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A TRIPLOID SNAKE.—The occurrence of triploid individuals within normally diploid populations has been reported in a wide variety of vertebrates, although differential viability has been noted in the affected animals. Triploid mammals generally die in utero but triploidy can occur with less dire consequences in other vertebrates such as the domestic chicken, *Gallus gallus domesticus*, in which an adult triploid has been described (Ohno et al., 1963). Gold and Avise (1976) reported spontaneous triploidy in a cyprinid fish, and within the salmonids (a group characterized by ancestral tetraploidization) adult triploids of rainbow trout, *Oncorhynchus mykiss*, have been reported (Cuellar and Uyeno, 1972; Thorgaard and Gall, 1979).

Populations of allotriploids that reproduce by

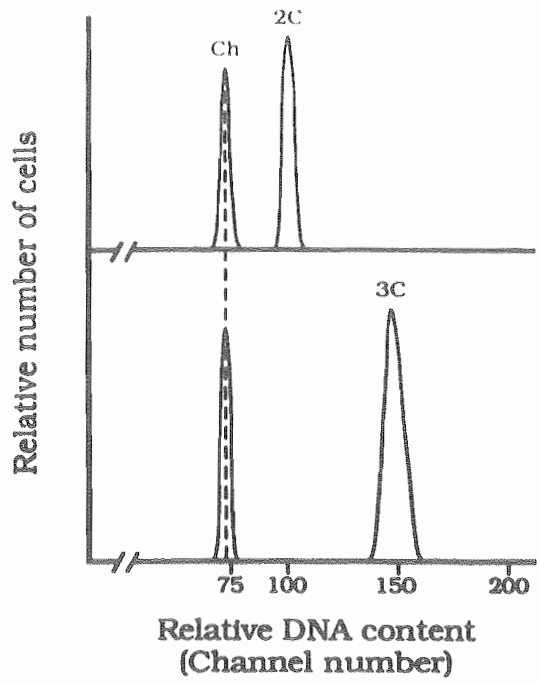

Fig. 1. Nuclear DNA fluorescence distributions of erythrocytes from chicken (Ch), and diploid (2C) and triploid (3C) *Agkistrodon piscivorus* analyzed by flow cytometry. Channel number is directly proportional to DNA content. The percent coefficient of variation was less than 2.65% for all peaks.

gynogenesis, parthenogenesis, or hybridogenesis have been described in fishes (e.g., Schultz and Kallman, 1968; Rasch et al., 1970), amphibians (e.g., Fankhauser, 1945; Uzzell, 1964), and reptiles (e.g., Bogart, 1979; Bickham, 1983). Within snakes, the unisexual brahminy blind snake *Ramphotyphlops braminus* may be triploid in relation to the diploid state of other typhlopid species (Wynn et al., 1987).

Autotriploids usually do not differ from diploids in external appearance. However, the gonads of spontaneous triploids can be undeveloped or abnormal (Ohno et al., 1963; Thorgaard and Gall, 1979). Among the reptiles, spontaneous triploidy has been reported in turtles and lizards but not in snakes, crocodilians, or tuatara (Bickham et al., 1985). Here we describe a western cottonmouth, *Agkistrodon piscivorus leucostoma*, which to our knowledge, is the first spontaneously triploid snake to be identified.

Materials and methods.—The specimen was one of 24 *A. piscivorus leucostoma* collected 20 June 1989 at the Ames Plantation (Tiersch et al., 1990), which lies in the headwater basin of the North Fork Wolf River watershed in Hardeman and Fayette counties of western Tennessee. The snakes were killed and measured, and within five minutes blood was collected from the tail into a syringe containing sodium citrate (from Becton-Dickinson vacutainer #4606). The snakes were frozen and dissected three weeks later to examine gut contents and the gonads. Blood samples were refrigerated, and analyzed within 24 hours as described elsewhere (Tiersch et al., 1989).

The DNA content of blood cells was estimated with an EPICS V flow cytometer (Coulter Electronics, Hialeah, Florida) that was aligned using fluorescent beads and human leukocytes to obtain highest resolution. The blood cells of snake and thawed blood cells of a male domestic chicken, *G. gallus*, were analyzed as a mixture in 0.5 ml of lysis-staining buffer containing 25 μl buffered RNase (1.0 mg/ml), 0.1% sodium citrate, 0.1% Triton X 100, and 25 μg propidium iodide. The fluorescence values of at least 40,000 stained nuclei (in stages G_0 and G_1 of the cell cycle) from snake and chicken were digitized individually and used to calculate the DNA content of the snakes in relation to an assigned value of 7.0 picograms DNA for fresh human leukocytes that had been analyzed as a mixture with the chicken cells. Percent coefficient of variation (CV) was calculated at 50% maximal height of the fluorescence peak for each snake sample.

