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18 July 1991

Mr. Carl Becker
Illinois Department of Conservation
Division of Natural Heritage
524 South Second Street
Springfield, IL 62701-1787

Dear Mr. Becker:

Enclosed please find the final report for the Nongame Wildlife Conservation Fund project; Reproduction, Survival, and Genetic Constitution of the Polyploid Mole Salamanders of Kickapoo State Park. In addition, find eleven color slides and a single page of slide descriptions. If you require any additional information, do not hesitate to contact us.

Sincerely,

Christopher A. Phillips

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Postdoctoral Research Associate

Christina M. Spolsky
Thomas Uzzell

Final Report for Nongame Wildlife Conservation Fund Project; Reproduction, Survival and Genetic Constitution of the Polyploid Mole Salamanders of Kickapoo State Park

submitted by
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Introduction

The silvery salamander, *Ambystoma platineum*, is a triploid, nearly all-female group of salamanders distributed throughout Indiana, Ohio, S. Michigan, and S. Ontario with isolated populations in New Jersey, Massachusetts, and Connecticut. They are thought to be the product of a mating between an *A. jeffersonianum* - *A. laterale* female hybrid (JL) and an *A. jeffersonianum* male (Uzzell and Goldblatt, 1967; Sessions, 1982). Silvery salamanders are thought to utilize gynogenesis, a mode of reproduction in which females lay chromosomally unreduced eggs and sperm is required to induce the egg to divide but does not donate any genetic material (Macgregor and Uzzell, 1964). The resulting offspring are genetically identical to their mother. Occasionally, however, the sperm exclusion mechanism breaks down and tetraploids are formed that posses a set of chromosomes from the male parent. Because females are the heterogametic sex in *Ambystoma*, and thus carry the female determining chromosome, these tetraploids are all-female as well. Because of their peculiar reproductive mechanism, *A. platineum* must live in association with a suitable sperm donor. For most populations, this host is *A. jeffersonianum*, however, a few populations have been discovered that have made a successful host shift and now utilize *A. texanum* as sperm donors (texanum dependent populations).

This report deals with a recently described population of *A. platineum* from Vermilion County, Illinois (Morris, 1974). This population is unique in that it is a texanum dependent population with an unusually large proportion of tetraploids (4n; *A. platineum* x *A. texanum*). The objectives of this study were:

1) to determine what proportion of the polyploid population is tetraploid and to calculate how this distribution has changed since the 1981 estimate (Morris and Brandon, 1984);

2) to determine whether or not the tetraploids represent a continuing clonal lineage, i.e. do tetraploids produce tetraploid offspring or any offspring at all?

3) to verify, using biochemical markers, the genomic constitution of the triploids and tetraploids proposed by Morris and Brandon (1984);

4) to monitor the recruitment of juveniles into the population.

Methods

The study area consists of two ridge top breeding ponds (referred to as large and small ponds) in the Middle Fork Woods Nature Preserve, Vermilion County, Illinois. The following physical parameters of the large pond were measured on several occasions: ice cover, water temperature, pH, maximum depth, and maximum width. Both ponds were checked for spermatophores and egg masses during February and March.

The large pond was partially surrounded by a drift fence and 34 exterior pitfall cans (Fig. 1) The fence was completed on February 18th, 1991 and removed on March 26th, 1991 after several rainy nights with no *A. texanum* or polyploid immigration. Standing water, especially on the southeast side, prevented us from fencing the entire pond. A small span of drift fence with 4 pitfall cans was constructed on the northwest side of the small pond on 21 February and removed on March 26th, 1991 (Fig. 1).

With the exception of an extremely cold period, 22-25 February and two extended dry periods, 8-11 March and 14-17 March, the fence was checked daily for the presence of immigrating salamanders. Species, sex and can number were recorded for each immigrant mole salamander encountered at the fence. *A. platineum* and tetraploids were not distinguished in the field but were taken to the laboratory in Urbana, Illinois were they were anesthetized with tricaine methanesulfonate (MS-222, Sigma Chemical, St. Louis, MO). A small amount of blood was taken from a cut toe (usually right hind foot, middle digit) for flow cytometric determination of ploidy. In addition, the distance from the snout to the posterior angle of the vent (SVL) was measured to the nearest mm with a transparent ruler and internarial distance (IND) was measured to the nearest .1 mm with the ocular micrometer of a dissecting microscope. Most of the *A. texanum* encountered were also treated in this manner. All other species were released over the fence immediately.

Minnow traps were set in both ponds on five occasions in order to capture additional salamanders for ploidy determination. These animals were treated as above.

Male *A. texanum* were paired with *A. texanum* or polyploid females and placed in salamander-proof screenwire cylinders (mating cages) in the small pond at the study sight. Adults for the crosses came from the study sight drift fence or from a drift fence at Trelease Woods in Champaign County, Illinois. Some males were used in more than one cross. The mating cages were checked daily for seven days starting the morning after they were set up. The number of spermatophores and eggs were recorded and the males were removed if eggs were present. A cross was scored as either failure, if no spermatophores or their remains were present and no eggs were laid; spontaneous, if the female laid a clutch of eggs in the absence of any evidence of spermatophores; courtship, if spermatophores were present but no or infertile (non-dividing) eggs were laid by the female; or as fertilization, if spermatophores or their remains were present and the female laid a clutch of eggs that divided. All egg masses were taken to the laboratory were they were held in pond water at 4° C until they reached the limb bud stage. They were then transferred to a bench top in the laboratory (temperature range was 14-24° C) where they were kept through metamorphosis. As the larvae hatched they were gradually moved to separate (one larvae each) plastic containers (10 x 10 x 8 cm) of commercial spring water where they were fed wild caught *Daphnia sp.*, live pet store tubifex worms (known as bloodworms or blackworms in the pet trade) or small chunks of beef liver approximately every other day. Metamorphosed larvae were held on wet paper towelling until they achieved an adult color pattern (usually dark brown to black as opposed to green to light brown for larvae and newly metamorphosed juveniles). They were then sacrificed and a small amount of blood was taken for flow cytometric analysis of ploidy. Number of eggs laid, percent fertilized, percent hatching, and percent of larvae metamorphosing were recorded for all egg masses. Morphological deformities were also noted.

One *A. platineum* and one tetraploid were sacrificed in order to verify the genomic constitution proposed for these taxa by Morris and Brandon (1984). Liver and skeletal muscle were removed and stored in 20-50 ml of 0.1 M EDTA at -80 °C until they were homogenized in 0.1 M Tris/10 mM DTT, pH 7.2. Horizontal starch gel electrophoresis was carried out using a morpholine citrate buffer system, pH 6.5 (Clayton and Tretiak, 1972). Using skeletal muscle, we scored the mobility of proteins encoded by two loci: aconitate hydratase (ACOH; EC 4.2.1.3) and creatine kinase (CK; EC 2.7.3.2); using liver, we scored superoxide dismutase mobilities (SOD; EC 1.15.1.1). The mobilities of the bands were defined relative to the mobility of the most common allelic product of *A. laterale* (100), which was used as a standard on most gels.

The pond was visited almost weekly after the fence was removed in order to check on the progress of the eggs, make a visual assessment of the size of the larval population, and determine the chances for larval metamorphosis. During this time, both ponds were dip-netted or seined and a small number of larvae were inspected with a hand lens.

