

QUANTITATIVE VARIABILITY OF THE CONSTITUTIVE HETEROCHROMATIN (CBG) CONTENT IN THE KARYOTYPE OF THE BLUE FOX (*ALOPEX LAGOPUS*)

VARIABILIDAD CUANTITATIVA DEL CONTENIDO DE HETEROCROMATINA CONSTITUTIVA (CBG) EN EL CARIOTIPO DEL ZORRO AZUL (*ALOPEX LAGOPUS*)

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Additional keywords

Heterochromatin. Karyotype. Blue fox.

Palabras clave adicionales

Heterocromatina. Cariotipo. Zorros.

SUMMARY

In the karyotype of the blue fox ($2n=50$) there are twenty chromosomes (ten pairs) with one arm entirely heterochromatic. Size variability of these arms was revealed on the CBG-banded chromosomes (Makinen *et al.*, 1981) and using synaptonemal complex analysis (Switonski *et al.*, 1991).

In the present study, CBG banding was carried out for 49 animals. Four types of the karyotype were recognised. In the first one, all twenty chromosomes had heterochromatic arms (group 1; 26 animals); in the second one a lack of the heterochromatic arm on a single chromosome was found (group 2; 15 animals); in the third type, two chromosomes did not have these arms (group 3; 5 animals) and in the fourth one, three chromosomes missed heterochromatic arms (group 4, 3 animals). The aim of the study was revealing whether the total size of CBG arms of these chromosomes is constant or variable in this species. Length measurements of heterochromatic and euchromatic arms of ten chromosome pairs were performed with the use of Multiscan 4.01. computer system (Computer Scanning Systems, Ltd, Poland), connected with a Jenamed microscope. Three metaphases for each

animal were analysed. The total relative length of the CBG arms for each metaphase was calculated as the proportion of the total length of the euchromatic arms of these chromosomes and then - the mean value for each individual was obtained. The total relative length of the CBG arms varied among the animals from 0.53 to 1.04. One way analysis of variance showed that this variability was significant ($p<0.01$). The average total relative length of the CBG arms in four distinguished groups were: 0.84, 0.75, 0.65 and 0.67, respectively. The application of the contrast analysis showed that differences among these groups, with the exception of group 4 *versus* group 3, were also significant ($p<0.01$ or $p<0.05$).

RESUMEN

El cariotipo del zorro azul ($2n=50$) tiene veinte cromosomas (diez parejas) con un brazo enteramente heterocromático. Se ha descrito la variabilidad de tamaño en estos brazos en cromosomas de bandeado CBG (Makinen *et al.*, 1981) y utilizando

análisis complejo sinaptonémico (Switonski *et al.*, 1991).

En el presente trabajo, 49 animales han sido bandeados con CBG. Se describen cuatro cariotipos distintos. En el primero los veinte cromosomas tenían brazos heterocromáticos (grupo 1; 26 animales); en el segundo se encontró una carencia de brazo heterocromático en un solo cromosoma (grupo 2; 15 animales); en el tercero, dos cromosomas no presentaron este brazo heterocromático (grupo 3; 5 animales); y en el cuarto, en tres cromosomas faltaban brazos heterocromáticos (grupo 4; 3 animales). Nuestra meta en el presente trabajo fue esclarecer si el tamaño total de brazos CBG de estos cromosomas es constante o variable en esta especie. La longitud de los brazos heterocromáticos y eucromáticos de 10 parejas de cromosomas han sido medidos con el sistema de ordenador Multiscan 4.01 (Computer Scanning Systems Ltd, Poland), que estaba conectado a un microscopio Jenamed. En cada animal han sido analizadas tres metafases. La longitud relativa total de brazos CBG varió, entre los animales, de 0,53 a 1,04. Un análisis de varianza unimodal demostró que esta variabilidad era significativa ($p < 0,01$). La longitud total media de brazos CBG en cuatro grupos distintos era: 0,84, 0,75, 0,65 y 0,67 respectivamente. La aplicación del análisis de contraste demostró que la diferencia entre estos grupos, con la excepción de grupo 4 vs grupo 3, era significativa ($p < 0,01$ o $p < 0,05$).

INTRODUCTION

In the karyotype of the blue fox ($2n=50$) there are ten chromosome pairs with entirely heterochromatic arms. It was found that in this species there are animals with C-band heteromorphism due to the lack of the heterochromatic arm in a single chromosome pair (Mäkinen, Lohi and Juvonen, 1981; Switonski, 1985 b).

