A C-band Polymorphism of the X Chromosome in *Akodon azarae* (Rodentia, Cricetidae)

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Accepted July 15, 1998

Summary *Akodon azarae* is one of several rodent species in whose populations XY fully fertile females are found. The chromosomes of 83 specimens (40 females, 43 males) from three localities were studied. A high incidence of XY females was observed: 21 of the females presented an XY karyotype. The X chromosome relative length corresponded to 8.2% of the genome, but a significative portion of it consisted of C-positive material, giving to the euchromatic part a relative length of 5.07%, in agreement with the estimated size of the eutherian X. Four C-banding patterns (I–IV) of the X chromosomes were observed, one of them found in only one specimen. The X chromosome of males was type I, and XY females showed an X of type II or III. XX females presented one X of type I and the other of type I, II, III or (in only one animal) of type IV. The significance of this polymorphism with respect to the XY female phenotype is briefly discussed.

Key words *Akodon azarae*, XY females, X chromosome polymorphism, Sex determination, C-banding polymorphism.

A number of rodent species are characterized by the existence of fully fertile XY females in their populations (revision in Fredga 1988, 1994). In several species of the South American cricetid genus *Akodon*, fertile females are found that carry an heteromorphic sex pair formed by an X and a small element that has been considered either as a greatly deleted X or as an Y chromosome (Bianchi *et al.* 1971, 1989, Lobato *et al.* 1982, Pinna Senn *et al.* 1993, Vitullo *et al.* 1986). In *Akodon mollis*, this small chromosome has been identified cytologically as the Y (Lobato *et al.* 1982). In the heteromorphic females of *A. azarae*, where this cytological identification is not possible, the presence of the Y specific *Zfy* and *Sry* genes has been demonstrated (Bianchi *et al.* 1989, 1993). Strikingly, the XY females of *A. azarae* manifest an enhanced reproductive performance with respect to XX females (Espinosa and Vitullo 1996). This paper reports the analysis of a C-band polymorphism of the X chromosome in this species.

Materials and methods

Cytogenetical studies were performed on 83 specimens of *A. azarae* from three localities in the Southern region of the province of Córdoba: Chucul (32°55'S, 64°10'W), La Aguada (32°57'S, 64°39'W) and Tosquita (33°49'S, 64°28'W). Standard air-dried preparations from bone marrow were made after injection of 0.20–0.30 ml (according to size) of a 60 µg/ml colchicine solution, 2 hr before sacrifice. C-banding was obtained according to Sumner (1972).

The relative length of the X chromosome, expressed as percentage of the female genome, was determined in 10 unbanded karyotypes from 6 males from the same locality (Chucul). The relative amount of heterochromatin on the X was measured on projected photographic negatives of 10 male

C-banded metaphases. To search for possible length differences between the different X types (see below), 12 X chromosomes of each type were measured in C-banded cells and their lengths expressed in relation to the length of chromosome 1.

The process of digitalization and posterior analysis of X chromosomes images was carried out on photographic negatives obtained with a Zeiss Axiophot microscope. The negatives were recorded in digital format by means of a Rex transiluminator (Erlangen), a CCD camera model SVHS Hi 8 (Sony) and an image analyzer VIDAS ver. 2.1 (Kontron Elektronik). The image capture format was 512×512 pixels, 8 bits per pixel (b/w). Subsequent operations included: enhancement; segmentation of the X chromosome in 24 equal, successive segments, perpendicular in each point to the chromosome axis; measuring of the medium optical density (MOD) and calculation of the relative MOD in each segment with respect to each chromosome maximum value; storage and graphic display of the data. For each of the three principal X types, 10 images were captured. An average graph of each of the C-banding patterns was obtained.

Results and discussion

We studied 83 specimens of *A. azarae* from three localities in the Southern region of the province of Córdoba: 35 males and 28 females from Chucul, 5 males and 6 females from Tosquita, and 3 males and 6 females from La Aguada. All the specimens showed a 38-chromosomes karyotype fitting previous descriptions (Bianchi and Contreras 1967, Bianchi *et al.* 1971) and composed of 17 telocentric and a small metacentric autosomal pairs, and the sex pair; the X chromosome is a subterminal element, and the Y is a small biarmed chromosome. Only the first pair, the small biarmed autosomal pair and the gonosomes can be identified in unbanded cells. The relative length of the X, measured in male cells, corresponded to $8.20 \pm 0.64\%$ of the genome, a value that is coincident with that reported by Bianchi *et al.* (1971).

Several females presented an heteromorphic sex pair, considered an XY pair (Bianchi *et al.* 1989, 1993). Of the 28 females from Chucul, 17 (60.7%) were heteromorphic (XY). Although the animals here analyzed cannot be considered a representative sample of the population (they were captured during several years), the data do point to a high frequency of XY females in it. Also, 3 of the 6 females from La Aguada and 1 of the 6 from Tosquita showed an XY constitution.

