: UMMZ 123352 (4), 123353, 123354 (11), 123355 (8), 123357 (6), 123362 (4), 123363 (2), 123364; Silver Lake: FLD (24).

Ambystoma, Bass Islands: OHIO: Ottawa Co.: North Bass Island: Preserved specimens examined: UMMZ 122577 (52), 122578 (32), OSU-SL 916-21; specimens examined alive: UMMZ 148078-90, 148095-115. Middle Bass Island: Preserved specimens examined: UMMZ 147114-24, UMMZ field series CFW 4193-4200, OSU-SL 927-28; specimens examined alive: UMMZ 148018-23, 148028-77, 148091-94; *A. texanum* examined alive: UMMZ 148016-17, 148024-27, 148057-58. South Bass Island: Preserved specimens examined: UMMZ 100238, OSU-SL 759, 915.

SUMMARY

Populations of ambystomatid salamanders on Middle Bass and North Bass Islands in the western basin of Lake Erie consist largely of female unisexuals. On both islands, diploid unisexuals are more abundant than triploid unisexuals. The diploid bisexual *A. texanum* is present on Middle Bass Island, but bisexual species are apparently absent from North Bass Island.

The diploid unisexuals combine characteristics of *A. laterale* and *A. texanum*. Certain features, such as head shape, mean standard length and tail length, and the proportions of certain bones of the skull, are intermediate between those of the two bisexual species. In other features, such as the number of costal grooves, some unisexuals agree with *A. laterale* and others with *A. texanum*. The characteristics of the tongue and the relative length of the lower jaw in the unisexuals agree with *A. texanum* in some specimens, with *A. laterale* in others, and are intermediate in still others. The prevomers of the two bisexuals are highly distinctive, and the variable nature, including asymmetry, of that bone in the unisexuals is particularly indicative of their hybrid status. The blue pigment of *A. laterale* is present in the unisexuals, but its distribution in lichen-like blotches and diffuse frosting is reminiscent of the pattern of *A. texanum*.

The triploid unisexuals are superficially indistinguishable from the diploid unisexuals, but as a group show greater affinities to *A. texanum* than do the diploids. They are more like *A. texanum* in size, width of snout, number of costal grooves, condition of the tongue, and in the nature of the prevomers.

The electrophoretic patterns of the serum proteins in the unisexuals support the morphological evidence that the diploid unisexuals possess one set of chromosomes from *A. laterale* and one set from *A. texanum*, and the triploids one set from *A. laterale* and two sets from *A. texanum*. Diploid unisexuals tested from the two islands have equivalent electrophoretic patterns, but the *A. texanum* component of triploids from Middle Bass Island is homozygous and that for triploids from North Bass Island heterozygous.

Information on the breeding biology of the island unisexuals is limited. The apparent absence of bisexual species on North Bass Island argues for a parthenogenetic mode of

reproduction. Logistical problems have prevented the availability of island females captured during the spring breeding period, and thus limited breeding experiments to females that had been maintained in the laboratory for various lengths of time. Nonetheless, some such females have elicited courtship from males of *A. texanum* and *A. laterale*. On Middle Bass Island, where *A. texanum* occurs with the unisexuals, the latter may therefore have a gynogenetic or hybridogenetic relationship with males of the diploid bisexual. The variability shown by certain characters indicates that recombination is occurring, and favors an interpretation of hybridogenetic, rather than clonal, reproduction.

The existence of naturally-occurring hybrids between *A. laterale* and *A. texanum* is noteworthy since the parental species are not considered closely related. Because they are allodiploids rather than autodiploids, the presence of the diploid unisexuals lends support to the explanation of the origin of allotriploid *Ambystoma* proposed by Uzzell and Goldblatt (1967), in which the allotriploid genome is established by backcrossing after an initial hybridization. On the Bass Islands, the triploids are the apparent result of backcrossing between allodiploids and *A. texanum*.

The distributional limits of the hybrid unisexuals are not known, but the geographic ranges of such populations almost certainly extend onto the mainland of northwestern Ohio. Other populations in northern Ohio may include hybrids combining genetic contributions from *A. texanum* and *A. jeffersonianum*. Until these complex relationships are more clearly understood, formal taxonomic recognition of the hybrids is deemed inadvisable.

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