THE ECOLOGICAL RELATIONSHIP OF THE SALAMANDER *AMBYSTOMA LATERALE* TO ITS ALL-FEMALE, GYNOGENETIC ASSOCIATE

HENRY M. WILBUR

Museum and Department of Zoology, University of Michigan, Ann Arbor, Michigan 48104

The *Ambystoma jeffersonianum* complex consists of two bisexual, diploid forms and two all-female, triploid forms intermediate between the two diploid species in morphology and ecology. Uzzell (1964a) and Uzzell and Goldblatt (1967) have discussed the zoogeography and systematics of the complex and have suggested mechanisms for the origin and maintenance of the triploid populations. The two diploid forms, *A. laterale*, a smaller and more northern species, and *A. jeffersonianum*, a larger and more southern species, are thought to have arisen from Pleistocene isolates of a common ancestor. The triploid populations are presumed to have originated from the union of sperm from the parent population with unreduced eggs of diploid *jeffersonianum* × *laterale* hybrids. Uzzell (1964a) assigned the name *tremblayi* to the triploid form associated with *laterale* and the name *platineum* to the form associated with *jeffersonianum*. The triploid *tremblayi* has two sets of chromosomes derived from *laterale* and a third set derived from *jeffersonianum* (Uzzell and Goldblatt, 1967). The mechanism for the maintenance of triploidy in the *jeffersonianum* complex is a mitosis without cytokinesis prior to meiosis I. The sister chromatids form "pseudo-bivalents" with numerous chiasmata (Macgregor and Uzzell, 1964). With this mechanism of oogenesis triploid populations would be expected to consist of a series of clones in which all offspring are genetically identical to their mother. Diploid males provide sperm necessary to initiate cleavage but do not contribute genetic material to the genome of the embryo.

Because of this gynogenetic mode of reproduction, triploid forms are sexual parasites on the diploid populations. A similar complex exists in poecilliid fishes (Schultz, 1969).

Because of the obligatory sympatry of the triploid with the diploid form, one might expect that the sex ratios of demes would become increasingly biased as more and more triploid females successfully mate with the diminishing number of diploid males (Clanton, 1934). Populations would become extinct when, by sampling error, the remaining males court only with triploid females producing exclusively female offspring.

Adult ambystomatid salamanders in Michigan are generalized carnivores of the forest floor. Soon after the ground thaws adults migrate to temporary woodland ponds to breed. In southeastern Michigan the breeding season extends from the third week in March to the middle of April. The eggs hatch late in April. The larval salamanders are predators of any aquatic animal they can overpower; primarily: *Daphnia*, culicid fly larvae, and ostracods. Ambystomatid larvae are preyed upon by large dytiscid larvae, some odonate nymphs, and larger *Ambystoma tigrinum* larvae. The leech *Batracobdella picta* is a very common parasite of *Ambystoma* larvae in mid-June.

The primary purpose of this study was to investigate the competitive interactions of diploid and triploid larvae as a factor in the maintenance of the complex through ecological time. In spite of the genetic and morphological similarities between the diploid and triploid forms, there are dis-

TABLE 1. *Life history parameters of the* Ambystoma jeffersonianum *complex in southeastern Michigan.*

		Diploid	Triploid
Adult female body size (total length in mm)[1]	mean	104	120
	range	82–134	93–142
	sample size	23	66
Eggs per clutch[1]	mean	216	142
	range	120–300	50–216
	sample size	23	66
Number of spermatophores elicited per courtship[2]	mean	19.3	9.8
	range	1[3]–32	6–13
	sample size	4	6
Length of larval period[4]	mean	94.6	95.8
Relative survivorship[4]	%	84.4	64.1
Body size at metamorphosis[4] (trunk length in mm)	mean	15.5	17.5

[1] Data from Clanton (1934).
[2] Data from Uzzell (1969).
[3] This mating resulted in no eggs.
[4] Data from experimental populations, single-species populations with an initial density of 64 larvae (see Table 2).

tinct ecological differences (Table 1). Clanton (1934) and Uzzell (1964a) have shown that relative to *laterale* the triploid *tremblayi* has a larger body, a lighter coloration, fewer (but larger) eggs per clutch, and larger larvae. My study shows that there are differences in the length of the larval period, body size at transformation, and the response to crowding.