In the study of Bianchi *et al.* (1971) on animals from Buenos Aires province, 8 of the 25 females analyzed showed this constitution (at the time interpreted as an heterozygosity for a deletion of most of the long arm of the X), and another 5 presented other X chromosome anomalies. The repeated finding of a high proportion of XY females in natural populations of *A. azarae* indicates that no serious effect on fertility or viability is associated with this condition. In fact, it has been shown that, at least in the laboratory, XY females start to reproduce earlier, do it more frequently and stop reproduction later than XX females, showing similar litter sizes and a lesser preweaning mortality of young (Espinosa and Vitullo 1996).

We analyzed the C-banding pattern in 46 animals from Chucul (28 males, 6 XX and 12 XY females), 11 from Tosquita (5 males, 5 XX and 1 XY females), and 9 from La Aguada (3 males, 3 XX and 3 XY females). Centromeric bands were observed in the autosomes, with the exception of the small metacentric pair, and the Y chromosome was heterochromatic; the X chromosome showed several heterochromatic bands of different sizes (Figs. 1–3). The C-heterochromatic bands of the X (measured in male cells) amounted to $38.16 \pm 3.95\%$ of its length (3.13% of the genome), giving to the euchromatic portion of this chromosome a relative value of 5.07, in agreement with the estimated size of the conserved eutherian X (Ohno 1967).

According to the characteristics and position of the heterochromatic bands, four C-banding patterns could be determined in the X chromosomes of the animals studied (I, II, III, IV, Figs. 1–3). Type IV was found only in one XX specimen from Tosquita. A comparison of the relative lengths

Fig. 1. C-band polymorphism of the X chromosome in *Akodon azarae*. A) Male; B and F) XY females (partial photographs); C–E) XX females (partial photographs); G) the four X chromosome types at higher magnification. X types are indicated by numbers. The bar corresponds to 10 μm in A–F and to 5 μm in G.

of the three principal types, expressed as percent of the length of chromosome 1, did not reveal significant differences between them (type I, 0.75 ± 0.05 , type II, 0.76 ± 0.04 , and type III, 0.76 ± 0.07 ; $F = 0.0093$).

As can be seen in Table I, in all the males studied (24 from Chucul, 5 from Tosquita and 3 from La Aguada), the X chromosome corresponded to type I. In XX females, one X was always of type I, which is, obviously, consistent with the paternal origin of one of the X chromosomes in these females; the other was of type I, II or III, with the previously indicated exception of an animal from Tosquita, where it was of type IV. All XY females, on the other hand, showed an X chromosome of type II or III.

A digital image analysis was performed on a sample of 10 chromosomes of each of the types I, II and III. The method employed allowed an objective comparison of the different densitometric patterns observed (Fig. 3).

C-band polymorphism is a frequent cytogenetical observation, usually without phenotypic expression. The polymorphism of the X-chromosome here described does not seem to conform to this rule, but no simple explanation of the data is evident. At first glance, it could be thought that only type I X chromosome is compatible with male differentiation, the other types probably carrying a mutation responsible for the sex reversal, resembling the situation found in the XY females of *Myopus schisticolor* (Fredga *et al.* 1976, Herbst *et al.* 1978). However, *A. azarae* XY females are born

Fig. 2. Schematic representation of the X chromosome types in *Akodon azarae*.

Fig. 3. Graphic representation of *Akodon azarae* X chromosome types I, II and III on the basis of medium grey values in each chromosome segment.

Table 1. Number of *Akodon azarae* specimens of different chromosome constitutions with respect to X chromosome types

Locality	Males			Females			
	X ₁ Y	X ₂ Y	X ₃ Y	X ₁ X ₁	X ₁ X ₂	X ₁ X ₃	X ₁ X ₄
Chucul	28	6	6	1	2	3	—
Tosquita	5	1	—	1	2	1	1
La Aguada	3	—	3	—	1	2	—

only from other XY females (Lizarralde *et al.* 1982); they should also be produced by normal XX female×male crosses if an X-linked mutation were responsible *per se* for the sex reversal. Given this mode of inheritance, a mutation on the Y chromosome was postulated as the cause of the XY female phenotype in *A. azarae* (Bianchi *et al.* 1993). However, no difference between males and XY females was detected in the nucleotide sequence of a partial open reading frame of the conserved region of *Sry* (Bianchi *et al.* 1993). An analysis of the hereditary transmission of the different X types is under way.

Acknowledgements

We wish to thank Dr. Jaime Polop for providing the animals here studied. This work was supported by a grant from the Secretaría de Ciencia y Técnica, UNRC.

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