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Short Communication

Analysis of cytogenetic differentiation in selected species of the *Camelidae* family: *Vicugna pacos* and *Vicugna vicugna*

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The aim of this study was analysis of cytogenetic differentation in two species: *Vicugna pacos* and *Vicugna vicugna*. Cytogenetic analysis based on blood culture technique. Blood cultures were set up in culture medium Limphogrow and Lymphochrome. The cells growth, after 71 hours, was kept in metaphase using colcemid. Metaphase chromosomes were treated with hypotonic solution (KCl) for 30 minutes and fixed in a mixture of methanol and glacial acetic acid (3:1). After that, chromosomes were stained with Giemsa. Chromosomes were analyzed under microscope using Optica Vision pro-3 and MultiScan programs. Both species have 2n = 74 and showed close homology between autosomes and sex chromosomes. Analysed karyotypes consist of 19 pairs acrocentric, 9 pairs metacentric, 5 pairs subtelocentric and 3 pairs submetacentric autosomes. Sex chromosomes X was metacentric and Y was acrocentric.

Keywords: cytogenetic, chromosome, karyotype, *Vicugna pacos*, *Vicugna vicugna*

1 Introduction

Camelids family belongs to the herbivorous, placental mammals of the order *Artiodactyla*. It includes 6 species: camel dromedary (*Camelus dromedarius*), camel bactrian (*Camelus bactrianus*), llama (*Lama glama*), guanaco (*Lama guanicoe*), alpaca (*Vicugna pacos*) and vicuna (*Vicugna vicugna*) (Czerwiński and Krop-Warotek, 2005). Family of *Camelidae* can be divided into individuals of the Old World and New World. New World camelids are two domesticated species – llama and alpaca and two wild species - vicuna and guanaco, while the dromedary camel and bactrian represent the Old World (Alhadrami, 2003; Fernandez-Baca, 1994). Study of the *Camelidae* family are subject to a number of considerations, because the species belonging to this family have many behavioral, physiological and morphological similarities. In addition, individuals within the whole family can interbreed with each other, giving fertile offspring. All six species of *Camelidae* also shows similarities with the number of chromosomes karyotype 2n = 74 (Morales, 2010). The aim of the study was to analyse the variability of cytogenetic in two species of *Camelidae*: *Vicugna vicugna* and *Vicugna pacos*. The cytogenetic analysis takes into account the size of the chromosomes (including the surface area of autosomes and sex chromosomes), and the ratio of the short arm of chromosome (p) to the long arm (q).

2 Material and methods

Blood samples, were obtained from the vicuna male kept in Wroclaw Zoological Garden and the alpaca and vicuna males kept in Zoological Garden in Opole. Blood samples were collected using S-Monovette tube, and then the material was transported to the cytogenetic laboratoryin the Department of Genetics at the University of Environmental and Life Sciences in Wroclaw.

2.1 Cell culture of blood lymphocytes

The cell culture was set up during the 4 hours of blood collection on two liquid media – Lymphochrome and Lymphogrow. These media with biological material were placed into incubator for 72 hours in 5% CO_2 concentration and 80% humidity, at a temperature of 37.5 °C. After 71 h, 2 drops of colcemid

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(10 μg ml^{-1}) were added to inhibit the growth of cells in metaphase. After 72 h, the culture was poured into sterile tubes and centrifuged (1000 rpm per min for 10 minutes). Next, supernatant was treated with KCl (4 ml) to dissolve the chromosomes in metaphase plates. Then, the chromosomes were fixed by adding 4 ml of Carnoy's fixative at a temperature of -20 °C. and the whole mixture was centrifuged 5 minutes (1000 rpm per min.). A fixing process was repeated twice, then the samples were stored for 7 days in a refrigerator.

2.2 Dyeing preparations

From each cell culture six preparations were prepared for both species. Preparations were made on glass slides. After application, the slides were dried using artificial light. The preparations were stained with Giemsa. After that preparations were washed under running water and dried in vertical position at room temperature.

