Persistent High Levels of Tetraploidy in Salamanders of the Ambystoma jeffersonianum Complex


Abstract.—Almost all unisexual members of the Ambystoma jeffersonianum complex of salamanders are triploid, although occasional tetraploid individuals have been observed in a few populations. A population characterized by a high percentage of tetraploids (7–27%) exists at Kickapoo State Park, Vermilion Co., Illinois. This high frequency of tetraploids has been maintained over a period of 12 years. Tetraploids in this population were originally formed by fertilization of unreduced A. platineum ova by A. texanum sperm. Tetraploids at Kickapoo State Park are, however, capable of obtaining sperm from the sexual host, Ambystoma texanum, and producing tetraploid offspring. Because tetraploids can be produced two ways, they might be expected to out-reproduce and replace triploids. However, these tetraploids also produce abundant pentaploid offspring as a result of fertilization of their ova by A. texanum sperm. A high incidence of physical abnormalities among pentaploid larvae and their scarcity as breeding adults suggests that pentaploids have reduced viability. Production of pentaploid larvae may thus be the selective mechanism that prevents tetraploids from replacing triploids in this population. Higher than normal water temperature may be causing increased fertilization rates in this population; this would account for its higher frequency of tetraploids compared to other populations, as well as for the high number of pentaploid larvae that tetraploids produce.

The Ambystoma jeffersonianum complex consists of two diploid, bisexual species, A. jeffersonianum and A. laterale, and two essentially all-female triploid species (Appendix). A. platineum and A. tremblayi (Clanton, 1934; Minton, 1954; Uzzell, 1963). On the basis of their intermediate morphology and distribution, the two triploid species were postulated to have arisen by hybridization between A. jeffersonianum and A. laterale (Minton, 1954; Uzzell, 1964). Biochemical and karyological studies demonstrated that A. platineum has two chromosome sets from A. jeffersonianum and one from A. laterale (JLL), whereas A. tremblayi has the reverse, one chromosome set from A. jeffersonianum, two from A. laterale (JLL; Uzzell and Goldblatt, 1967; Sessions, 1982). Evidence for gyngenetic reproduction in these salamanders was provided by Macgregor and Uzzell’s (1964) observation of 42 bivalents, equivalent to hexaploidy, in oocytes of one A. platineum and five A. tremblayi, indicating the occurrence of an endoduplication, presumably premeiotic, followed by formation of pseudobivalents (paired sister chromosomes). The 42 pseudobivalents undergo a normal meiosis to yield ova with the exact chromosome complement of the mother. Sperm from spermatozoa deposited by males of the diploid, parental species, which normally serve as the hosts in these systems of sexual parasitism, activate the unreduced eggs of these females but normally do not fertilize them.

Several exceptions to this scenario have been reported, including: (1) populations of A. platineum that are sexual parasites on A. texanum rather than on either of the two parental species (Uzzell and Goldblatt, 1967; Morris and Brandon, 1984; Spolsky et al., 1992) and (2) populations of unisexual hybrids that include, in addition to the usual triploids, tetraploid individuals that result from fertilization rather than simple activation of unreduced triploid ova (Swayze, 1979; Bogart, 1989; Lowcock et al., 1991; Spolsky et al., 1992; Lowcock, 1994).

A salamander population at Kickapoo State Park in Vermilion County, Illinois, exhibits both of these exceptions (Morris and Brandon, 1984). This population, which consists almost exclusively of triploid A. platineum, tetraploid unisexuals, and A. texanum, is perhaps unique among

5 Present Address: Illinois Natural History Survey, Center For Biodiversity, 607 East Peabody Drive, Champaign, Illinois 61820, USA.
6 Present Address: Academy of Natural Sciences, Philadelphia, Pennsylvania 19103, USA.
7 Present Address: Applied Ecological Services, 17921 Smith Rd. P.O. Box 256, Broadhead, Wisconsin 53520, USA.
and development of flow cytometry techniques. Erythrocyte area measurement followed the procedures outlined by Macgregor and Uzzell (1964), except that dried blood cells were measured with the ocular micrometer of a dissecting microscope. Morphological measurements were described by Spolsky et al. (1992); flow cytometric techniques are those described by Lowcock et al. (1992).

