W-Sex Chromatin Fluorescence in Snakes[1]

While in the primitive non-poisonous snakes of the family Boidae the sex chromosomes are homomorphic, different degrees of ZW heteromorphism were found in other families of Serpentes[2].

Male Z-sex chromatin, analogous to the female X-sex chromatin of mammals, does not occur in reptiles[3]. This was confirmed by autoradiographic chromosome replication studies. The absence of a late replicating chromosome favours the hypothesis that no mechanism of sex dosage compensation is active in ophidians[4].

In the females, however, a sex-chromatin body was reported, in some species of snakes, that coincides with a late replication of the W[5,6].

Using the fluorescent staining technique by which a Y-chromatin body was demonstrated[7], we have been able to detect a characteristic fluorescent body in female interphase cells of some ophidians, which probably corresponds to the W-sex chromosome.

Cytological preparations were obtained by blood smears and by the squash technique of hypotonic pretreated fragments of spleen, intestine and gonads of specimens previously inoculated with colchicine[8]. The slides were hydrolysed in 1 *N* HCl at 60 °C for 10 min, stained in an 1% aqueous solution of quinacrine dihydrochloride (Atebrin) for 5 min, washed in running tapwater for 3 min, rinsed in sodium acetate buffer, pH 5.5 and mounted in buffer. Somatic metaphases were mostly studied in preparations without HCl hydrolysis. The preparations were photographed in a Zeiss photomicroscope with a 40 × or 100 × oil immersion objective; the light source was an HBO 200 W mercury vapour lamp with a 3.0 mm BG 12 excitor filter and a 530 nm barrier filter.

The following species were studied: *Boa constrictor amarali* (Stull) of the family Boidae; *Bothrops jararaca* (Wied) of the family Viperidae; *Clelia clelia plumbea* (Wied) and *Clelia occipitolutea* (Duméril, Bibron and Duméril) of the family Colubridae.

The karyotype of *B. constrictor amarali* shows $2n = 36$ chromosomes, comprising 16 macro and 20 microchromosomes, no heteromorphic chromosomes being detected in either sex. *B. jararaca* presents also $2n = 36$ chromosomes (16M + 20m) but exhibits, as do all the Viperidae, a striking heteromorphic ZW sex pair in the female[9]. *C. occipitolutea* and *C. clelia plumbea* show similar karyotypes with $2n = 50$ (14M + 36m). Their karyotype is unique among snakes; besides showing the highest diploid number so far reported in Serpentes, their W-sex chromosome is exceptionally large, about twice the Z, and the largest of the complement. While the W of *C. occipitolutea* is acrocentric, the one of C. *clelia plumbea* is submetacentric, a pericentric inversion probably being responsible for the difference in the centromere position[10].