Results

Physical parameters of the large pond are given in Table 1. Ice cover was complete until 19 February when open water was present at the edge of the pond. The maximum diameter of the large pond was 40 meters on 2 February 1991. This corresponded to a maximum depth of 48 cm and did not change significantly until early June when it dropped to 30 cm in only eight or ten days. The small pond had dried completely by 13 June and the large pond by 20 June, 1991. Spermatophores and eggs were first noticed on 3 March with new eggs masses appearing on 5 March.

The raw data for immigrants encountered at the drift fence is presented in Table 2. The total number of Ambystomatid salamanders encountered at the fence was 76 polyploids (all female), 26 *A. texanum* (17♀, 9♂), and 93 *A. maculatum* (30♀, 63♂). Figure 1 shows the location of the 1991 fence and cans relative to those utilized in past years (Morris and Brandon, unpublished). There were only two significant migration events for the polyploids during 1991, the nights of 18-19 February and 1-2 March when 38% and 46% of the polyploids were caught, respectively. The most significant immigration event for *A. texanum* was the night of 1-2 March when 58% of the total were recorded. The relationship of temperature and precipitation to immigration of the polyploids is given in Figure 2. The two significant polyploid immigration events were both accompanied by rainfall and a mean daily temperature of 8-12° C. The distribution of immigrant polyploids at the 38 drop cans is given in Figure 3. Similar distributions are given for *A. texanum* (Fig. 4) and *A. maculatum* (Fig. 5).

An additional 17 polyploids were taken from the large pond with minnow traps on 2 March (1), 13 March (1), 20 March (9) and 21 March (7). As these animals did not have a toe-clip, it is certain they had not been previously recorded. This gave a grand total of 93 polyploids available for ploidy determination. For all but two of these salamanders, ploidy determined by a plot of SVL vs. IND (Fig. 6) agreed with that obtained from flow cytometry. These two individuals had flow cytometry readings one would expect if a second *A. texanum* genome were added to a tetraploid to yield a pentaploid (Fig. 7). Re-evaluation of the SVL and IND of these individuals (Fig. 6) agrees with this scenario. We recorded 62 triploids, 29 tetraploids, and 2 pentaploids; all of which were female. The snout-vent length distributions for these individuals is given in Figures 8 and 9.

Twenty-three crosses were attempted with triploid and tetraploid females taken at the drift fence at Middle Fork Woods (Table 3). *A. texanum* males put down more spermatophores for conspecific females ($\bar{x}=20$) than they did for either triploid ($\bar{x}=6$) or tetraploid ($\bar{x}=9$) females and there was a slight trend toward more spermatophores for tetraploids than triploids. Ten of these crosses yielded viable eggs that were raised in the laboratory. Survivorship rates for these crosses (Table 4) show a trend towards a higher percentage of hatchlings in the *A. texanum* control crosses than in the *A. texanum* x tetraploid crosses. The latter cross type also had a high percentage of morphological abnormalities whereas no abnormalities were observed in the *A. texanum* control larvae.

Results of the protein electrophoresis show that the one triploid salamander examined has one genome of *A. laterale* and two of *A. jeffersonianum* and therefore is assignable to *A. platineum.* The tetraploid individual has, in addition to the compliment of *A. platineum*, an added *A. texanum* genome. These results are in agreement with the morphological determination of Morris and Brandon (1984). University of Illinois Museum of Natural History (UIMNH) catalogue numbers for these specimens are given in Appendix 1.

Egg masses were monitored on 3, 10, and 17 April. Most of the polyploid masses contained a large proportion of dead eggs and several had no living eggs at all. The few *A. texanum* masses observed had no visible mortality nor did a large number of *A. maculatum* egg masses. All *A. texanum* and polyploid eggs had hatched by 10 April but the *A. maculatum* eggs had not hatched by 17 April. Larval salamanders were sampled on 17 and 24 April, 14 and 24 May, and 5, 13, and 18 June. Polyploids were the most common larvae in the samples until they started to metamorphosis on 24 May; after this, *A. maculatum* larvae gradually replaced them in importance. On 18 June, when the large pond was reduced to puddle less than a meter long and 5cm deep, we searched the leaf litter and debris in the pond basin for less than five minutes and uncovered five newly metamorphosed polyploids (SVL; 30-35 mm).

Discussion

We have identified four main areas of discussion that pertain to the viability of the populations of *Ambystoma platineum*, *A. texanum* and their hybrids at Middle Fork Woods Nature Preserve:

Current size of the breeding population of *A. texanum*
Even though the number of *A. texanum* intercepted by drift fence this year (26) is the highest yet recorded, it should still be considered the most pressing problem at the Middle Fork Woods pond for two reasons. First, the polyploids are entirely dependent on *A. texanum* males for their reproductive success, and second, the small size of the *A. texanum* population makes it susceptible to genetic problems such as inbreeding depression as well as stochastic factors such as disease and climatic events (for example, a sudden freeze during the breeding migration could easily kill all the male *A. texanum*).
Recommendations:
The size of the breeding population of *A. texanum* should be followed for the next several years. This can be best accomplished by drift fence or minnow traps but observations of the percentage of dead eggs per egg mass will also help to track the size of the breeding population of *A. texanum* since the number of egg masses with 100% mortality (=unfertilized) is an indication of the scarcity of males. If the *A. texanum* population does not increase dramatically in the next few breeding seasons, translocations, either importing *A. texanum* egg masses to Kickapoo or exporting fertile polyploid egg masses to a pond with a large *A. texanum* population, should be considered. Translocations should be attempted only with eggs (or possibly small larvae) as some species of *Ambystoma* exhibit extreme breeding pond fidelity.

Current size of breeding population of polyploids
Fewer polyploids were caught by drift fence this year (76) than in any previous attempt (1980, 350; 1981, 258; 1990, 106). While this may reflect a reduction in the breeding population, other factors such as differences in fencing materials, fence placement and timing should be considered as variables when comparing between years. In addition, the 1991 fence did not completely surround the large pond, but left a portion unfenced that was identified by Morris (1981) as being an important corridor for polyploid migration.

Recommendations:
Any decline in the numbers of polyploids is undoubtedly tied to the scarcity of male *A. texanum* so attempts to remedy the latter will be the most beneficial to the system.

Ploidy composition
Of additional interest is the increasing proportion of tetraploids. The proportion of polyploids that are tetraploid has increased significantly from the only other known estimates of 1980 and 1981 (Morris, 1981). Table 5 gives the chi-square contingency table for the 1981 to 1991 comparison. It is clear from the results of the crosses that tetraploids are capable of obtaining sperm from male *A. texanum*, at least when paired one on one. The few progeny of these crosses that have been analyzed by flow cytometry are tetraploid indicating that the size of the adult tetraploid population does not depend entirely on fertilization of triploid (*A. platineum*) eggs. In other words, tetraploids can give rise to tetraploids and as such are in some control of their population size. Add to this the fact that some portion of the eggs of a triploid are normally fertilized to produce tetraploids and a situation arises in which tetraploids may be increasing at the expense of triploids. In this regard, it should be noted that there is laboratory evidence that the rate of fertilization (as opposed to activation) is higher at warmer water temperatures (Bogart et al., 1989). This means that at higher water temperatures, *A. platineum* may produce mostly tetraploids. If water temperatures at the pond fluctuate from year to year because they are tied to water depth and thus precipitation levels, there may be an environmental basis for cycles of ploidy composition. With only two periods in time sampled, it is possible that this increase in tetraploids represents only a temporary tetraploid peak in a normally fluctuating cycle of ploidy composition.
Recommendations:
Ploidy composition should be determined each year for a random sample of adults collected by drift fence or minnow traps. Ploidy can easily be determined using the morphological parameters in Figure 5. The SVL distribution of these same adults can be used to document the appearance of new recruits (SVL class I individuals, see below).