Moreover, karyotype polymorphism, caused by a centric fusion between

acrocentric chromosomes from pairs 23 and 24, is widely distributed in this species (Christiansen *et al.*, 1987; Mäkinen and Lohi, 1987; Moller *et al.*, 1985; Switonski, 1985a). Due to the centric fusion polymorphism, there are three karyotype forms with diploid chromosome numbers 50, 49 or 48. Animals with $2n=50$ have four acrocentric chromosomes, those with $2n=49$ have two, and those with $2n=48$ have a pair of biarmed chromosomes instead of two pairs of acrocentrics.

The aim of the present study was quantitative analysis of the variability of the total length of heterochromatic arms in four groups of animals demonstrating the following C-band heteromorphism:

H 20 - twenty chromosomes with heterochromatic arms.

H 19 - nineteen chromosomes with heterochromatic arms.

H 18 - eighteen chromosomes with heterochromatic arms.

H 17 - seventeen chromosomes with heterochromatic arms.

MATERIAL AND METHODS

CBG banding was carried out for 49 animals, according to the method described by Sumner (1972). Length measurements of heterochromatic and euchromatic arms of ten chromosome pairs were performed with the use of Multiscan 4.01 computer system (Computer Scanning Systems, Ltd, Poland) connected with a Jenamed microscope. Three metaphases for each animal were analysed. Total real length of heterochromatic and euchromatic arms was measured. The total relative

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length was calculated as follows:

$$TRLH = \frac{\sum_{i=1}^{20} h_i}{\sum_{i=1}^{20} e_i}$$

TRLH - total relative length of the heterochromatin blocks.

h_i - real length of the heterochromatic arm of the i^{th} chromosome.

e_j - real length of the euchromatic arm of the j^{th} chromosome.

RESULTS AND DISCUSSION

In the studied groups of animals, four types of the karyotype with regards to the heterochromatin heteromorphism, were recognized (**figure 1 a-d**).

The total real and relative lengths of

heterochromatic arms varied among the studied groups of animals from 27.74 μm to 31.22 μm and from 0.65 to 0.84, respectively (**table I**). It was found that this variability was significant in the whole group and also within the group H 20 (real and relative length) and H 19 (real length). The application of the contrast analysis showed that differences among the distinguished groups, with the exception of the comparison group 4 versus 3, were also significant ($p < 0.01$ or $p < 0.05$) (**table II**).

It can be concluded that variability of the total length of heterochromatic arms exists in the karyotype of the blue fox as a consequence of the heteromorphism. Since chromosome preidentification (i. e. QFQ) was not applied, it was impossible to indicate which chromosome pairs were heteromorphic. In earlier study, including CBG and GTG banding techniques, it was suggested that chromosome pair 13, according to the standard karyotype of the blue fox

Table I. Variability of the length of heterochromatin arms in four groups of animals representing four types of the karyotype. (Variabilidad de la longitud de los brazos de heterocromatina en cuatro grupos de animales representantes de cuatro tipos de cariotipo).

GROUP OF ANIMALS		HETEROCHROMATIN BLOCKS			
Type of the karyotype	Number of animals	Real length (μm)		Relative length	
		x	S	x	S
H 20	26	31.22	3.88	0.84	0.14
H 19	15	28.40	5.14	0.75	0.12
H 18	5	28.17	2.34	0.65	0.10
H 17	3	27.74	3.21	0.67	0.10
Together	49	29.80	4.39	0.78	0.14

significant interindividual variability within the group at $p < 0.01$

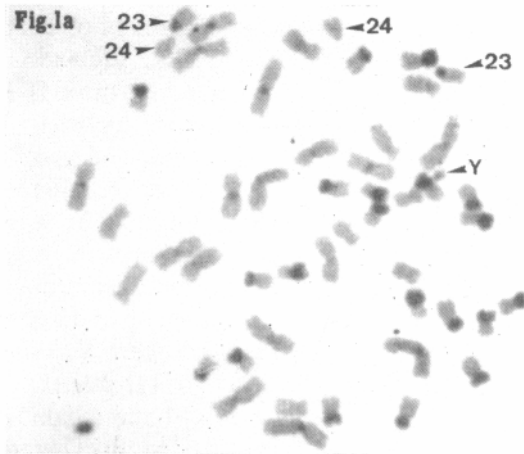


Figure 1a. CBG banded metaphase spread: male; $2n=50$; 20 chromosomes with heterochromatic arms (group H 20). Chromosome Y and four acrocentric chromosomes (pair 23 and 24) are indicated. (Desarrollo de una metafase con bandas CBG: macho; $2n = 50$; 20 cromosomas con brazos heterocromáticos (grupo H20). Están indicados el cromosoma Y y cuatro cromosomas acrocéntricos (pares 23 y 24)).