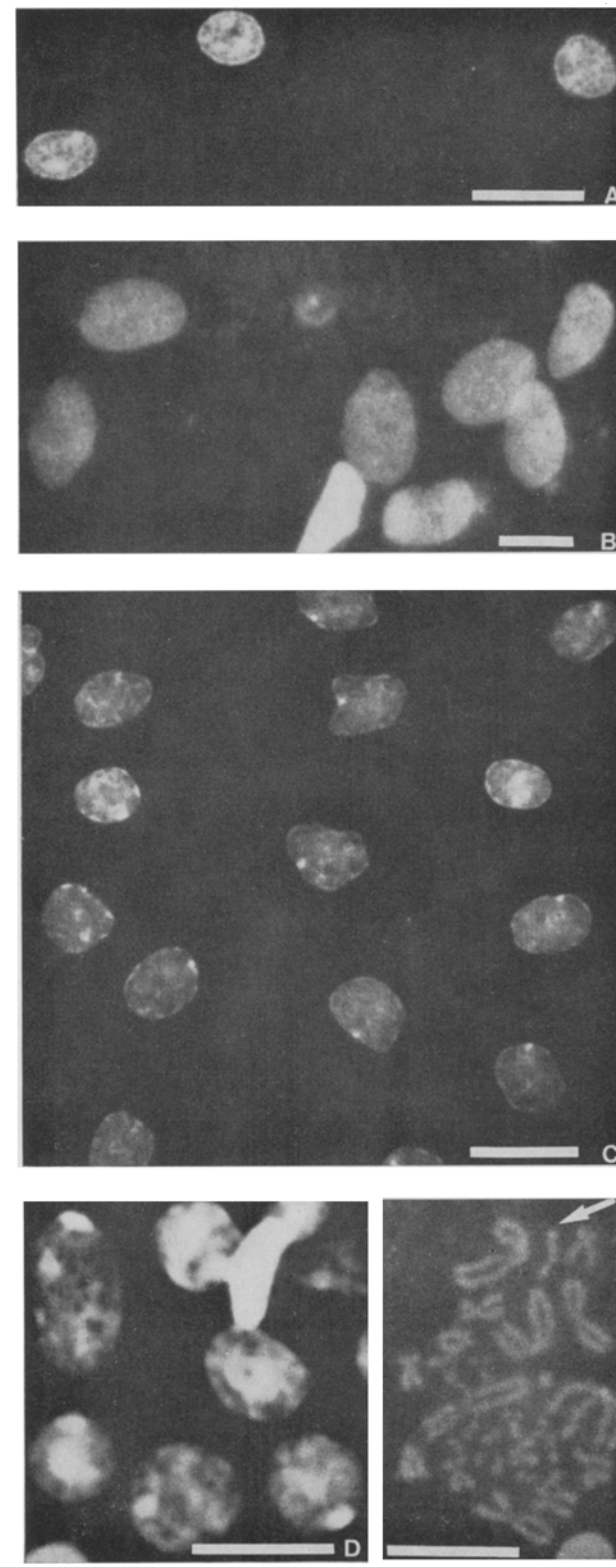

Fig. 1. A) Erythrocytes of the *Boa constrictor amarali* female without fluorescent chromatin. B) Testicle interphase nuclei of *Bothrops jararaca* showing absence of the fluorescent body. C)–D) Erythrocytes and spleen interphase nuclei of *B. jararaca* females exhibiting the bright fluorescent W-sex chromatin. E) Fluorescent metaphase chromosomes from spleen of a *B. jararaca* female; the W is indicated. The bars correspond to 10 μm.

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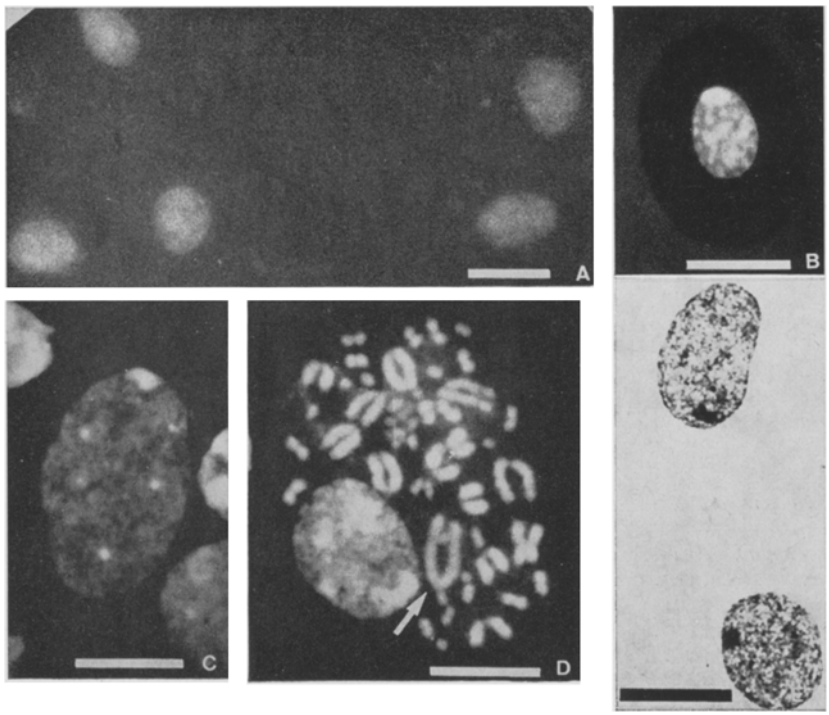

Fig. 2. *Clelia occipitolutea*. A) Spleen nuclei of a male specimen without the fluorescent chromatin. B)–C) Erythrocytes and spleen nuclei of female specimens showing the bright fluorescent W-sex chromatin. D) Metaphase chromosomes from spleen of a female snake showing fluorescence; the W is indicated. E) Spleen interphase nuclei of a female specimen exhibiting the W-sex chromatin; Giemsa stained. The bars correspond to 10 μm.