Larval metamorphosis
Since the first signs of polyploid metamorphosis were observed 25 days before the large pond dried and newly metamorphosed polyploids were abundant in the dried pond basin, it can be assumed that most of the polyploid larvae were successful in leaving the breeding pond. However, no quantitative assessment was attempted. The few measurements recorded for newly metamorphosed polyploids in 1991 are similar to those

taken by Morris (1981) suggesting that they did not metamorphose at a sub-optimum size as a result of the early pond drying in 1991. If Morris' (1981) laboratory estimate of two years as the time required for juveniles to reach sexual maturity can be applied to natural populations then one would expect to see individuals of age class I (72-84 mm SVL) in the breeding migration of 1993. Since no individuals of this age class were caught at the drift fence in 1991 we can assume that there was little or no metamorphosis in 1989. However, the appearance of age class I individuals in the 1990 sample (Szafoni, 1990) indicates that there may have been metamorphosis three to four years ago.

Recommendations:

The water levels of both ponds should be noted at regular intervals throughout the entire year, with a concentration of effort from February through August. This would provide a rough estimate of the chances for metamorphosis in a given year. If possible, the pond should be dip-netted to verify that larvae are present. The SVL distribution obtained from the above recommendation can be used to document the first appearance of successful metamorphs from past years. In this way a better estimate of the time from metamorphosis to first breeding can be obtained.

Appendix I
University of Illinois Museum of Natural History (UIMNH) Catalogue Numbers

Species	UIMNH#	Comments
Ambystoma platineum	95675	used in genetic identification
A. platineum x *A. texanum*	95676	" "
A. maculatum	95665	found dead in pond
Plethodon cinereus	95644	found dead in drop-can

Literature Cited

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Macgregor, H. C. and T .M. Uzzell, Jr. 1964. Gynogenesis in salamanders related to _Ambystoma jeffersonianum_. Science 143:1043-1045

Morris, M. A. 1974. An Illinois record for a triploid species of the _Ambystoma jeffersonianum_ complex. J. Herpetol. 8:255-256.

Morris, M. A. 1981. Taxonomic status, reproductive biology, and larval life history of two unisexual forms of _Ambystoma_ from Vermilion County, Illinois. MA Thesis, Southern Illinois University, Carbondale, Illinois. 88 pp.

Morris, M. A. and R. A. Brandon. 1984. Gynogenesis and hybridization between _Ambystoma platineum_ and _Ambystoma texanum_ in Illinois. Copeia 1984:324-337.

Morris, M. A. and R. A. Brandon. Unpublished Report: Methods for monitoring the _Ambystoma platineum_ population at Kickapoo State Park, Vermilion County, Illinois.

Sessions, S. K. 1982 Cytogenetics of diploid and triploid salamanders of the _Ambystoma jeffersonianum_ complex. Chromosoma 84:599-621.

Szafoni, R. E. 1990. Unpublished Report: Report of the 1990 survey of spring-breeding _Ambystoma_ salamanders at Middle Fork Woods Nature Preserve Vermilion County, Illinois, with special reference to the silvery salamander, _Ambystoma platineum_.

Uzzell, T. M., Jr. and S. M. Goldblatt. 1967 Serum proteins of salamanders of the _Ambystoma jeffersonianum_ complex, and the origin of the triploid species of this group. Evolution 21:345-354.

Table 1. Physical measurements at the large pond, Middle Fork Woods Nature Preserve, Vermilion Co., IL, 1991.

Date	pH	water temp	max. depth
9 February	----	5-7° C	----
21 February	----	9-10° C	----
27 February	6.0*	2-4° C	----
28 February	----	8-9° C	----
1 March	----	7-8° C	48 cm
3 March	----	3-4° C	----
4 March	----	6-8° C	----
5 March	----	8° C	----
6 March	----	6° C	----
7 March	6.0*	8-9° C	----
12 March	----	4° C	48 cm
13 March	----	3° C	----
18 March	----	8° C	----
19 March	----	10-13° C	----
20 March	----	11° C	----
21 March	----	14° C	----
22 March	----	16° C	----
23 March	----	----	----
24 April	7.8#	----	----
14 May	7.2#	----	----
24 May	6.6#	----	----
5 June	6.4#	----	30 cm
13 June	----	----	10 cm
18 June	----	----	5 cm

*=taken with Hydrion pH paper.

#=taken with a laboratory pH meter.

Table 2. Salamander migration by pitfall can and date for all salamanders encountered at the drift fence during the 1991 breeding season at Middle Fork Woods Nature Preserve, Vermilion Co., Illinois. p= *Ambystoma* polyploids (all are female), t= *A. texanum*, m= *A. maculatum*.

Can#	19 Feb	21 Feb	27 Feb	1 Mar	2 Mar	3 Mar	6 Mar	12 Mar	13 Mar	18 Mar	Totals
1	1 p				*				* 1 t (♀)	*	1 p 1 t
2					1 p					1 m	1 p 1 m
3					1 m					1 m	2 m
4									*		
5					2 p				2 m		2 p 2 m
6	1 p				1 p			1 m		1 m	2 p 2 m
7	2 p				*					*	2 p
8			1 t (♀)		6 p 10 m					2 p 2 m	8 p 1 t 12 m
9	3 p 1 t (♂)		1 p		2 p 10 m						6 p 1 t 10 m
10					1 p 10 m					3 m	1 p 13 m
11	1 p				15 m					3 m	1 p 18 m
12										1 m	1 m
13	2 p				1 m				1 p	1 m	2 p 2 m

Table 2. (continued)

Can#	19 Feb	21 Feb	27 Feb	1 Mar	2 Mar	3 Mar	6 Mar	12 Mar	13 Mar	18 Mar	Totals
14	1 p				3 m				*		1 p 3 m
15										3 m	3 m
16					*				2 m*	*	2 m
17	2 p 1 m				5 p 1 m						7 p 2 m
18	2 p				1 t (♀)						2 p 1 t
19	1 p				1 p 2 m					1 m	2 p 3 m
20	1 p				1 t (♀) 1 m				*	1 p 2 m	2 p 1 t 3 m
21				1 p	2 p 1 t (♀) 4 m				1 p		4 p 1 t 4 m
22				1 t (♂)	1 t (♀)						2 t
23	3 p				3 p 1 m				1 m		6 p 2 m
24	4 p 1 t (♂)		1 t (♂)		1 p 2 t (♂)						5 p 4 t
25	2 p 1 t (♀)	1 t (♀)			2 p 1 t (♀)		1 p		1 p		6 p 3 t
26	1 p				2 t (1 ♀)	1 t (♀)			*		1 p 3 t
27					*				*		
28	1 p				1 p					*	2 p
29					1 p						1 p

Table 2. (continued)

Can#	19 Feb	21 Feb	27 Feb	1 Mar	2 Mar	3 Mar	6 Mar	12 Mar	13 Mar	18 Mar	Totals
30	1 p				1 t (♀)						1 p 1 t
31								1 p			1 p
32											1 t
33									1 m	1 t (♂)	1 m
34								1 p			1 p
35	NA	NA			3 t (♀) 1 m					1 m	3 t 2 m
36	NA	NA			1 p 2 m					2 m	1 p 4 m
37	NA	NA			5 p 2 t (1 ♀)			1 p			6 p 2 t
38	NA	NA							1 t (♀) 1 m		1 t 1 m
Totals	29 p 3 t (1♀) 1 m	1 t (♀)	1 p 2 t (1 ♀)	1 p 1 t (♂)	35 p 15 t (11♀) 62 m	1 t (♀)	1 p	3 p 1 m	3 p 2 t (♀) 7 m	3 p 1 t (♂) 22 m	76 p 26 t (17♀) 93 m

*=these cans were flooded with water on these dates.
NA=these cans were not added until 21 February

Table 3. Crosses set up at Middle Fork Woods Nature Preserve-1991. (n) = the mean number of spermatophores laid in that cross type.