These ecological differences are an example of Gause's axiom that sympatric species cannot co-exist if they have identical ecological requirements. That two closely related forms have different ecological requirements is not surprising. It is of greater interest to ask how much the two forms overlap in their ecological requirements (Slobodkin, 1961, p. 125; Hutchinson, 1959; Levins, 1968, p. 50). Field experiments permit the investigation of such questions. These experiments were designed to test the alternative hypotheses that: (I) the two forms have identical ecological requirements or, (II) they do not overlap in their ecological requirements. Acceptance of the first hypothesis requires that, in a mixed population, species

A responds to an increase in the density of species B just as it does to an equal increase in the density of conspecifics. Acceptance of the second hypothesis requires that an increase of density of species B in a mixed population has no effect on species A. In the terminology of Hutchinson (1957) acceptance of the first hypothesis implies that the niches of the two forms are identical; acceptance of the second would imply that the niches are mutually exclusive. If the results are between the two extremes then the niches overlap.

METHODS AND MATERIALS

The study was conducted on the E. S. George Reserve, a University of Michigan research area located four miles west of Pinckney, Livingston County, Michigan. Adult salamanders were collected as they migrated to breeding ponds by encircling the ponds with low fences of window screening. Pitfalls (no. 10 cans) were placed flush with the ground at about two meter intervals along the outside of the fence. The cans were checked at fre-

quent intervals during the nights that migration occurred. By this method almost the entire breeding population is trapped (Shoop, 1965; Husting, 1965). Sex was determined by the enlarged vents and exaggerated lateral compression of the tails of mature males. Triploid females may be recognized by the larger size of their erythrocytes (Uzzell, 1964a). Dried blood smears made in the field and stored allowed ploidy determinations of virtually all females captured.

The larvae used in competition experiments were raised from eggs from females of known ploidy. All courtships and matings took place in breeding ponds within wire baskets about 25 cm in diameter. The adults were obtained from two ponds on the Reserve. Diploid females were caged with males from the same population to preserve any local genetic coadaptation. The larvae of each clutch were assigned in the same frequency to each experimental population. This procedure equalized the intraspecific genetic variability in the experimental populations. Experimental populations were contained in $2 \times 2 \times 8$ foot pens constructed of 2×2 inch wooden frames covered with fiberglass window screening (18 meshes per inch). Sheets of steel-reinforced plastic formed the bottoms of the pens. The window screening permitted the circulation of water and the entry of small invertebrates, but it excluded the larger instars of predaceous insects. Leeches freely entered the pens, but they were not a serious source of larval mortality. The pens were the same cages as the "screen-bottom pens" used by Brockelman (1969) in his study of population regulation in *Bufo* tadpoles (see his discussion of the microhabitat characteristics of these enclosures). The pens were placed in Burt Pond, a permanent woodland pond (described by Brockelman), on March 28, 1968. On April 13 approximately three bushels of litter from the floor of a nearby oak-hickory woodlot were evenly spread over the bottom of each pen to provide cover for

the salamander larvae and their prey, and to simulate the substrate of natural breeding ponds. This litter was thoroughly waterlogged when experimental populations of one- to three-day-old larvae were introduced on April 27. On the date of introduction the pens protruded from the water 6 to 10 cm at the offshore end and 10 to 20 cm at the inshore end. As the level of the pond dropped during the summer the inshore end of the pens became exposed.

There were twelve experimental populations; two replicates of six treatments. The pens were linearly arranged into two adjacent blocks along the southern edge of the pond. The experiment was of a $2 \times 2 \times 2$ factorial, 2-block design for variance analysis in which all combinations of the factors, initial density of larvae (32 or 64), and species composition (each species alone or in a mixed population) were represented once in each block (Table 2). Treatments were randomly assigned to pen positions. The pens were examined each day as long as transforming individuals were found under boards at the emergent, inshore end of the pens. The first transformed individuals were captured on July 8, the last on October 4. The length of the larval period was defined as the time between the day of introduction as one- to three-day-old larvae and the day of capture. When captured, larvae had the characteristic morphology of the late stages of metamorphosis such as reduced gill stubs, remnants of the larval color pattern, poorly developed integumentary glands etc. Larvae from mixed populations were identified by examination of blood smears. The data for each pen consisted of the number of individuals of each species surviving to transformation, the length of the larval period, and the body size of the recently transformed individuals. Each of these three variables was considered a measure of competitive success. Body size was measured as the distance between the fore and hind limb insertions. This metric can be converted