Measurements of the lengths and widths of cells and nuclei were made to compare the dimensions of erythrocytes among snakes. Smears were prepared from fresh blood of the triploid snake and from five diploid snakes chosen at random. The smears were fixed in absolute methanol for 2 min, stained for 30 min in 10% Giemsa (in 0.2 M phosphate buffer, pH 6.75), and rinsed for 2 min in the same buffer (Beck and Biggers, 1983). Cell and nuclear dimensions were measured with a Filar ocular micrometer. Volumes were calculated by the formula for an oblate spheroid, $4/3\pi ab^2$, where a is the major semiaxis, and b is the minor semiaxis.

Results and discussion.—The utility of flow cytometry in ploidy determination has been demonstrated in numerous studies (e.g., Thorgaard et al., 1982; Allen, 1983; Tank et al., 1987). This study revealed 23 snakes with a mean nu-

TABLE 1. NUCLEAR AND CELL SIZES OF ERYTHROCYTES FROM DIPLOID AND TRIPLOID *Agkistrodon piscivorus leucostoma*. Measurements of length and width (mean ± SD) were made on 10 cells from each of five diploid snakes and on 25 cells from a triploid snake. The triploid cells and nuclei were significantly larger than the diploid cells and nuclei in length, width and volume (t-test; $P < 0.001$). All length and width measurements given in μm; the volumes were calculated by the formula: $4/3\pi ab^2$.

Ploidy	Nucleus		Cell		Nuclear volume	Cell volume	Ratio of nuclear volume : cell volume
	Length	Width	Length	Width			
Diploid	5.7 ± 0.09	4.6 ± 0.06	18.5 ± 0.17	11.1 ± 0.13	63.15	1193.48	0.05
Triploid	8.4 ± 0.12	5.2 ± 0.08	22.2 ± 0.16	13.1 ± 0.14	118.93	1994.78	0.06

clear DNA content of 3.47 ± 0.05 pg (SD) and a single specimen with an exceptional value of 5.15 pg. This value, 1.48 times greater than the mean value of the other snakes, indicated a triploid (3C) DNA content (Fig. 1).

No mosaicism (as indicated by additional fluorescence peaks or shoulders) was detected for any snake. Small aneuploid variations in the DNA content of subpopulations of cells resulting from gain or loss of chromosomes or parts of chromosomes can cause an increase in CV values (Otto et al., 1981). The mean CV was 2.23 ± 0.17% for all 24 snakes (range 1.98–2.63%). The triploid snake had a CV of 2.31. The snakes were frozen, and tissues other than blood were not obtained at the time of collection. Flow cytometry was accordingly performed on blood cells only and cytogenetic analysis was not attempted. Thus, the possibility of mosaicism or ploidy differences in tissues other than blood could not be ruled out.

Measurements of erythrocytes (RBC) from five diploids and the triploid agreed with the results from flow cytometry. Length, width and volume of the nuclei and cells of the triploid were significantly larger than those of the diploids (Table 1). Nuclear length of the triploid RBC was 1.47 times greater than the nuclear length of the diploid RBC. Likewise the nuclear volume was 1.67 times greater in the triploid. In contrast, the ratio of nuclear volume to cell volume was consistent in the triploid and diploids with values of 0.06 and 0.05, respectively. This constant relationship between nuclear and cell size regardless of ploidy (the "nucleocytoplasmic ratio") has been reported for other species (e.g., Swarup, 1959; Pedersen, 1971; Beck and Biggers, 1983).

The triploid was ordinary in appearance and was 53.5 cm in length. All but one snake was longer; lengths ranged from 38 to 114.5 cm (mean = 77.0 ± 17.5 cm). The triploid was the darkest specimen collected, but was not unusually melanistic for the species (R. Semlitsch, pers. comm.). The belly was black, and dorsal brown markings were indistinct. Yellow coloration, characteristic of juveniles, was not visible on the tail. Examination of the gut contents revealed digested anuran remains. Hemipenes could not be everted by injection of fluid, nor were they found in dissection. Gonads were not observed and were apparently undeveloped because the gonads were easily identified in all other snakes, including those of similar or smaller size.

Possible modes for the production of triploidy are diandry, double fertilization of an ovum by two spermatozoa; digyny, the union of a single male pronucleus with two female pronuclei, or aneugamy (produced by mitotic error of the germ cell precursors) which can result in the fusion of a pronucleus and an unreduced diploid gamete (Austin, 1960). Triploidy has been produced experimentally in animals by application of thermal shock, hydrostatic pressure or chemicals to fertilized eggs. These treatments block the second meiotic division or extrusion of the second polar body, each of which normally occurs shortly after fertilization (Purdom, 1983). Environmental factors such as temperature have been implicated in the natural production of polyploidy in some vertebrates (Allen and Stanley, 1978; Bogart et al., 1989). Although the fertilization of an unreduced gamete by a normal sperm cell is a probable mechanism for triploidy in lower vertebrates (Thorgaard and Gall, 1979), the etiology of triploidy in this snake is uncertain.

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