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ZZW autotriploidy in a Blue-and-Yellow Macaw

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Abstract

We describe genome size (nuclear DNA content), and cellular and nuclear dimensions of erythrocytes in a triploid Blue-and-Yellow Macaw (*Ara ararauna*) and its diploid parents. The genome size of the triploid (4.23 pg) was 1.5 times greater than the genome size of the mother (2.80 pg) and the father (2.89 pg). The sex chromosome composition was ZZW, and was predicted correctly based on the genome size of the parents. Erythrocytes of the triploid were significantly larger than the erythrocytes of the parents. Because polyploidy has been reported only in one other family of birds (Phasianidae), the parrots and their relatives might prove to be useful in the study of avian triploidy.

Introduction

Although triploidy can be induced experimentally in certain animals (e.g. Purdom, 1983), natural occurrence of triploidy appears to be uncommon, especially in the higher vertebrates (Ohno *et al.*, 1963; Thorgaard & Gall, 1979). Polyploids with some diploid tissues are observed more often than completely triploid animals (Abdel-Hameed & Shoffner, 1971; Chu *et al.*, 1964). Evidently the presence of diploid cells can enhance the viability of some heteroploid organisms.

Studies of triploidy in birds have been confined to the domestic chicken (*Gallus domesticus*). Genome size and cellular dimensions of diploids and triploids have been analyzed in chickens (e.g. Abdel-Hameed *et al.*, 1971; Thorne *et al.*, 1987) but comparison of these values in diploids and triploids has not been possible for other bird species. In this study we describe genome size, cellular measurements and karyotype in a triploid Blue-and-Yellow Macaw (*Ara ararauna*) which to our knowledge is the first non-galliform triploid bird reported.

Materials and methods

We studied a parrot described as triploid after cytogenetic analysis (undertaken for the identification of sex; Valentine, 1990). Blood samples from this bird and its parents were collected and refrigerated until analysis. Nuclear DNA content was estimated by flow cytometry. Blood cells from individual parrots were mixed with blood cells from a male domestic chicken (used as an internal reference) in 0.5 ml of a 50 mg/l solution of propidium iodide containing 25 μl RNase (1.0 mg/ml), 0.1% sodium citrate, and 0.1% Triton X 100, and analyzed in a Coulter 753 flow cytometer according to the method detailed in Tiersch *et al.* (1989). At least 25,000 cells were scored for each mixture of cells from chicken and parrot.

Measurements of length and width of cells and nuclei were made to compare erythrocyte dimensions among the parrots. Smears were prepared from fresh blood, 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 & 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.

Metaphase chromosomes were prepared from feather pulp of the triploid using the techniques of Van Tuinen and Valentine (1982). The number of macrochromosomes, including sex chromosomes, was determined for 14 spreads.

Results

Genome size was determined by flow cytometry for the parents and their progeny (Table 1). Cells of the progeny had a DNA content 1.5 times that of the parents; populations of cells with non-triploid DNA content were not detected. Haploid genome size, including either the Z or the W chromosome, was calculated from parental nuclear DNA contents (Table 2) and the expected DNA content for a triploid, based on various sex chromosome combinations, was calculated. The genome size of the progeny (4.23 pg) approximated the value predicted for a triploid with a ZZW sex chromosome composition (4.24 pg; Table 2).

Measurements of length, width, and volume of cells and nuclei (Table 3) revealed that the red blood cells of the progeny were significantly larger than those of the diploid parents (t-Test; $P < 0.001$). The red blood cell sizes of the progeny corresponded to those values expected for a triploid.

In each metaphase spread of the progeny examined, three haploid sets of macrochromosomes were present (Fig. 1). The sex chromosome composition was ZZW.

Table 1. Nuclear DNA content[a] and sex chromosomes of a triploid *Ara ararauna* and parents.

Bird	Ploidy	DNA content (pg)	Sex chromosomes
Mother	2N	2.797±0.007	ZW
Father	2N	2.890±0.005	ZZ
Progeny	3N	4.228±0.004	ZZW

[a] Mean ± SD of three measurements of DNA content for each bird.

Table 2. Expected nuclear DNA content of triploid based on possible combinations of sex chromosomes from parents[a].

Pronuclei	Zygote	DNA content (pg)
ZZ + Z	ZZZ	4.335
ZW + Z	ZZW	4.242
ZZ + W	ZZW	4.242
WW + Z	ZWW	4.149

[a] Values calculated from parental nuclear DNA contents: haploid genome including Z = 1.445 pg (ZZ/2 = 2.890/2); haploid genome including W = 1.352 pg (ZW-Z = 2.797–1.445).

Table 3. Nuclear and cell sizes of erythrocytes from a triploid *Ara ararauna* and the diploid parental birds[a].