TABLE 2. *Summary of competition experiments.*

Pen	Initial Diploids	Density Triploids	Number Metamorphosed	Body Size		Larval Period		Corr. coef.
				Mean	SD Dv	Mean	SD Dv	
3	64	0	57	15.94	1.18	91	17	0.677
8	64	0	51	15.01	0.92	100	16	0.575
5	32	0	29	17.46	1.20	86	9	0.616
9	32	0	29	16.69	0.96	89	12	0.247
4	0	64	43	17.23	0.77	96	23	0.289
10	0	64	39	17.78	1.22	96	18	−0.008
6	0	32	25	18.02	1.48	91	22	−0.001
12	0	32	27	19.11	1.13	94	22	0.038
2	32	−	22	16.32	1.00	91	13	0.616
11	32	−	17	16.47	1.18	108	13	0.750
1	16	−	15	18.20	1.67	86	13	−0.057
7	16	−	12	17.12	1.19	92	10	0.667
2	−	32	22	17.43	1.31	89	26	0.032
11	−	32	23	17.41	1.50	90	21	0.087
1	−	16	16	18.50	1.54	87	19	−0.348
7	−	16	16	17.56	1.21	84	11	−0.491

to the more conventional snout to vent measure of body size by the relationship.

Snout-vent length = $(1.586 \pm .098) \times$ Trunk length + 3.466 ± 1.254 mm. Diploid and triploid larval measurements have the same regression line. Many workers have used survivorship data as a measure of the results of competition. I assumed that within a species both an early date of transformation and a large body size at transformation increase the probability of survival to the age of first breeding and therefore are components of fitness. An early date of transformation is an escape from the temporary pond community where in most years food organisms are becoming increasingly rare and the probability of the complete evaporation of the pond is increasing during the summer. A large size at transformation would be expected to increase the probability of survival to the age of first breeding. Survivorship was measured as the percentage of the number of larvae introduced which transformed. The survivorship of each population was normalized by extracting the arcsine of the square root of the per cent survivorship, the recommended transformation for data taken as percentages (Bartlett, 1947).

Although the mean body size and the mean length of larval period were unrelated across pens, within single pens the two variables were often positively correlated (Tables 2 and 3). Correlations between body size and length of larval period have been reported in laboratory studies (Uhlenhuth, 1919) and in field studies (Dempster, 1933). Because of this correlation an analysis of covariance was performed on the body size data with the length of the larval period treated as a covariate according to a linear hypothesis in which the effects of Table 3 were assumed to be additive.

With respect to diploid larvae, hypothesis I (identical niches) predicts the survivorship, mean body size, and length of larval period of the low density, pure-diploid population will be the same as the outcome of the diploid component of the low density, mixed-species population and also that the high density, pure population would have the same outcome as the diploid component of the high density, mixed-species population. Hypothesis II (no niche overlap) would be accepted with respect to diploid larvae if the outcome of the low density, pure population was the same as the diploid component of the

TABLE 3. *Analysis of covariance body size adjusted for length of larval period.*

Source of variation	Degrees Freedom	Regression Coefficient	Mean Square	F Ratio
Mean	1	15.940	3903.000	2934.697
Density	1	0.614	134.051	100.794**
Block	1	0.152	8.372	6.295*
Species	1	−0.629	144.313	108.510**
Mixing	1	−0.124	5.602	4.212*
Density × Block	1	0.077	2.149	1.616
Density × Species	1	0.170	10.518	7.909**
Density × Mixing	1	0.947	3.276	2.463
Block × Species	1	0.237	20.247	15.224**
Block × Mixing	1	−0.119	5.147	3.870*
Species × Mixing	1	−0.231	19.333	14.537**
Density × Block × Species	1	−0.029	0.305	0.230
Density × Block × Mixing	1	−0.165	9.983	7.506**
Density × Species × Mixing	1	−0.016	0.095	0.717
Block × Species × Mixing	1	0.195	13.912	10.460**
Density × Block × Species × Mixing	1	0.005	0.008	0.006
Length of larval period	1	0.014	28.254	21.245**
Error	426		1.330	

* $.01 < P < .05$
** $P < .01$

high density, mixed-species population. Therefore, with respect to each species, in each block there are two independent tests of hypothesis I and one test of hypothesis II for each of the measures of competition. One set of tests compared the survivorships of pure and mixed-species populations by Fisher's exact probability of the observed or a more extreme 2 × 2 contingency table of the number from the initial population which transformed or died in each population. Another set of tests compared by one-way analyses of covariance the regressions of body size at transformation on the length of the larval period of the two populations.