2.3 Analysis of karyotypes

Cytogenetic diagnosis performed in mitotic metaphase stage. Quantitative analysis was performed based on the MultiScan program. For *Vicugna vicugna* 11 metaphase plates were received and for *Vicugna pacos* – 46. The Optica Vision Pro-3. program were used to photograph obtained karyotypes. Morphological type was classified based on morphologica

l descriptions and values adopted by Świtoński et al. (2009). For each chromosome, 3 measurements of arm length and surface area were performed, and then calculating the mean used Microsoft Excel. The relative size of the surface area of the sex chromosomes, expressed as a percentage, was calculated based on the sum of the areas of sex chromosomes to the autosomes, while the share of sex chromosomes are expressed relative to the total area of all the chromosomes in metaphase plate (Bogdzińska and Ziółkowska, 2009).

3 Results and discussion

Both analyzed species belonging to the family *Camelidae* showed great similarity in karyotypes and the same number of chromosomes (2n = 74). Karyotype contained 36 pairs of homologous chromosomes and a pair of sex chromosomes (XY). In both species, among the 72 autosomes, 19 pairs of acrocentic chromosomes, 9 pairs metacentric, 5 pairs subtelocentric and 3 pairs of submetacentric chromosomes were observed. The Y chromosome, in both species, was acrocentric and much smaller than chromosome X. Analyzed the morphological types of chromosome in the species measured the length of the short (p) and long (q) arm, and then calculating their mean values (Table 1).

Table 1 Average lengths of(p) and (q) arm in different morphological types of chromosome in *V.pacos* and *V. vicugna*

Species	Acrocentric autosomes [μm]	Metacentric autosomes [μm]	Subtelocentric autosomes [μm]	Submetacentric autosomes [μm]
Vicugna pacos	p = 0.06 q = 0.52	p = 0.35 q = 0.44	p = 0.21 q = 0.81	p = 0.21 q = 0.38
Vicugna vicugna	p = 0.06 q = 0.51	p = 0.31 q = 0.39	p = 0.21 q = 0.76	p = 0.21 q = 0.38

From these results it can be concluded that the vicuna and alpaca characterized by very similar length of autosomes arms (Table 1), and arms in the sex chromosome X (Table 2).

Table 2 Arm length of X sex chromosome in *V. pacos* and *V. vicugna*

Species	Length of p arm [μm]	Length of q arm [μm]	SDR ratio
Vicugna pacos	0.53	0.63	1.19
Vicugna vicugna	0.51	0.52	1.10

Table 3 The surface area of chromosome in *V. pacos* and *V. vicugna*

Species	Sum of the autosomes area [µm^2]	Surface area of allosomes [µm^2]	Average area of autosomes [µm^2]	Average area of allosomes [µm^2]
Vicugna pacos	104.58	3.25 [X] 0.95 [Y]	1.45	2.10
Vicugna vicugna	83.88	2.56 [X] 0.67 [Y]	1.16	1.62

All species belonging to the family *Camelidae* have almost identical sets of chromosomes but with differences in the size (Johnson and Perelman, 2007). The study indicated that the analyzed species *Vicugna pacos* and *Vicugna vicugna* are cytogenetically similar, which is also reflected in the origin of these species (Kadwell and Fernandez, 2001). They show a similar type of surface morphology and the sex chromosomes. Their very similar karyotype, despite the geographical separation and diverse living environment, suggests that camelids karyotype remained unchanged for millions of years of evolution (Di Benardinio et al., 2006). In the literature there are no data of the chromosomes surface area of the family *Camelidae*, hence for more accurate analysis of individual chromosomes and the confirmation of the results would broaden the scope of these studies.

4 Conclusions

Vicugna pacos and *Vicugna vicugna* showed the same number of chromosomes (2n = 74) and great similarity in karyotype.

Among all autosomes distinguished19 pairs of acrocentic chromosomes, 9 pairs metacentric, 5 pairs subtelocentric and 3 pairs of submetacentric chromosomes.

The length of X chromosome arms, the surface of autosomes and sex chromosomes was similar in both species.

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