Male A. texanum were paired with A. texanum or polyploid females and placed in screen wire cylinders (mating cages) in the pond at the study site. No male was used in more than one mating trial. Polyploid females came from the study site drift fence. *Ambystoma texanum* came from the study site drift fence or from a drift fence at Trelease Woods in Champaign County, Illinois, approximately 50 km west of KSP. This was necessary because very few A. texanum migrated to the drift fence at KSP during this investigation. The mating cages were checked daily for seven days beginning the morning after the experiments were started. The number of spermatophores and presence of egg masses were recorded; males were removed if eggs were present. Crosses were scored as failure (no spermatophores or remains present; no eggs laid), infertile eggs (a clutch of eggs, always nondividing, laid in the absence of any spermatophores), spermatophores only (spermatophores present but no eggs or non-dividing eggs laid), or fertilization (spermatophores or remains present; eggs laid that divided). All egg masses were taken to the laboratory where they were held in pond water at 4–7 °C until they reached the limb bud stage. As the larvae hatched they were moved to separate plastic containers of commercial spring water ranging from 14–24 °C. They were fed wild caught *Daphnia* sp., tubifex worms, or small chunks of beef liver approximately every other day. Metamorphosed individuals were maintained on wet paper towels 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 DNA content. Number of eggs laid, percent fertilized, percent hatching, and percent of larvae metamorphosing were recorded for all egg masses. Morphological deformities were also noted.

**RESULTS**

In the 1990 breeding season, we measured erythrocyte area for 44 polyploids out of a total of 106 that were captured at the drift fence. Forty-one (93%) were triploid and three (7%) were tetraploid. The range of erythrocyte areas ($\mu^2$) was 790 to 1070 for triploids and 1230 to 1260

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### Table 1. Numbers and percentage of tetraploids observed in *Ambystoma texanum*-dependent populations of hybrid salamanders at Kickapoo State Park and four sites in Indiana.

<table>
<thead>
<tr>
<th>Site</th>
<th>Year</th>
<th>% tetraploid/total</th>
<th># of polypliods</th>
</tr>
</thead>
<tbody>
<tr>
<td>KSP</td>
<td>1980</td>
<td>14</td>
<td>14/99</td>
</tr>
<tr>
<td>KSP</td>
<td>1990</td>
<td>7</td>
<td>7/44</td>
</tr>
<tr>
<td>KSP</td>
<td>1991</td>
<td>28</td>
<td>27/95</td>
</tr>
<tr>
<td>KSP</td>
<td>1992</td>
<td>13</td>
<td>28/210</td>
</tr>
<tr>
<td>Clinton Co., IN</td>
<td>1989</td>
<td>6</td>
<td>1/17</td>
</tr>
<tr>
<td>Delaware Co., IN</td>
<td>1989</td>
<td>0</td>
<td>0/5</td>
</tr>
<tr>
<td>Clay Co., IN</td>
<td>1990</td>
<td>0</td>
<td>0/5</td>
</tr>
<tr>
<td>Montgomery Co., IN</td>
<td>1990</td>
<td>0</td>
<td>0/5</td>
</tr>
</tbody>
</table>

1 Morris and Brandon 1984; 2 this study; 3 Spolsky et al. 1992.
for tetraploids (the range for diploid *A. texanum* was 630 to 650). In the 1991 breeding season we used both IND-SVL plots and FCM to infer ploidy of all 95 polyploids encountered. Sixty-six (70%) of the hybrids were triploid, 27 (28%) were tetraploid, and two (2%) were pentaploid. Ploidy inferred by morphology agreed with the FCM results in all cases except that the two pentaploid individuals were initially judged to be tetraploid by IND-SVL. Fig. 1 shows the IND-SVL plots for the polyploids and *A. texanum* sampled at KSP in 1991. For the 1992 breeding season, IND-SVL plots indicated that out of a total of 210 polyploids encountered at the fence, 182 (87%) were triploid and 28 (13%) were tetraploid. The percentage of tetraploids breeding at KSP has thus fluctuated significantly since 1981 and over the past three years (2 × 4 contingency table, $\chi^2 = 18.47, P < 0.001$), although it has remained consistently higher than the percentage found in other populations of *A. platinum* (Spolsky et al., 1992).