RESULTS

The results of the analysis of covariance of body size at transformation adjusted for the length of the larval period are presented in Table 3. The difference between effects can be compared by the estimates of the parameter values; the significance of these effects are tested by the F ratios. The difference between the body sizes of the two kinds of larvae is highly significant. Both forms had a highly significant response to density. At the high density the length of larval period was increased and the larvae transformed at a smaller size. The effect of the microhabitats (blocks) was significant at a lower level. The effect of mixing was also significant at the 5% level indicating that the larvae did respond to the presence of the other species. Several of the factors interacted significantly in their effect on body size. The highly significant interaction between the factors blocks and species indicates that the two forms reacted differently to different microhabitats. For example, if the block effect was caused by temperature differences the significant interaction term would imply that the two forms had different temperature response curves. The highly significant interaction between the species and mixing factors implies that the two forms reacted quite differently to the presence of the other form. This result is primarily because at the low density the diploid larvae increased their body size and triploid larvae decreased their body size in response to the presence of the other species. At the high density the triploid larvae did not respond

TABLE 4. *Survivorship* ($Arcsin \sqrt{\%}$) *to metamorphosis.*

Source of Variation	Degrees of Freedom	Regression Coefficient	Mean Square	F Ratio
Mean	1	65.35	68333.88	97424.11**
Density	1	8.22	1081.92	1542.49**
Block	1	1.83	53.55	76.34
Species	1	−0.79	10.10	14.39
Mixing	1	−1.17	21.97	31.33
Density × Block	1	−0.48	3.64	5.19
Density × Species	1	−2.84	129.11	184.07*
Density × Mixing	1	−4.11	271.34	386.86*
Block × Species	1	2.19	76.78	109.47
Block × Mixing	1	−1.00	16.06	22.90
Species × Mixing	1	6.17	609.97	869.63*
Density × Block × Species	1	0.32	1.66	2.36
Density × Block × Mixing	1	−1.51	36.51	52.05
Density × Species × Mixing	1	1.33	28.12	40.09
Block × Species × Mixing	1	−1.14	20.91	29.81
Error[a]	1		0.70	

[a] The four-way interaction term was used as an estimate of the error mean square.

* $.05 < P < .01$

** $P < .01$

to the other form; diploid larvae again increased their body size. It is more difficult to interpret the highly significant three-way interaction between the factors: blocks, species, and mixing. This interaction implies that the difference in the relative responses of the two species to competitors is modified by microhabitat conditions. The remaining significant effect is the interaction between blocks and mixing. This effect again implies that competition is modified by microhabitat.

The results of the analysis of variance of the survivorship in each population are presented in Table 4. Survivorship varied inversely with density in both species. The significant interactions between density, species and mixing resulted from the relatively high survivorship of diploid larvae at the high density and of the triploid larvae at the low density. The carrying capacity of the pen environment is larger for diploids; at least 57 compared with 43 for triploid larvae. The high survivorship of triploid larvae in the low density, mixed populations may be due to the occasional predation by the larger triploid larvae on smaller diploid larvae.

The tests of the alternate hypotheses of complete niche overlap or nonoverlap (Table 5) examined more closely the interaction between the two forms. With respect to diploid larvae all tests rejected the hypothesis of no niche overlap at high densities. Three of the four tests also rejected the hypothesis of complete overlap at the high density. All four tests of complete overlap at low densities were accepted. The design did not permit testing of nonoverlap at the low density. These results demonstrate that diploid larvae are affected significantly by the presence of triploid larvae but the effect is significantly less than that of an equal increase in the density of conspecifics. With respect to triploid larvae there was one rejection of the test of nonoverlap, the analysis of covariance of body size on length of larval period at high density in block II. There were no rejections of the hypothesis of complete overlap at the high density. Three of the four tests rejected the hypothesis of complete overlap at the low density. These results indicate that at the high density triploid larvae have a variable response to diploid larvae. At