We analyzed interphase nuclei and somatic metaphases in both sexes of the 3 genera. The preparations of both sexes of *Boa* and of *Bothrops* and *Clelia* males did not show any typical bright fluorescent material (Figures 1 and 2). But the females of *Bothrops* and of the 2 *Clelia* species exhibited, in blood smears and in squash preparations of spleen and intestine, a characteristic fluorescent body at the periphery of most interphase nuclei (Figures 1, 2 and 3). This fluorescence coincides with the condensed heteropycnotic body observed in similar interphase nuclei stained with Giemsa, after hydrolysis in 1 *N* HCl at 60 °C (Figures 2 and 3).

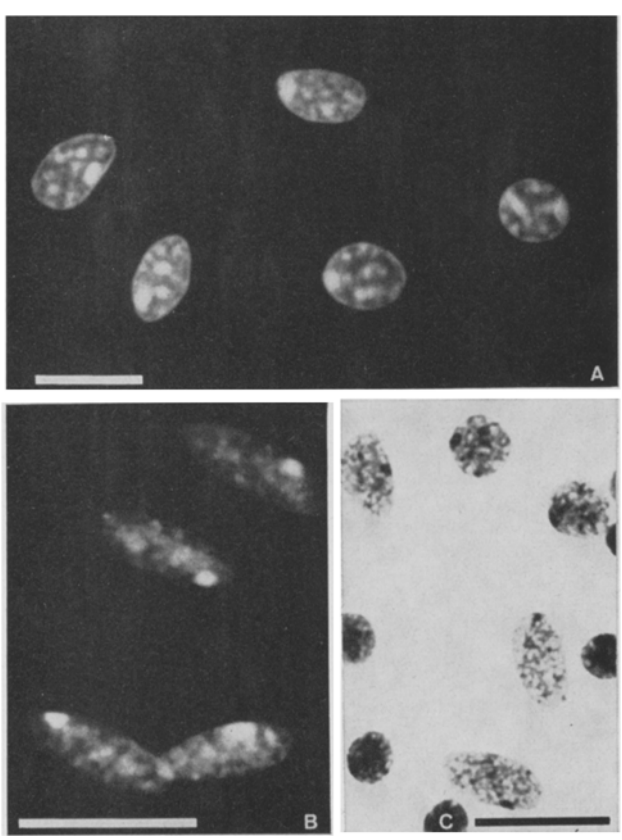

Fig. 3. *Clelia clelia plumbea*. A)–B) Erythrocytes and intestine nuclei of female specimens showing the fluorescent W-sex chromatin. C) Spleen interphase nuclei of a female snake exhibiting the W-sex chromatin; Giemsa stained. The bars correspond to 10 μm.

All chromosomes of the somatic metaphases show fluorescence, which is however weaker in the W-sex chromosome and in part of the microchromosomes (Figures 1 and 2).

The typical fluorescent body of the *Bothrops* and *Clelia* females apparently corresponds to a part or to the whole of the W-chromosome. The *Boa* snake, in which sex chromosomes are still morphologically undifferenciated, showed neither W-sex chromatin nor W-sex chromatin fluorescence.

The heterogeneous behavior of the bright fluorescent material, i.e. its presence in the interphase nuclei of the ZW heteromorphic snakes and its absence in the somatic metaphases, may well reflect the nature of this phenomenon. We may suggest that the intensity of fluorescence depends upon the functional DNA stage. The same region might show a bright fluorescence in one phase, and absence of a specific pattern in others.

Resumen. En los ofídios, las hembras presentan, en los núcleos interfásicos teñidos por la quinacrina, un corpúsculo fluorescente brillante. Este coincide con él condensado heteropicnótico de preparaciones comunes y corresponde al cromosoma W. Sin enbargo, él W no presenta fluorescencia intensa en las metafases somaticas.

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