Twenty-three crosses, all using *A. texanum* males, were attempted with triploid and tetraploid females taken at the drift fence at KSP as well as with conspecific females (Table 2). *Ambystoma texanum* males deposited significantly more spermatophores for conspecific females ($\bar{x} = 20$) than they did for either triploid ($\bar{x} = 6$) or tetraploid ($\bar{x} = 9$) females (Table 3). Among crosses in which males deposited spermatophores, most conspecific crosses (3 of 4; 75%) and over half of the crosses with tetraploid fe-

**Table 2.** Outcome of crosses set up at Kickapoo State Park in 1991. All crosses involved *Ambystoma texanum* males.

<table>
<thead>
<tr>
<th>Female</th>
<th>N</th>
<th>Failure</th>
<th>Inertile eggs</th>
<th>Spermatophores only</th>
<th>Fertilization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. texanum</em></td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3 (75.0)</td>
</tr>
<tr>
<td>4n unisexual</td>
<td>12</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>7 (58.3)</td>
</tr>
<tr>
<td><em>A. platinum</em></td>
<td>6</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

**Table 3.** Numbers of spermatophores deposited by *Ambystoma texanum* males when confined in breeding cages with *Ambystoma texanum* females, tetraploid females, or *Ambystoma platinum* females. N = number of crosses. Values on the diagonal are ranges and means; values in the lower left are probabilities of a significant difference in number between the combinations compared (Mann-Whitney U test).

<table>
<thead>
<tr>
<th>Female</th>
<th>N</th>
<th><em>A. texanum</em></th>
<th>4n unisexual</th>
<th><em>A. platinum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. texanum</em></td>
<td>4</td>
<td>15–35 (22.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4n unisexual</td>
<td>12</td>
<td>0.008</td>
<td>0–20 (5.75)</td>
<td></td>
</tr>
<tr>
<td><em>A. platinum</em></td>
<td>5</td>
<td>0.01</td>
<td>0.433</td>
<td>0–7 (2.8)</td>
</tr>
</tbody>
</table>
males (7 of 12; 58.3%) resulted in dividing eggs, whereas none of the courtships with A. platineum resulted in dividing eggs (Table 2). Four A. texanum × A. texanum controls and four A. texanum × tetraploid crosses yielded viable eggs that were raised in the laboratory. Because of space limitations, only a subset of the control larvae were raised to metamorphosis.

Survivorship rates for the various crosses (Table 4) show a nonsignificant (Mann-Whitney U, P = 0.569) trend towards a higher percentage of hatchlings in the A. texanum control crosses than in the A. texanum × tetraploid crosses. The latter crosses also had a high percentage of morphological abnormalities (13% of eggs) whereas no abnormalities were observed in the A. texanum control larvae (Table 4). Two abnormalities, coiling of the posterior tail and curvature of the body axis, were noticeable and frequent but confined to pentaploid larvae. These abnormalities persisted beyond metamorphosis. As expected, a sample of 50 larvae from the control crosses were all diploid. In contrast, 77% of the larvae of the tetraploid females were pentaploid (Table 4).

**DISCUSSION**

Variable ploidy, including persistently high levels of tetraploidy, have been reported for other populations of the Ambystoma jeffersonianum complex for several consecutive years (Lowcock, 1994). The data presented here, however, suggest that a high percentage of tetraploid hybrids has persisted at KSP for 15 years. It therefore becomes logical to ask how they are maintained. Electrophoretic analysis of one tetraploid from KSP (Spolsky et al., 1992) confirms the morphological evidence (Fig. 1) that the tetraploid unisexuals contain an A. texanum genome in addition to the usual triploid A. platineum complement, and thus originally result from fertilization of unreduced A. platineum ova by A. texanum sperm, as suggested by Morris and Brandon (1984). Although such fertilizations must account for the initial production of tetraploids at KSP and elsewhere, our mating trials also suggest that some KSP tetraploids can acquire sperm from male A. texanum and produce at least some tetraploid offspring.

The production of tetraploids by tetraploids could reflect either gynogenetic reproduction by tetraploids or a genome exchange involving loss of the A. texanum genome during oogenesis and its replacement by fertilization. Whether the tetraploid progeny produced by tetraploid females in our crosses represent activation of tetraploid oocytes or replacement of texanum chromosome
sets (oogenetic loss followed by fertilization) is not known, but allozyme markers demonstrate that chromosome exchange is very unlikely in four other texanum-dependent A. platineum populations (Spolsky et al., 1992). Because tetraploid hybrids can be formed both by fertilization of ova of A. platineum and by reproduction without increase in ploidy level by tetraploid females, their frequency in the population might be expected to increase relative to A. platineum.