TABLE 5. *Tests of niche overlap hypotheses.*

	No Overlap[a] High Density[b]	Complete Overlap High Density[c]	Low Density[d]
Diploid Larvae			
Body Size[e]			
Block I	$F**_{1,48}$=30.713	accept	accept
Block II	$F**_{1,43}$= 7.171	$F**_{1,66}$ = 24.865	accept
Survivorship[f]			
Block I	$P*$= 0.0398	$P*$ =0.0167	accept
Block II	$P**$=0.0009	$P**$=0.0076	accept
Triploid Larvae			
Body Size[e]			
Block I	accept	accept	accept
Block II	$F**_{1,47}$=19.768	accept	$F**_{1,40}$=17.723
Survivorship[f]			
Block I	accept	accept	$P*$=0.0457
Block II	accept	accept	$P*$=0.0099

[a] There were no tests of nonoverlap at the low density.
[b] Tests for no overlap compared 32–alone with 32 + 32–mixed.
[c] Tests for complete overlap at the high density compared 64–alone with 32 + 32 mixed.
[d] Tests for complete overlap at the low density compared 32–alone with 16 + 16 mixed.
[e] F tests of treatment means in one-way analyses of covariance of the regressions of body size on length of larval period.
[f] Fisher's exact probability tests of 2 × 2 contingency tables of number alive and number dead at the end of the experiment in each population.
* $.05 < P < .01$
** $P < .01$

the low density the effect of an increase in diploid larvae has little effect.

In summary, the two forms are quite different in their response to density and microhabitat differences. The two kinds of larvae interact significantly and the interaction is affected by the microhabitat and probably by the density of the population. The reciprocal effects of competition are not equal; diploid larvae are less adversely affected by the presence of triploid larvae than are triploid larvae to the presence of diploid larvae.

DISCUSSION

The association now existing is certainly a transitory stage in an evolutionary sequence leading to either the extinction of the triploid forms or to the independence of the triploid forms from the diploid populations. Extinction would follow perfection of the discrimination of diploid

males against triploid females or by the competitive exclusion of the triploid form. The competitive exclusion of the diploid form by the triploid form would also result in the extinction of the complex. This paper examines the relationship of the two forms during this transitory period.

The preponderance of females in the populations from southeastern Michigan presented in Table 6 are all significantly different from equal sex ratios. Uzzell (1964a) has discussed the sex ratios of the complex throughout its geographical range. The peculiar nature of the *A. jeffersonianum* complex was suspected for a long time prior to Uzzell's definitive studies (Humphrey, 1938; Clanton, 1934; Bishop, 1941; Minton, 1954) because of this unbalanced sex ratio. Clanton (1934) was the first to suggest the potential instability of the system caused by the increasingly biased sex ratios resulting from

TABLE 6. *Sex and ploidy ratios of populations of* Ambystoma laterale *and* A. tremblayi.

	Year	Males	Females	Total	Sex Ratio	Ploidy Ratio[a]
Washtenaw Co.						
Scio Twp.	1928–32[b]	322	692	1014[c]	2.14	
	1959[d]	4	52	56	13.00	
Pittsfield Twp.	1928–32[b,e]	4	205	209[c]	51.25	
Lodi Twp.	1928–32[b,e]	2	104	106[c]	52.00	
Northfield Twp.	1959[d]	22	79	101	3.59	
Livingston Co.						
Putnam Twp.[f]						
S–21	1959[d]	17	119	136	7.00	
S–21	1967	17	106	123	6.23	
S–21	1968	5	42	47	8.40	18.00
E–7	1968	6	85	91	14.16	6.07
E–7	1969	49	329	378	6.71	3.21
P–25	1968	7	23	30	3.28	1.56

[a] Triploid/diploid females
[b] Sample combined for five years; not captured by fencing pond
[c] Data from Clanton (1934)
[d] Data from Uzzell (1964a)
[e] Many newly transformed individuals in the sample
[f] Localities are positions on the 1963 grid of the E. S. George Reserve

the decreasing probability of diploid males encountering diploid females in breeding aggregations. At some critical ratio of triploid to diploid females the probability of this event would become so low that there would be local extinctions due to the lack of male production. Clanton noted a positive relation between the number of specimens from a locality and the equality of the sex ratio. There is not a significant correlation between the population size and sex ratio in the collections from fenced ponds (Table 6) in southeastern Michigan. Using the relative egg numbers of diploid and triploid females and assuming equal survivorships, equal sex ratios in diploid clutches, and the same age of first breeding in both forms, Uzzell (1964a) calculated that in 13 generations the ratio of triploid to diploid females could increase from 1.0 to 54.5. He presents evidence that the sex ratios of local populations may rapidly change. Minton (1954) used the argument of unstable sex ratios as evidence of reproductive isolation and as a justification for the recognition of *jeffersonianum* and *laterale* as distinct species.