Cross Type (♂ x ♀)	Failure	Spontaneous	Courtship	Fertilization	Total
A. jeff. x 3n	1				1
A. tex. x A. tex.	1			3 (20)	4
A. tex. x 3n	3		3 (6)		6
A. tex. x 4n		5		7 (9)	12
					23

Table 4. Survivorship of eggs from successfull crosses set up in 1991 at Middle Fork Woods Nature Preserve. A. tex. = *Ambystoma texanum.*

Cross Type (♂ x ♀)	# eggs laid	% Fertilized (#)	% Hatched (#)	% Deformed (#)	% Metamorphosed
A. tex. x A. tex.					
#22	320	98 (315)	89 (286)	0	NA
#41	280	?	30 (82)	0	NA
#42	434	84 (366)	66 (286)	0	NA
A. tex. x 4n					
#23	70	40 (28)	14 (10)	6 (4)	3 (2)
#24	132	94 (114)	52 (68)	14 (19)	NA
#25	119	96 (114)	62 (74)	18 (21)	NA
#27	100	43 (43)	4 (4)	1 (1)	0 (0)
#30	128	89 (114)	24 (31)	23 (29)	6 (7)
#33	90	49 (44)	39 (35)	16 (14)	9 (8)
#49(241/2)	137	84 (115)	54 (74)	15 (21)	NA

NA - all larvae from these crosses have not yet metamorphosed

Table 5. Contingency table for *Ambystoma* ploidy composition differences between 1981 and 1991 at Middle Fork Woods pond.

	3n	4n	
1981	85	14	99
1991	66	27	93
	151	41	192

$$\chi^2=\frac{192(|85\cdot27-66\cdot14|-96)^2}{151\cdot41\cdot99\cdot93}$$

$=5.51 \quad p<.025$

Figure 1. Map of the study site showing the location of the 1991 fence and drop-cans relative to the 1980-81 fence and drop-cans (modified from Morris and Brandon, unpublished report).

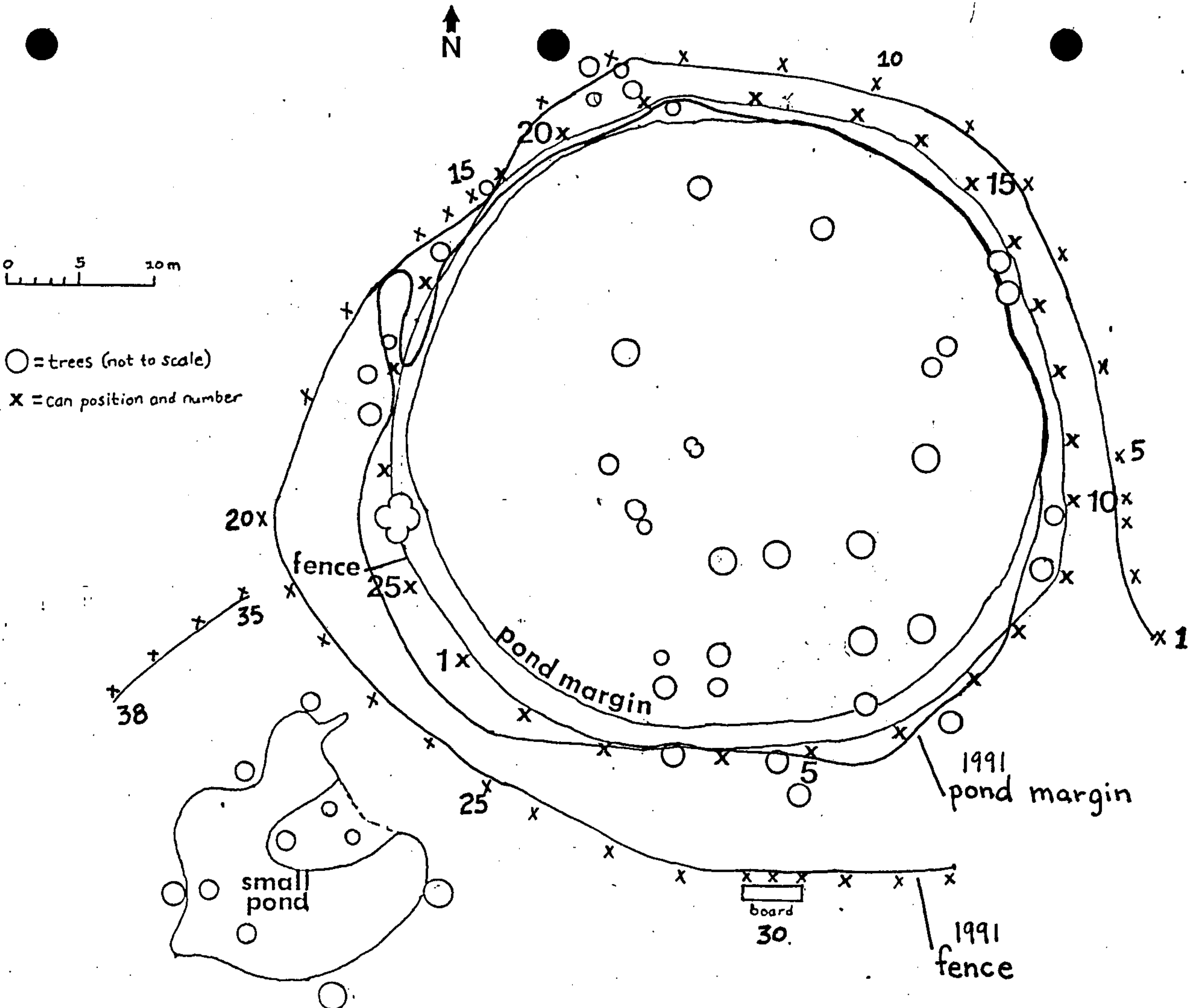
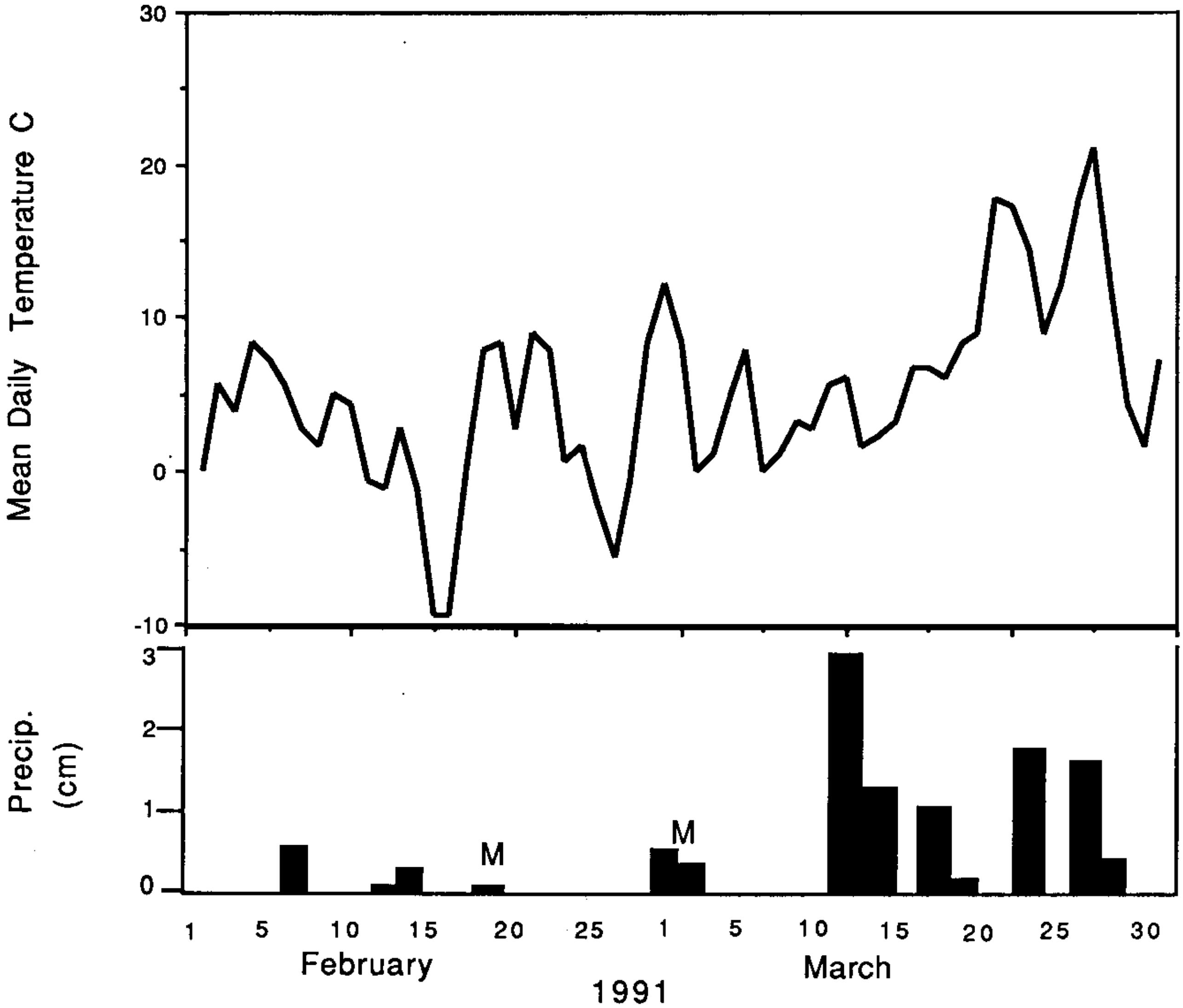