	Nucleus		Cell				
Ploidy	Length	Width	Length	Width	Nuclear volume	Ratio of Cell volume	nuclear volume: cell volume
Diploid	4.38±0.32	2.98±0.31	9.04±0.79	7.53±0.85	20.36	268.10	0.08
Triploid	7.38±0.42	4.30±0.30	10.69±0.32	8.73±0.43	71.41	425.67	0.17
3C/2C	1.68	1.44	1.18	1.16	3.51	1.59	2.12

[a] Measurements of length and width (mean ± SD) were made on ten cells from each of the three parrots. 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: $4/3\ \pi\ ab^2$.

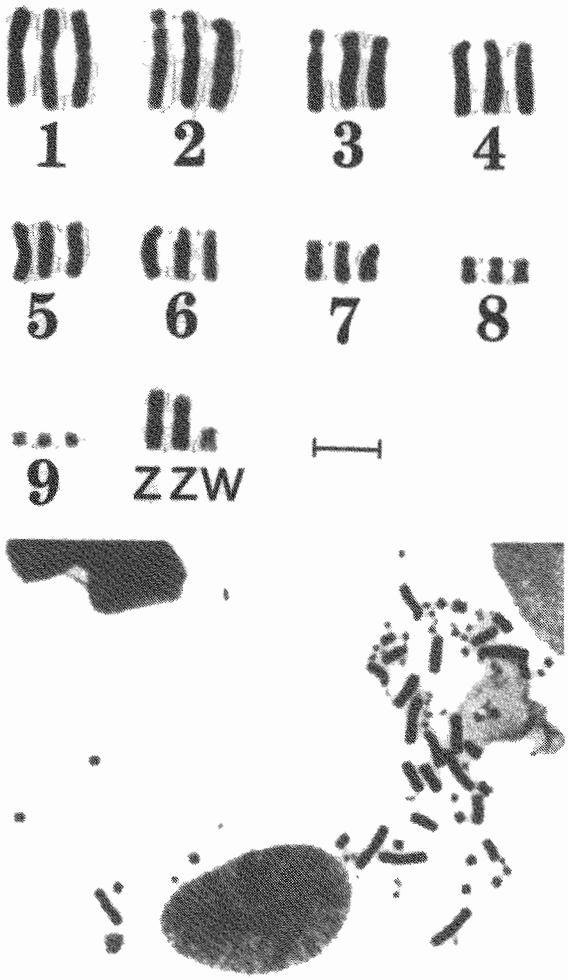

Fig. 1. Karyotype of the macrochromosomes and the chromosome spread of a triploid *Ara ararauna*. Bar equals 5 μm.

Discussion

The diploid chromosome number of *A. ararauna* is 70 including nine pairs of biarmed macrochromosomes (Van Dongen & De Boer, 1984). All autosomal macrochromosomes were present in triplicate in the progeny of the diploid parents. Because the triploid macaw described here possessed a ZZW sex chromosome composition, it was not possible to determine which parent had contributed the extra haploid set of chromosomes. Triploidy could have been generated by union of a haploid Z-bearing sperm and a diploid ZW egg (due to failure of reductional division in meiosis I). Alternatively, a diploid sperm or two haploid sperm could have fertilized a haploid W-bearing egg. In the domestic chicken, diploid sperm or dispermy are rare as sources of triploidy (Fechheimer, 1981); the most common cause in the chicken is an error of meiosis during oogenesis (Fechheimer & Jaap, 1978).

Mosaicism in ploidy level has been reported in chickens (e.g. Thorne *et al.*, 1987) and in other vertebrates (Chu *et al.*, 1964; Dawley & Goddard, 1988). All observations of feather pulp and blood cells indicate that the macaw in this study was probably completely triploid. When observed in the chicken, complete triploidy was associated with an intersex phenotype (Abdel-Hameed & Shoffner, 1971; Ohno *et al.*, 1963). The triploid parrot studied here was normal in appearance. Because this species is sexually-monomorphic, however, an intersexual phenotype would not be detectable. Intersexuality is well known and widespread in sexually-dimorphic species of birds (Taber, 1964). Given the observed relationship between intersexuality and triploidy, and our report of a triploid parrot, polyploidy may be more widespread in birds than present observations would suggest.

The small but detectable difference in the genome sizes of the parental macaws could have been due to size difference between the Z and W sex chromosomes. Elias *et al.* (1988) and Kent *et al.* (1988) used flow cytometry to discriminate between male and female of the human and domestic horse, respectively, and Nakamura *et al.* (1990) used flow cytometry to identify sex in 29 species of birds that possess heteromorphic sex chromosomes. The present study indicates that genome size in birds may be inherited as a function of parental haploid genome size as occurs in interspecific hybrids of cyprinid fishes (Dawley & Goddard, 1988).

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