Even permitting multiple origins of triploid populations, the wide range of the complex (see Uzzell and Goldblatt, 1967), the more than 40-year history of the Scio Township population near Ann Arbor (Clanton 1934; Uzzell, 1964a), and the remarkable agreement between the 1959 and 1967 data for pond S-21 (Table 6) on the George Reserve suggest that there are factors tending to stabilize local populations. Such factors could operate at any time during the life cycle by affecting the probability of courtship of a triploid female, the survival of triploid eggs to hatching, the survival of eggs to metamorphosis, the adult survivorship, the age of first breeding or the clutch size of triploid females.

Discrimination of diploid males against triploid females would be a powerful mechanism for the extinction of triploids. Mosimann (1958) has shown that theoretically mate-finding may become a limiting condition in sparse populations. The selective advantage of the rejection of a triploid female by a diploid male who later used the spermatophores in courtship

with a diploid female is obvious. There is no evidence for extended sperm storage or true parthenogenesis in populations in southeastern Michigan. Triploid females do not deposit fertile eggs unless they have picked up spermatophores (personal observation). An unusually high incidence of infertile eggs has been observed in many populations throughout the range of the complex (Piersol, 1910, 1929; Clanton, 1934; Wacasey, 1961; Uzzell, 1964a). I observed 61% inviability in 101 clumps of triploid eggs in Pond S-21 on the George Reserve in 1967. In other ambystomatid salamanders infertile eggs are a rarity (personal observation about *A. tigrinum* and *A. maculatum*). The length of time that a male remains in the breeding pond, the number of females he engages in courtship, the total number of spermatophores he can produce, and the distribution of the spermatophores among courtships are all relevant to the effective sex ratio of populations. Uzzell (1969) records single *laterale* males depositing 1–32 spermatophores (mean = 19.3) and reports (1964a) that one female was fertilized by a single spermatophore and two females by as few as five. Clanton (1934) tested six males with "an excess" of females and found that the average number of spermatophores per male was 4.83 ± 0.88. Storez (1969) reported that two to four spermatophores per courtship bout are usual. Clanton (unpub. ms) reported that if a female picks up a spermatophore early in the courtship she may lose interest and fail to elicit further spermatophore depositions by the male. If triploid females pick up spermatophores more readily than diploid females because of a lower arousal threshold, they may not require as many spermatophore depositions as diploid females to complete a courtship bout. This is an alternative explanation for Uzzell's (1969) hypothesis of male discrimination against triploid females based on fewer spermatophores deposited in courtship with triploid females. A test of these alterna-

tive hypotheses would require a detailed observation of courtship bouts to distinguish the relative importance of male discrimination and female response. Clanton (unpubl. ms) also noted that females may pick up spermatophores remaining from previous courtships without an actual courtship. If this is a common occurrence even strong male discrimination would fail to prevent breeding by triploid females. Clanton (1934) noted that many females do not breed successfully and resorb their ovarian eggs. He also had evidence that the percentage of fertilized eggs decreases as the breeding season progresses perhaps due to an "over-ripening" of the ovarian eggs. Clanton's counts of ovarian eggs of females from Washtenaw County indicated that the mean clutch size was 216 eggs for diploid females and 142 eggs for triploid females. The reduced fecundity of triploid females is balanced, in a demographic sense, by the fact that all progeny are females.

Eggs I have reared in the laboratory have had very high survivorships; there are no data on survivorship of eggs in nature. Triploid females lay their eggs in small clumps (mean = 4.84 ± .27 eggs, $n = 200$ clumps) attached to twigs and leaves. Diploid females lay their eggs singly or in twos in the leaf litter on the pond bottom (Clanton, 1934; Uzzell, 1964a; personal observation). The strategy of laying the eggs in clumps would provide better protection from predators such as leeches which bore through the jelly envelopes. The leech *Macrobdella decora* is known to prey heavily on *Rana sylvatica* eggs but is unable to penetrate the outer envelope of *Ambystoma maculatum* or *A. tigrinum* egg masses (Cory and Manion, 1953). I have never observed leech predation on the hundreds of *Ambystoma* egg clumps that I have observed in the field although leeches are commonly seen in the ponds later in the summer. The deposition of eggs singly, as by diploid females, would be an effective strategy

against predators consuming entire egg masses. The newt *Notophthalmus viridescens* is known to eat *Ambystoma* eggs (Hamilton, 1932, *maculatum*; C. F. Walker, pers. comm., *jeffersonianum*); however newts have been found in only one of the 14 ponds used by *laterale* and *tremblayi* on the George Reserve.