N
0 5 10 m
= trees (not to scale)
x = can position and number
10
20x
15
x 15
x 5
x 10
20x
fence
25x
35
1x
pond margin
38
x 1
5
25
1991
pond margin
small
pond
board
30.
1991
fence

Figure 2. Relationship of temperature and precipitation to 1991 immigration of polyploid *Ambystoma* at Middle Fork Woods Nature Preserve. M indicates significant polyploid immigration.



Mean Daily Temperature C
30
20
10
0
-10
Precip. (cm)
3
2
1
0
M
M
1
5
10
15
20
25
1
5
10
15
20
25
30
February
March
1991

Figure 3. Distribution of immigrant polyploid *Ambystoma* at the 38 drop-cans of the 1991 drift fence at Middle Fork Woods Nature Preserve.

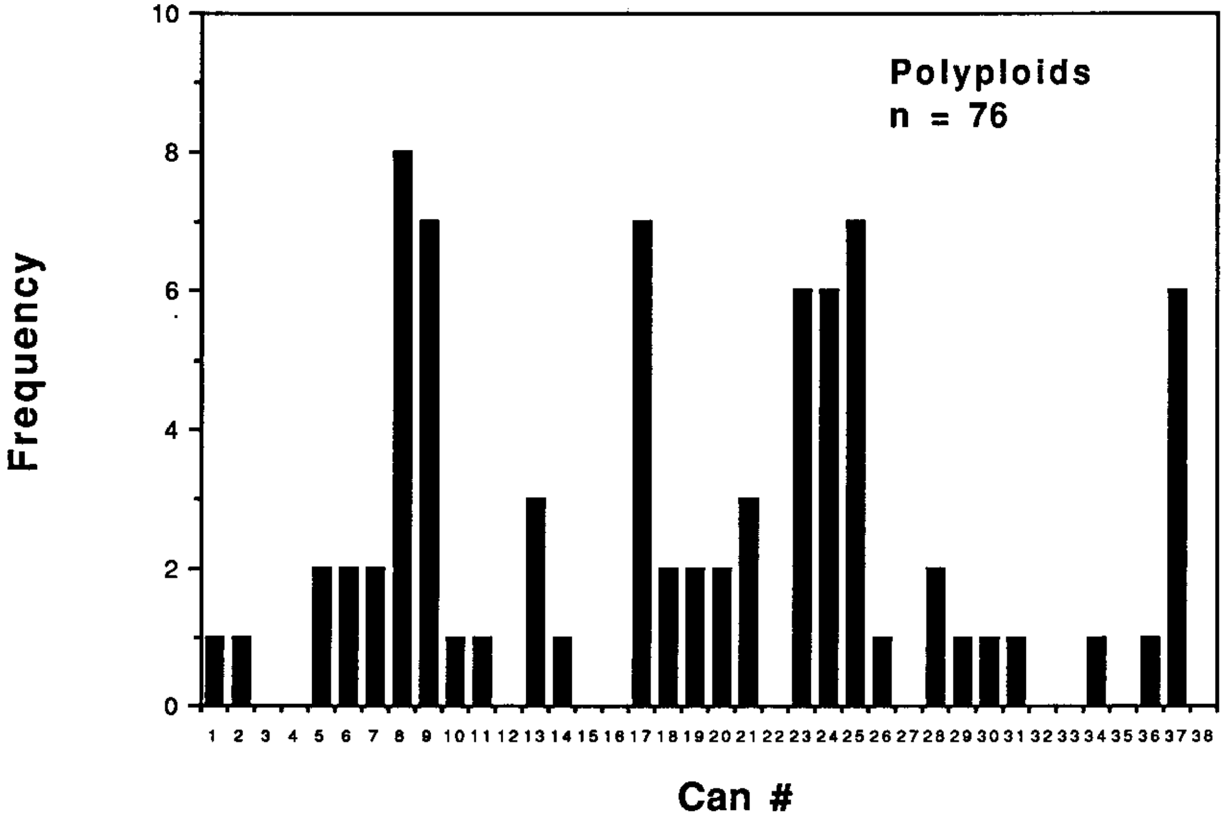
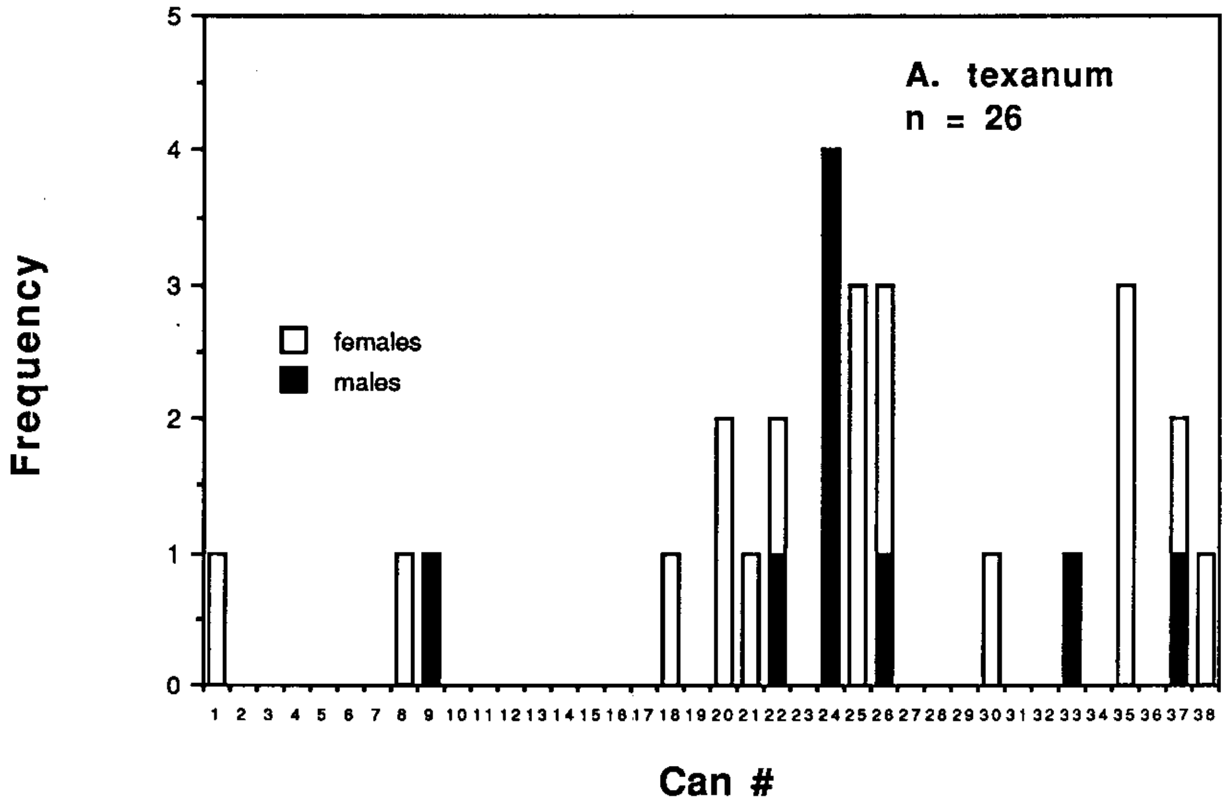

Polyploids
n = 76
Frequency
0
2
4
6
8
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38
Can #

Figure 4. Distribution of immigrant *Ambystoma texanum* at the 38 drop-cans of the 1991 drift fence at Middle Fork Woods Nature Preserve.



A. texanum
n = 26
Frequency
females
males
Can #
0
1
2
3
4
5
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38

Figure 5. Distribution of immigrant *Ambystoma maculatum* at the 38 drop-cans of the 1991 drift fence at Middle Fork Woods Nature Preserve.

