

mentally that Barn Swallows could not distinguish between their own young up to 11 days of age and Burt (1977) showed that Barn Swallows do not start to recognize their young until the time of fledging. Furthermore, Barn Swallows have been reported to feed unrelated fledgling Barn Swallows that settle in their nests (Ball 1982). Our data suggest that adult Barn Swallows also do not recognize young of alien species during at least part of the nestling period.

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Literature Cited

- Ball, G. 1982. Barn Swallow fledgling successfully elicits feeding at a non-parental nest. *Wilson Bulletin* 94: 362-363.
- Beecher, M. D., I. M. Beecher, and S. Hahn. 1981. Parent-offspring recognition in Bank Swallows (*Riparia riparia*): II. Development and acoustic basis. *Animal Behaviour* 29: 95-101.
- Burt, E. H., Jr. 1977. Some factors in the timing of parent-chick recognition in swallows. *Animal Behaviour* 25: 231-239.
- Butler, R. W. 1980. Appropriation of an American Robin nest by Dark-eyed Juncos. *Canadian Field-Naturalist* 94: 197.
- Cullen, R. 1957. Adaptations in the Kittiwake to cliff nesting. *Ibis* 99: 275-302.
- Erskine, A. J. 1971. Buffleheads. Canadian Wildlife Service Monograph Series Number 4. Ottawa. 240 pp.
- Erskine, A. J. 1979. Man's influence on potential nesting sites and populations of swallows in Canada. *Canadian Field-Naturalist* 93: 371-377.
- Grzybowski, J. A. 1979. Responses of Barn Swallows to eggs, young, nests, and nest sites. *Condor* 81: 236-246.
- Payne, R. B. 1969. Nest parasitism and display of Chestnut Sparrows in a colony of Gray-capped Social Weavers. *Ibis* 111: 300-307.
- Timbergen, N. 1953. The Herring Gull's world. Collins, London.

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Evidence for the Requirement of Sperm in Unisexual Salamander Hybrids (Genus *Ambystoma*)

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A fast and easy method is described which can be used to detect insemination in hybrid female salamanders of the genus *Ambystoma*. Finding sperm in the cloacae of these females provides evidence of the presence of males, which are often rare. Of 70 females examined on Pelee Island, only those females which were found to be sperm positive gave rise to free-swimming larvae. It is suggested that true parthenogenesis does not occur in the sampled populations.

Key Words: Amphibia, Caudata, *Ambystoma*, hybrids, sperm, parthenogenesis, *Ambystoma laterale*, Blue-spotted Salamander, *Ambystoma texanum*, Smallmouth Salamander.

Unisexual hybrids of mole salamanders, *Ambystoma*, occur in the Great Lakes region of eastern North America. Hybridization has occurred between *A. jeffersonianum* (Jefferson Salamander) and *A. laterale* (Blue-spotted Salamander) to produce the triploids, "*A. platineum*" and "*A. tremblayi*" (Uzzell 1963, 1964); between *A. laterale* and *A. texanum* (Smallmouth Salamander) to produce diploid and polyploid hybrids (Downs 1978; Kraus

1985a; Bogart et al. 1985; Bogart and Licht 1986); between *A. laterale*, *A. texanum* and *A. tigrinum* (Tiger Salamander) to produce the triploid trihybrid "*A. nothogenes*" (Kraus 1985b).

Owing to the continued existence of these hybrids, and the fact that they are essentially all-female, it has been hypothesized that they reproduce either by parthenogenesis (Uzzell 1969; Downs 1978; Kraus 1985a, 1985b), a mode in which sperm is not necessary

at all, or by gynogenesis (Uzzell 1964; Macgregor and Uzzell 1964; Morris and Brandon 1984), in which sperm from a male is used for initiation of cleavage. The male is presumed to be from a sympatric, normally bisexually reproducing species. In gynogenesis, the male genome is not incorporated into the egg nucleus and this mode may also be considered as a form of parthenogenesis (Lynch 1984; Moore 1984).

In a study of *Ambystoma* egg masses in Ontario, Bogart (1982) found some egg masses which had mixed genotypes and ploidy ($2n$ and $3n$). This suggests that certain females are neither parthenogenetic nor gynogenetic. But, in the same study, a single female was found to produce 28 larvae which were genetically identical for two polymorphic electrophoretic loci. This female's cloaca did contain sperm and, based on the available information, reproduction by gynogenesis remains a possibility. True parthenogenesis, without sperm to initiate development, has yet to be proven for any population of *Ambystoma*.