The difference in survivorship of the two kinds of larvae was investigated in the experimental section of this study. These experiments were designed to demonstrate the existence of competition and to estimate the magnitude of the interaction at the two initial densities. They were not designed to explain the mechanism of the interaction. The density effect on body size suggests that food is in short supply and there is competition for food both within and between species. The experimental demonstration of this mechanism requires the manipulation of the quantity of food available in the pens.

Diploid larvae have a higher survivorship and a shorter larval period than triploid larvae. The length of the larval period is an important factor in the life history of temporary pond forms. As the vernal ponds dry during the summer the salamander larvae become increasingly crowded while, at the same time, the major food source of *Daphnia* and culicid larvae becomes less abundant, and more rapidly growing *A. tigrinum* larvae become increasingly important as predators in some ponds. In short, the aquatic environment is rapidly deteriorating; those larvae which can escape through metamorphosis and continue to grow in the terrestrial environment are favored by selection. There are frequent reports of ponds drying early in the summer causing the death of large members of *Ambystoma* larvae (c.f. Hamilton, 1942; Bishop, 1941). Studies designed to measure quantitatively the overlap of the food habits and microhabitat preferences of the two forms and the ecological relationship of

the complex to the other sympatric species of *Ambystoma* are in progress.

Tremblayi inherits some of its ecological characters from *jeffersonianum*. The extent of the modification of differences between *laterale* and *tremblayi* which has occurred in sympatry cannot be inferred without extensive studies of geographic variation of ecological characters.

Uzzell (1969) suggested that some populations of *tremblayi* in northern Illinois may be parthenogenetic. He found large breeding populations apparently without males but which produced viable eggs. In the teiid lizard genus *Cnemidophorus* and in a complex of lacertids there are examples of true parthenogenesis in unisexual populations (Maslin, 1968; Darevsky, 1966). Another possible mechanism for the independence of the triploid forms is the emergence of a tetraploid form from the union of a 3n egg of a triploid female and a 1n gamete from a diploid male. This tetraploid form could return to a bisexual reproductive system by producing diploid eggs by normal meiotic mechanisms. Such an evolutionary history may have been the case for the recently reported tetraploid population of the treefrog *Hyla versicolor* (Wasserman, 1970).

SUMMARY

In southeastern Michigan the all-female, gynogenetic salamander *Ambystoma tremblayi* is associated with a bisexual, diploid form, *A. laterale*. *A. laterale* has contributed two sets of chromosomes to the triploid genome of *A. tremblayi*. The present gynogenetic relationship between the two forms is probably a transitory stage in an evolutionary sequence that could result in the reproductive independence of the triploid line by the evolution of a parthenogenetic or of a bisexual, tetraploid form. Extinction of either form by competitive exclusion by the other form is also a possibility. Experimental larval populations in field enclosures tested the alternative hypotheses that the two forms do not overlap

at all in their ecological requirements or that they interact as ecological equals. The outcomes of the experiments were measured by the survivorship of the population, the body sizes at metamorphosis and the lengths of the larval period. The results indicated that the two forms have different ecological requirements but still interact significantly. This interaction is affected by the microhabitat and the density of the population. Diploid larvae are less adversely affected by the presence of triploid larvae than triploid larvae are to the presence of diploid larvae. The shorter larval period of the diploid larvae is advantageous in the uncertain vernal pond habitat.

LITERATURE CITED

BARTLETT, M. S. 1947. The use of transformations. Biometrics 3:39–52.

BISHOP, S. C. 1941. The salamanders of New York. N. Y. State Mus. Bull. No. 324. 365 p.

BROCKELMAN, W. Y. 1969. An analysis of density effects and predation in *Bufo americanus* tadpoles. Ecology 50:632–644.

CLANTON, W. unpublished manuscript. Univ. Mich. Mus. Zool. Herpetol. Div. Lib.