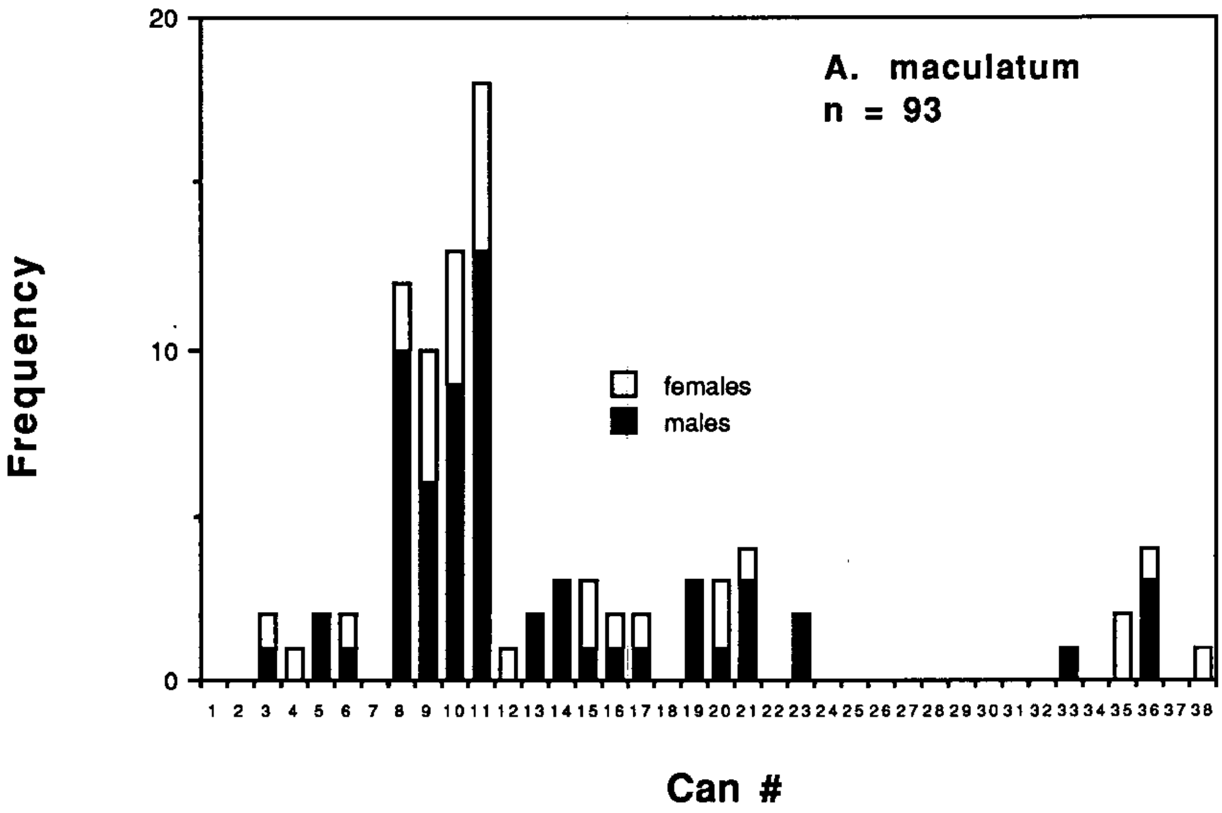
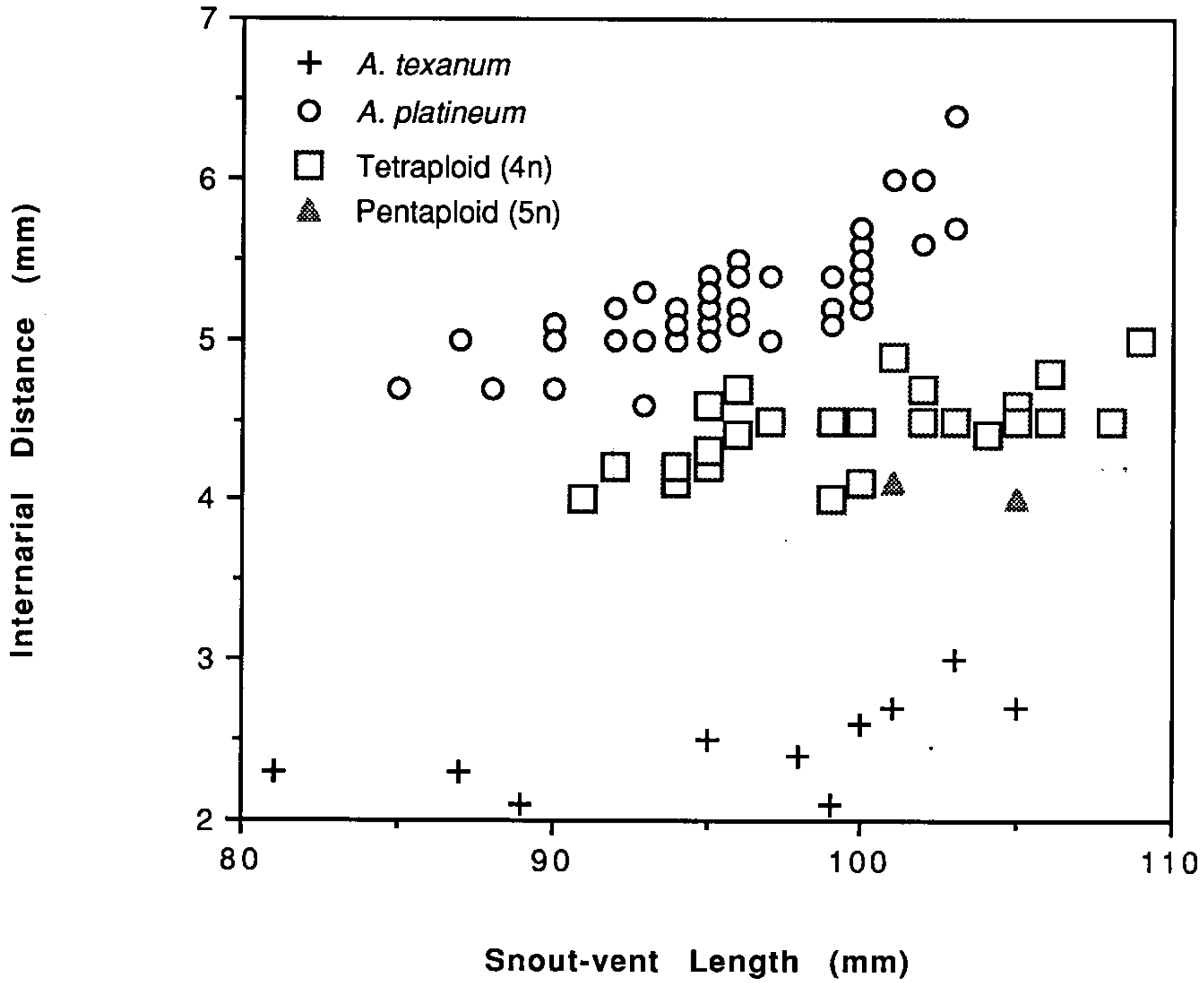

A. maculatum
n = 93
females
males
Frequency
0
10
20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38
Can #

Figure 6. Graph of snout-vent length vs. internarial distance for polyploid *Ambystoma* and *A. texanum* captured at Middle Fork Woods Nature Preserve in 1991.



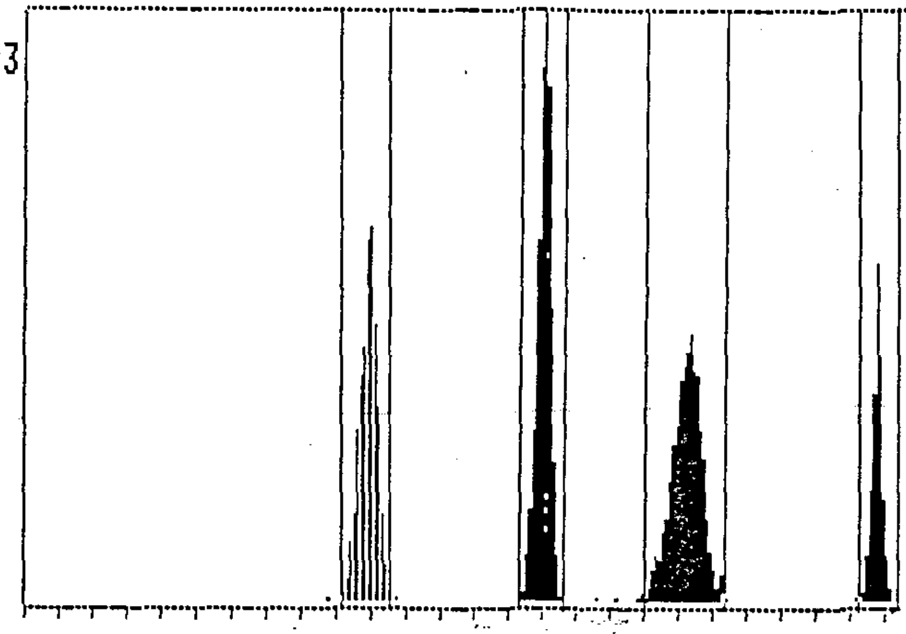
A. texanum
A. platineum
Tetraploid (4n)
Pentaploid (5n)
Internarial Distance (mm)
Snout-vent Length (mm)
2
3
4
5
6
7
80
90
100
110

Figure 7. Print-out of flow cytometric analysis of four *Ambystoma* captured at Middle Fork Woods Nature Preserve in 1991. Peaks are, from left to right, a diploid (*A. texanum*), a triploid (*A. platineum*), a tetraploid (*A. platineum* x *A. texanum*), and a pentaploid (thought to be a tetraploid x *A. texanum*).

