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Comparative cytogenetics in felids (Carnivora: Felidae): *Leopardus wiedii, Panthera onca, Puma concolor* and *Felis catus*

[*Citogenética comparativa em felídeos (Carnivora: Felidae)*: Leopardus wiedii, Panthera onca, Puma concolor *e* Felis catus]

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ABSTRACT

This study aims to conduct a cytogenetic analysis on four South American feline species: the jaguar (Panthera onca), the puma (Puma concolor), the ocelot (Leopardus wiedii), and the domestic cat (Felis catus). We discovered that the jaguar and the puma mainly differ in their fundamental number of chromosomes. Furthermore, we observed a morphological difference in a pair of chromosomes between the jaguar and the domestic cat. This suggests that, over the course of evolution, there was a pericentric inversion in a pair of B4 group chromosomes, leading them to become part of the A group in the jaguar. When analyzing the puma, we noticed the presence of a fourth pair of chromosomes in the E group, along with a deletion in the F group. Additionally, we observed the presence of an additional pair of chromosomes in the C group in the ocelot. Another interesting aspect of the study was the location of nucleolar organizer regions (NORs) on the chromosomes. To elucidate the origin of other chromosome pairs, we employed chromosomal banding techniques, allowing us to better understand the evolution of the C3 pair in the Leopardus genus and the E4 pair in the Puma genus.

Keywords: felidae, NOR banding, chromosome marker

RESUMO

*Este estudo tem como objetivo realizar uma análise citogenética em quatro espécies de felinos sul-americanos: a onça-pintada (*Panthera onca*), a suçuarana (*Puma concolor*), o gato-do-mato-pequeno (*Leopardus wiedii*) e o gato doméstico (*Felis catus*). Descobriu-se que a onça-pintada e a suçuarana diferem principalmente em seu número fundamental de cromossomos. Além disso, notou-se uma diferença morfológica em um par de cromossomos entre a onça-pintada e o gato doméstico. Isso nos sugere que, ao longo da evolução, houve uma inversão pericêntrica em um par de cromossomos do grupo B4, que passou a fazer parte do grupo A na onça-pintada. Quando se analisou a suçuarana, observou-se a presença de um quarto par de cromossomos no grupo E, juntamente com uma deleção no grupo F. Isso sugere que, ao longo de sua evolução, a suçuarana também passou por uma inversão pericêntrica. A principal diferença entre o gato-do-mato-pequeno e o gato doméstico reside na ausência de cromossomos no grupo F devido à fusão de dois pares de cromossomos telocêntricos. Além disso, notou-se a presença de um par adicional de cromossomos no grupo C no gato-do-mato-pequeno. Outro aspecto interessante do estudo foi a localização das regiões organizadoras nucleolares (NOR) nos cromossomos. Descobriu-se que, tanto na onça-pintada quanto na suçuarana, as NORs estão localizadas no braço curto do par de cromossomos E1. Para elucidar a origem de outros pares de cromossomos, foram empregadas técnicas de bandamento cromossômico, o que permitiu entender melhor a evolução do par C3 no gênero* Leopardus *e do par E4 no gênero* Puma.

Palavras-chave: felidae, banda NOR, marcador cromossômico

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INTRODUCTION

The family Felidae comprises a diverse group of at least 36 species of wild cats. They are characterized by morphological similarities, including round and flat faces, prominent whiskers, and large eyes and ears. This taxonomic family exhibits the broadest range of body sizes among all extant carnivorous families and has a global distribution, excluding Antarctica and Australia, where they were introduced by human activity (Lamberski, 2015). Felids possess binocular vision and share common features, such as a rounded skull with a robust zygomatic arch. Notably, larger species within this family are capable of emitting roars due to the presence of flexible cartilage replacing the hyoid bone, while smaller felids are known for their purring behavior. Additionally, they are equipped with powerful jaw muscles, which they employ to immobilize their prey by either choking or biting the neck, enabling the canines to penetrate the vertebrae and sever the spinal cord. Their digitigrade locomotion is facilitated by large, curved, and retractable claws, emphasizing their adaptability as stealthy hunters. Most members of the Felidae family are nocturnal creatures, renowned for their exceptional agility and their proficiency in stalking, attacking, or ambushing their prey (Montero and Autino, 2004).