We have been studying *A. laterale* x *A. texanum* hybrids on Pelee Island in Lake Erie, Ontario ($41^{\circ}45'N$, $82^{\circ}40'W$), where males are rare (less than 5%; Bogart et al. 1985). It was deemed important to determine the presence or absence of sperm in the reproductive tract of these females and to examine a large number of females. Such information is essential to distinguish between true parthenogenesis and gynogenesis, as well as being a first step toward elucidating alternative mechanisms. The following procedure allows for the detection of sperm from females taken from breeding populations.

Females were removed from breeding ponds on Pelee Island and maintained at $8^{\circ}C$ while transported to the laboratory. They were anesthetized in tricaine methane sulfonate (MS222), after which a small drop of physiological saline solution was inserted into the cloaca with a pasteur pipette. The cloaca was flushed using gentle pressure on the bulb of the pipette. A drop of the cloacal fluid was placed on a slide and a cover slip applied to the wet mount. The slide was examined for the presence of sperm using phase contrast optics under low power (16X objective: total magnification 160X).

Ambystoma sperm cells are large and easily observed under low power so the entire slide can be scanned before they die. The activity of the sperm cells makes it relatively easy to observe and photograph them (Bogart 1982). The technique is rapid and it is possible to examine a large number of females in a short period of time.

We used this technique to determine the presence or absence of sperm in the cloacae of 70 Pelee Island female *A. laterale* x *A. texanum* hybrids. Of these, 23 were found to be sperm negative and all others had

observable sperm. Sperm was observed from females prior to egg deposition as well as after the eggs had been deposited. Females maintained for up to two weeks at $8^{\circ}C$ after being removed from the breeding population still contained viable sperm. Post-ovulatory females kept at room temperature for two weeks or under refrigeration for longer periods of time (three weeks) showed no evidence of sperm even though some of these same females were sperm-positive in earlier examinations.

Of the initial 70 females examined, only the 47 sperm-positive females laid eggs which either showed late embryological stages or hatched. None of the sperm negative females produced eggs which hatched. We therefore suggest that sperm is essential for normal egg development among Pelee Island *Ambystoma* hybrids. We have observed sperm in females from breeding ponds where males have not yet been found. We also have evidence from the electrophoretic analyses of females and their offspring that genes from sperm are actually present in the zygotes, and this evidence suggests that neither parthenogenesis nor obligatory gynogenesis is operating on Pelee Island (Bogart and Licht 1986).

Survival time for spermatozoa varies in salamanders from a few weeks to more than two years (Boisseau and Joly 1975). Sperm survival in one species of *Ambystoma* (*A. opacum*) is at the low end of the range (Noble and Brady 1933). In his investigation of salamander breeding behaviour, Arnold (1976) examined the cloacae of *A. maculatum* and *A. tigrinum* and observed spermatophores which had just been deposited by males and retrieved by females during laboratory breeding trials. Arnold, however, did not describe his technique for the examination of the females nor did he look for actual spermatozoa.

Noble (1931) suggested that fertilization in salamanders occurs in oviducts, but more recent evidence (Boisseau and Joly 1975; Sever and Houck 1985) indicates that spermatozoa are extruded onto eggs in the cloaca just before they are laid and thus, spermatozoa may not be expected to occur in oviducts. In fact, the ascent of sperm into the oviduct of salamanders is only known to occur in the viviparous *Salamandra salamandra* (Boisseau and Joly 1975). This may explain the findings of Cuellar (1976) who, in his study of meiosis in triploid "*A. tremblayi*," did not examine the cloaca but noted the absence of sperm in oviducts and suggested the possibility of parthenogenesis.

An examination for sperm in the cloacae of breeding females of unisexual *Ambystoma* populations has only been described previously for one individual of the *Ambystoma jeffersonianum*

complex (Bogart 1982). In some other populations it was observed that spermatophores were needed for females to produce normal eggs (Wilber 1971). Using the technique of rapid detection of sperm in the cloacae of females described here, the presence or absence of males and the possible role of males in reproduction may be assessed. A determination of sperm dependency should help clarify the breeding systems used by the different populations of unisexual *Ambystoma* throughout their ranges.