An additional factor that may contribute to the reproductive advantage of tetraploids is discrimination by A. texanum males. Their spermatophore deposition was markedly lower when they were caged with polyploid females compared with conspecific females, but slightly higher when caged with tetraploids rather than A. platineum (Table 3). This slight preference for tetraploids may reflect the presence in them of an A. texanum genome. Similar preferential deposition of spermatophores by males of A. jeffersonianum and A. laterale already has been shown (Uzzell and Goldblatt, 1967; Uzzell, 1969). In those studies, spermatophore deposition was slightly lower when males were caged with triploids with two conspecific genomes compared with conspecific females, but was markedly lower when the triploids contained a single conspecific genome.

In addition to the male discrimination revealed by spermatophore numbers, discrimination by male A. jeffersonianum against A. platineum females has also been observed in choice experiments (Dawley and Dawley, 1986). Although such discrimination against clonal hybrids by males of the sexual host species is possibly the most important factor regulating the ratio of clonal hybrids to sexual hosts, in the case of tetraploid females that contain an additional genome from the sexual host, the genomic composition might lead to decreased discrimination by the males. The presence of a single conspecific genome in the KSP tetraploids could have such an effect on A. texanum males.

Given these possible advantages, some factors must restrain the production of tetraploids at KSP. Although a slight reduction in viability (Fankhauser, 1945) or fecundity (Bogart et al., 1987) of tetraploid salamanders may play a role, decreased viability associated with pentaploidy may be the key. The overwhelming majority (72%) of the offspring of tetraploid females in our crosses were pentaploid. In 1991, when FCM data allowed us to identify adult pentaploids unequivocally we detected only two. This, coupled with the severe physical abnormalities of the pentaploid larvae from our crosses and the occurrence of identical abnormalities in pentaploid larvae of Notophthalmus viridescens (Fankhauser, 1945) strongly suggest reduced viability of pentaploid salamanders. Therefore, even though higher than normal fertilization rates are responsible for the origin and early maintenance of tetraploids at KSP, they may also be the ultimate check on their increase.

Based on the discovery of two adult pentaploids out of 606 polyploids at Haliburton Beaver Pond, Lowcock et al. (1991) suggested that allopentaploids had an enhanced tolerance to multiple chromosome sets compared to the autotetraploids reported by Fankhauser (1945). Lowcock et al. (1991) presented no information on the ploidy of progeny produced by tetraploids, but if pentaploids were produced at Haliburton Beaver Pond at even half the rate documented in our crosses, observation of only two adult pentaploids would indicate a significant selective loss of pentaploids prior to their first breeding, probably caused by the multiple chromosome sets. Reduced viability associated with pentaploidy appears to be a wide-spread phenomenon in salamanders.

The persistent, high frequency of tetraploid unisexuals at KSP distinguishes this population from other A. platineum populations. Only Haliburton Beaver Pond in Ontario has comparable levels of tetraploidy. Although they were only documented for a five-year period (Lowcock, 1994), they quite possibly persist as well as KSP. Of the factors that seem to lead to high numbers of tetraploids at KSP (male discrimination and high fertilization rates), fertilization rates seem more likely to vary geographically and provide the difference between KSP and other texanum-dependent A. platineum populations. A similar phenomenon may occur at Haliburton Beaver Pond. Water temperature has been identified as an important variable controlling fertilization rates in unisexual Ambystoma; higher water temperatures resulted in higher fertilization rates (Bogart et al., 1989). If water temperatures have been unusually high at KSP, even if only for a few breeding seasons, this could explain the relative abundance of tetraploids at KSP compared to other texanum-dependent A. platineum populations.

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TETRAPLOIDY IN MOLE SALAManders

LITERATURE CITED


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APPENDIX

Although 18 distinctive hybrid genomic combinations have been noted in the genus Ambystoma (Vrijenhoek et al., 1989), few of them form standing populations; most of them, therefore, almost certainly make no contribution to on-going lineages. Such sporadic individuals, which constitute “hybrids as such” for the purposes of the International Code of Zoological Nomenclature, are best identified by genome-based labels. In contrast, the unisexual triploid mole salamander hybrids at Kickapoo State Park participate in on-going clonal lineages. We therefore use the appropriate species-level name (Ambystoma platineum Cope 1868) to refer to them (cf. Spolsky et al., 1992; Hotz et al., 1996).