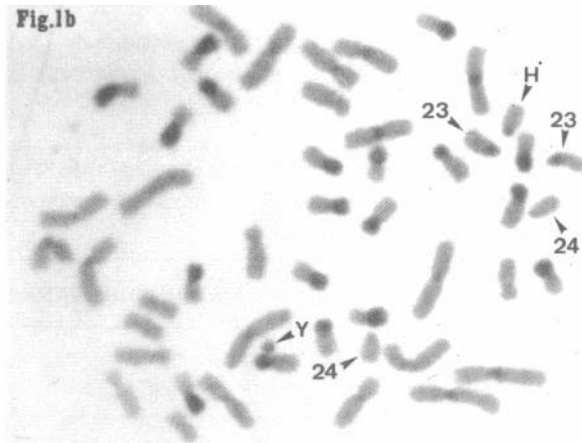


Figure 1b. CBG banded metaphase spread: male; $2n=50$; 19 chromosomes with heterochromatic arms (group H 19). Chromosome Y, four acrocentric chromosomes (pair 23 and 24) and one chromosome (H) without heterochromatic arm are indicated. (Desarrollo de una metafase con bandas CBG: macho $2n = 50$; 19 cromosomas con brazos heterocromáticos (grupo H19). Están indicados el cromosoma Y, cuatro cromosomas acrocéntricos (pares 23 y 24) y un cromosoma (H) sin brazo heterocromático).

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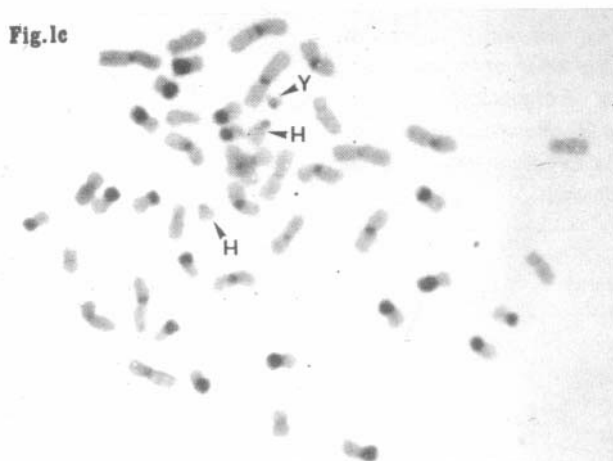


Figure 1c. CBG banded metaphase spread: male; $2n=48$; 18 chromosomes with heterochromatic arms (group H 18). Chromosome Y, and two chromosomes (H) without heterochromatic arms are indicated. (Desarrollo de una metafase con bandas CBG: macho $2n = 48$; 18 cromosomas con brazos heterocromáticos (grupo H18). Están indicados el cromosoma Y y dos cromosomas (H) sin brazos heterocromáticos).

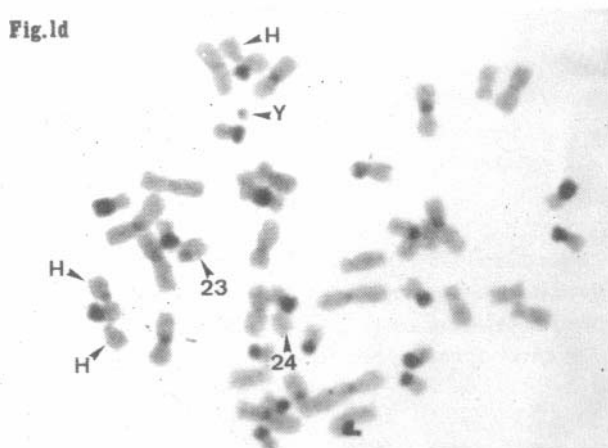


Figure 1d. CBG banded metaphase spreads: male; $2n=49$; 17 chromosomes with heterochromatic arms (group H 17). Chromosome Y, two acrocentric chromosomes (pair 23 and 24) and three chromosomes (H) without heterochromatic arms are indicated. (Desarrollo de una metafase con bandas CBG: macho $2n = 49$; 17 cromosomas con brazos heterocromáticos (grupo H17). Están indicados el cromosoma Y, dos cromosomas acrocéntricos (pares 23 y 24) y tres cromosomas (H) sin brazos heterocromáticos).

Table II. Contrast analysis showing differences of the relative length of heterochromatin arms among four groups of animals. (Análisis de contraste, mostrando las diferencias de longitud relativa de los brazos de heterocromatina entre cuatro grupos de animales).

CONTRAST	VALUE OF THE F-STATISTICS		
	Calculated	F _{0.05}	F _{0.01}
H20:H19	21.00**		
H20:H18	44.00**		
H20:H17	21.00**	3.94	6.90
H19:H18	11.00**		
H19:H17	4.20*		
H18:H17	0.39		

*significant variability among the groups at $p < 0.05$

**significant variability among the groups at $p < 0.01$

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