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Literature Cited

- Arnold, Steven J.** 1976. Sexual behavior, sexual interference, and sexual defense in the salamanders *Ambystoma maculatum*, *Ambystoma tigrinum* and *Plethodon jordani*. *Zeitschrift Tierpsychologisch* 42: 247–300.
- Bogart, J. P.** 1982. Ploidy and genetic diversity in Ontario salamanders of the *Ambystoma jeffersonianum* complex revealed through an electrophoretic examination of larvae. *Canadian Journal of Zoology* 60: 848–855.
- Bogart, J. P., L. E. Licht, M. J. Oldham, and S. J. Darbyshire.** 1985. Electrophoretic identification of *Ambystoma laterale* and *Ambystoma texanum* as well as their diploid and triploid interspecific hybrids (Amphibia: Caudata) on Pelee Island, Ontario. *Canadian Journal of Zoology* 63: 340–347.
- Bogart, J. P., and L. E. Licht.** 1986. Reproduction and the origin of polyploids in hybrid salamanders of the genus *Ambystoma*. *Canadian Journal of Genetics and Cytology* 28: 605–617.
- Boisseau, C., and J. Joly.** 1975. Transport and survival of spermatozoa in female Amphibia. Pages 94–104 in *The biology of spermatozoa. Edited by E. S. E. Hafez and C. G. Thibault. Proceedings of the INSERM International Symposium, Nouzilly (1973). S. Karger, Basel, New York.*
- Cuellar, O.** 1976. Cytology of meiosis in the diploid gynogenetic salamander *Ambystoma tremblayi*. *Chromosoma* (Berlin) 58: 355–364.
- Downs, F. L.** 1978. Unisexual *Ambystoma* from the Bass Islands of Lake Erie. Occasional Paper, Museum of Zoology, University of Michigan (685): 1–36.
- Kraus, F.** 1985a. A new unisexual salamander from Ohio. Occasional Papers, Museum of Zoology, University of Michigan (709): 1–24.
- Kraus, F.** 1985b. Unisexual salamander lineages in northwestern Ohio and southwestern Michigan: a study of the consequences of hybridization. *Copeia* 1985: 309–324.
- Lynch, M.** 1984. Destabilizing hybridization, general-purpose genotypes and geographic parthenogenesis. *Quarterly Review of Biology* 59: 257–290.
- Macgregor, H. C., and T. M. Uzzell, Jr.** 1964. Gynogenesis in salamanders related to *Ambystoma jeffersonianum*. *Science* 143: 1043–1045.
- Moore, W. S.** 1984. Evolutionary ecology of unisexual fishes. Pages 329–398 in *Evolutionary genetics of fishes. Edited by B. J. Turner. Plenum Publishing Corporation.*
- Morris, M. A., and R. A. Brandon.** 1984. Gynogenesis and hybridization between *Ambystoma platineum* and *Ambystoma texanum* in Illinois. *Copeia* 1984: 324–337.
- Noble, G. K.** 1931. *The biology of the amphibia.* McGraw-Hill Book Co., New York.
- Noble, G. K., and M. K. Brady.** 1933. Observations on the life history of the marbled salamander, *Ambystoma opacum* Gravenhorst. *Zoologica* 8: 89–132.
- Sever, D. M., and L. D. Houck.** 1985. Spermatophore formation in *Desmognathus ochrophaeus* (Amphibia: Plethodontidae). *Copeia* 1985: 394–402.
- Uzzell, T. M., Jr.** 1963. Natural triploidy in salamanders related to *Ambystoma jeffersonianum*. *Science* 139: 113–115.
- Uzzell, T. M., Jr.** 1964. Relations of the triploid and diploid species of the *Ambystoma jeffersonianum* complex (Amphibia: Caudata). *Copeia* 1964: 257–300.
- Uzzell, T. M.** 1969. Notes on spermatophore production by salamanders of the *Ambystoma jeffersonianum* complex. *Copeia* 1969: 602–612.
- Wilber, H. M.** 1971. The ecological relationship of the salamander *Ambystoma laterale* to its all-female, gynogenetic associate. *Evolution* 25: 168–179.

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