——. 1934. An unusual situation in the salamander *Ambystoma jeffersonianum* (Green). Occ. Pap. Mus. Zool. Univ. Mich. 290:1–14.

CORY, L. AND J. L. MANION. 1953. Predation on eggs of the wood-frog, *Rana sylvatica*, by leeches. Copeia 1953:66.

DAREVSKY, I. S. 1966. Natural parthenogenesis in a polymorphic group of Caucasian rock lizards related to *Lacerta saxicola* Eversmann. J. Ohio Herpetol. Soc. 5:115–152.

DEMPSTER, W. T. 1933. Growth in *Amblystoma punctatum* during the embryonic and early larval period. J. Exp. Zool. 64(3):495–511.

HAMILTON, W. J. 1932. The food and feeding habits of some eastern salamanders. Copeia 1932:83–86.

——. 1942. Crows feeding on larval amphibians. Auk 59:446.

HUMPHREY, R. R. 1938. Sex differentiation in gonads developed from transplants of the intermediate mesoderm of *Amblystoma*. Biol. Bull. 55:317–338.

HUSTING, E. L. 1965. Survival and breeding structure in a population of *Ambystoma maculatum*. Copeia 1965:352–362.

HUTCHINSON, G. E. 1957. Concluding remarks. Cold Spring Harbor Symposium Quantitative Biol. 22:415–427.

——. 1959. Homage to Santa Rosalia or why are there so many kinds of animals? Amer. Natur. 93:145–159.

LEVINS, R. 1968. Evolution in Changing Environments. Princeton Univ. Press. Princeton, N. J. 120 p.

MACGREGOR, H. C. AND T. M. UZZELL, JR. 1964. Gynogenesis in salamanders related to *Ambystoma jeffersonianum*. Science 143:1043–1045.

MASLIN, T. P. 1968. Taxonomic problems in parthenogenetic vertebrates. Syst. Zool. 17:219–231.

MINTON, S. A. 1954. Salamanders of the *Ambystoma jeffersonianum* complex in Indiana. Herpetologica 10:173–179.

MOSIMANN, J. E. 1958. The evolutionary significance of rare matings in animal populations. Evolution 12:246–261.

PIERSOL, W. H. 1910. Spawn and larvae of *Ambystoma jeffersonianum*. Amer. Natur. 44:732–738.

——. 1929. Pathological polyspermy in eggs of *Ambystoma jeffersonianum*. Trans. Roy. Can. Inst. 17:57–74.

SCHULTZ, R. J. 1969. Hybridization, unisexuality, and polyploidy in the teleost *Poeciliopsis* (Poeciliidae) and other vertebrates. Amer. Natur. 103:605–620.

SHOOP, C. R. 1965. Orientation of *Ambystoma maculatum*: movements to and from breeding ponds. Science 149:558–559.

SLOBODKIN, L. B. 1961. Growth and regulation of animal populations. Holt, Rinehart and Winston. 184 p.

STOREZ, R. A. 1969. Observations on the court-ship of *Ambystoma laterale*. J. Herpetol. 3:87–95.

UHLENHUTH, E. 1919. Relation between thy-roid gland, metamorphosis, and growth. J. Gen. Physiol. 1:473.

UZZELL, T. M., JR. 1963. Natural triploidy in salamanders related to *Ambystoma jefferson-ianum*. Science 139:113–115.

——. 1964a. Relations of the diploid and trip-loid species of the *Ambystoma jeffersonianum* complex (Amphibia: Caudata). Copeia 1964: 257–300.

——. 1964b. Gynogenesis in salamanders re-lated to *Ambystoma jeffersonianum*. Science 143:1043–1045.

——. 1969. Notes on spermatophore produc-tion by males of the *Ambystoma jefferson-ianum* complex. Copeia 1969:602–612.

UZZELL, T. M., JR. AND S. M. GOLDBLATT. 1967. Serum proteins of salamanders of the *Am-bystoma jeffersonianum* complex, and the origin of the triploid species of this group. Evolution 21:345–354.

WACASEY, J. W. 1961. An ecological study of two sympatric species of salamanders, *Am-bystoma maculatum* and *Ambystoma jeffer-sonianum*, in Southern Michigan. Ph.D. thesis Mich. State Univ. 117 p.

WASSERMAN, A. O. 1970. Polyploidy in the common tree toad *Hyla versicolor* Le Conte. Science 167:385–386.