STATISTICS

SCALE= 512 TOTAL= 2502

MEAN
192.83
SD
4.34
CV
2.25
LCV
2.58
RCV
1.87
HCV
2.13



PHILLIPS 13:15 04APR91.05202C
477
IGFL /IGFL ,PGFL ,MAP1

PRINT

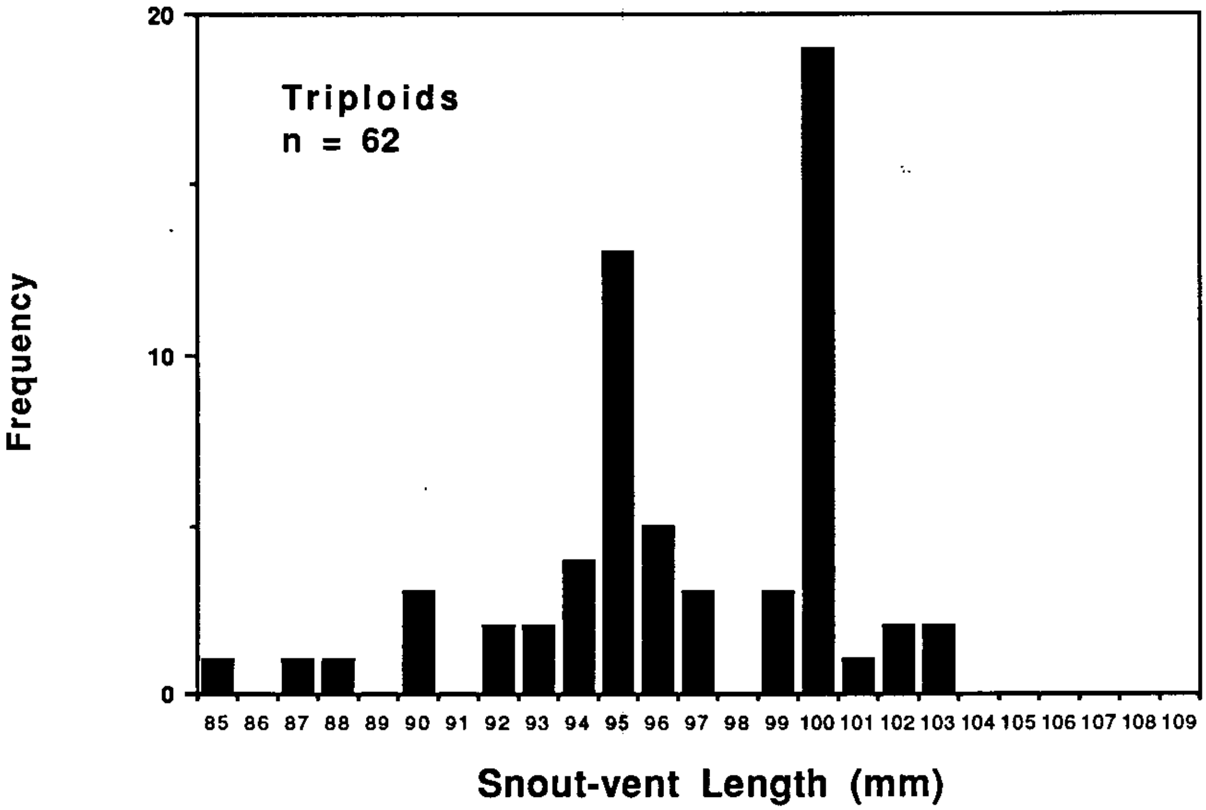
NEXT
CURSOR

CHANNEL 181 TO 204 INTEGRAL 2387
PEAK 233 AT 194 % IN INTERVAL 95.40

RETURN

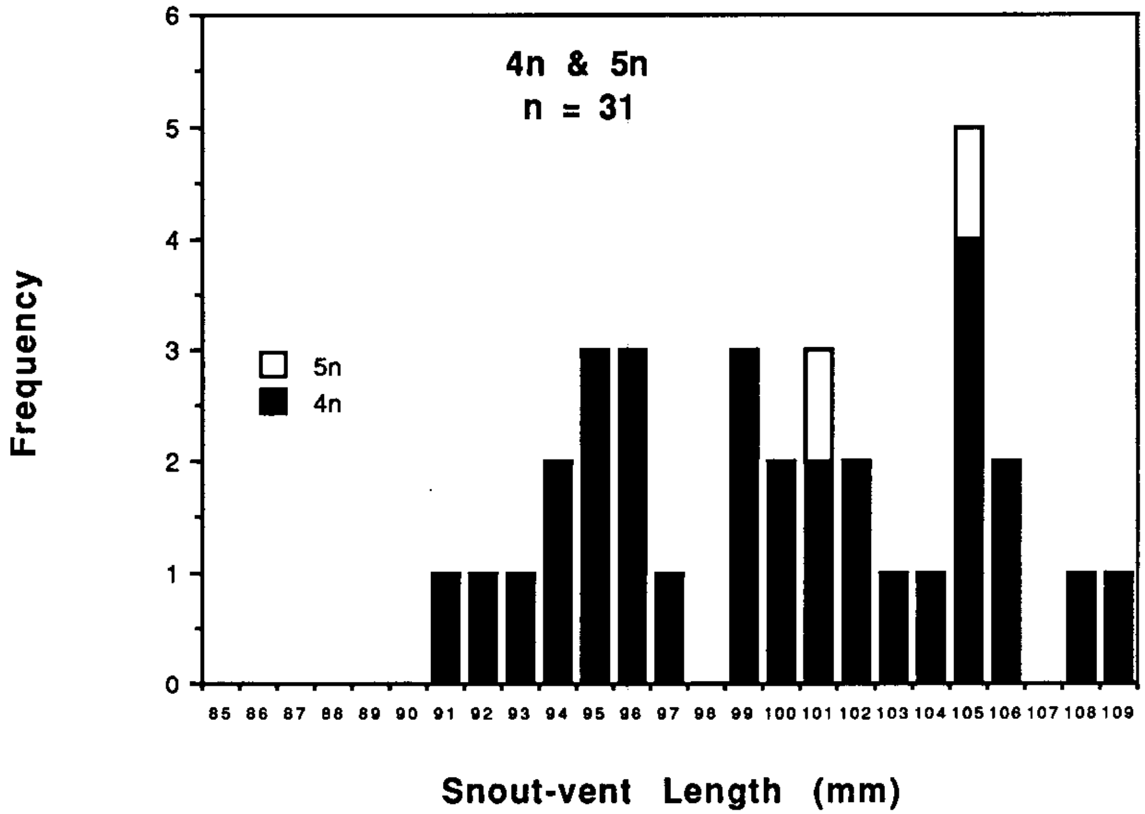
READY.ADJUST LOWER CURSOR

Figure 8. Snout-vent length distribution of *A. platineum* captured at Middle Fork Woods Nature Preserve in 1991.



Triploids
n = 62
Frequency
20
10
0
85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109
Snout-vent Length (mm)

Figure 9. Snout-vent length distribution of tetraploid and pentaploid *Ambystoma* captured at Middle Fork Woods Nature Preserve in 1991.



4n & 5n
n = 31
5n
4n
Frequency
0
1
2
3
4
5
6
85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109
Snout-vent Length (mm)

Color Slide Descriptions for the final report for the Nongame Wildlife Conservation Fund Project; Reproduction, Survival and Genetic Constitution of the Polyploid Mole Salamanders of Kickapoo State Park.

1-4) the large pond at Middle Fork Woods Nature Preserve, Vermilion Co., Illinois taken in February, March, April and June, 1991.

5) the 1991 drift fence at Middle Fork Woods Nature Preserve.

6) a fertilized (living) polyploid egg mass from the large pond, at Middle Fork Woods Nature Preserve.

7) a polyploid egg mass containing mostly dead eggs from the large pond, at Middle Fork Woods Nature Preserve.

8-11) a sample of the morphological deformities encountered in the larvae from crosses involving tetraploid females and *A. texanum* males:

- 8) blunt head; lethal even when hand fed.
- 9) curved body; lethal, even when hand fed.
- 10) bent body; sub-lethal, these larvae can be reared to metamorphosis with a great deal of effort.
- 11) an example of an older larvae with the "bent" deformity.