Cytogenetics encompasses studies related to isolated or common, condensed or dispersed chromosomes, including their development, variability, morphology, function, organization, and replication (Guerra, 1988). Members of the Felidae family are highly conserved in terms of diploid chromosome numbers and exhibit nearly constant morphology across all species within this group. Most cat species have been reported to have diploid numbers ranging from 2n=36 to 2n=38 and fundamental numbers between FN=66 and FN=74, with uniform sex chromosome structures (Wurster-Hill and Bernirshke, 1968). Most Felidae species have been cytogenetically studied using conventional techniques and chromosome banding, with G-banding being the most used method. In most species, the diploid number was found to be 38, and it's interesting to note that thirteen of the nineteen chromosome pairs in domestic cats are also found in other cat species, highlighting chromosome conservation, and serving as a model for comparisons within the family (Hsu *et al*., 1963; Wurster-Hill and Gray, 1975; Collier and O'Brien, 1985; Nie *et al*., 2002). Additionally, all members of the subfamily Felinae (except the genus Leopardus) and members of the subfamily Pantherinae share similar karyotypes, differing mainly in their morphological characteristics (Collier and O'Brien, 1985).”

A key chromosomal marker of the Felidae family, known as the 'carnivore chromosome,' is the E1 pair, which demonstrates the group's homology (Hsu *et al*., 1963). This chromosome is a small to medium-sized submetacentric one with a secondary constriction at the short arm level. Among all these families, the Felidae family exhibits the most uniform karyotypic pattern, and this chromosomal marker is remarkably consistent (Wurster-Hill and Benisrchke, 1968; Wurster-Hill and Gray, 1973, 1975; Wurster-Hill and Centerwall, 1982; Collier and O'Brien, 1985). It's important to note that the location of this chromosomal pair varies depending on the family analyzed. While the Felidae family is karyotypically homologous, this similarity does not apply to all chromosome groups between species. For instance, chromosome pairs like A1 in some species do not necessarily correspond to the pair in another species (Hsu *et al*., 1963). However, certain pairs, such as pair E1 (Ledesma *et al*., 2004), provide certainty about the group's uniformity.

Limited published studies using silver nitrate streaks in the Felidae family have been conducted in Felis catus (Pearson *et al*., 1979) and Panthera onca (Ledesma *et al*., 2004). Both authors demonstrated that the nucleolus organization region (NOR) is in the secondary constriction of the short arm of the E1 chromosome pair in both species. In 1965, the feline chromosome classification system was developed by Jones (1965) and was quickly adopted by geneticists at the International Conference on Standardization of Karyotypes of Domestic Animal Bands (Ford *et al*., 1980).

In the state of Misiones, the following species are found: Puma concolor, Puma yagouaroundi, Leopardus tigrinus, Leopardus wiedii, Leopardus pardalis, and P. onca (Massoia *et al*., 2012). One representative from each of these genera was selected for chromosomal analysis, along with F. catus, which served as the standard for

comparisons (Roubin *et al*., 1973). Therefore, this study aims to compare the karyotypes obtained with those published in previous years and align them with the new cat classification system proposed by the San Juan Convention. Some species' karyotypes were described before the International Conference on the Standardization of Karyotypes of Domestic Animals (Ford *et al*., 1980), during which this system was confirmed. Additionally, the homology of the NOR carrier pair was compared in three of the studied species.

MATERIAL AND METHODS

Blood samples were collected from confiscated animals in the rehabilitation process for reintroduction in different provincial parks, from 2008 to 2012, as well as from animals permanently exhibited in the *Parque Ecológico El Puma* (https://ecologia.misiones.gob.ar/parque-ecologico-el-puma/), under the jurisdiction of the *Subsecretaria de Ecologia e Desenvolvimento Sustentável do Ministério de Misiones Argentina*. *El Puma* is a natural area of the multipurpose reserve category, dedicated to research, self-sufficient production of native flora and fauna, natural spaces for outdoor recreation and experimental areas for models of sustainable use of natural resources. Chromosomes of species belonging to the family Felidae, which are part of the order Carnivora, were analyzed: a male of *L. wiedii*, a male of *P. concolor*, 2 males and 3 females of *P. onca*, and 1 male and 1 female of *F. catus*.

Long-term lymphocyte cultures were established for 72 hours in PBMax medium (Gibco) according to Moorhead *et al.* (1960) with modifications to obtain metaphases. The blood was previously defibrinated, and samples were transferred to flasks containing small glass beads and gently shaken for 10 to 15 minutes. The obtained metaphases were analyzed using conventional and differential techniques by NOR-banding according to Howel and Black (1980). Feline chromosomes were morphologically classified into their respective groups according to the San Juan de Costa Rica Conference (Jones, 1965): A (large submetacentric); B (large acrocentric); C (large metacentric); D (small submetacentric and subtelocentric); E (small metacentric); F (telocentric or acrocentric); X (medium submetacentric); and Y (small submetacentric).

An Olympus Cx31 microscope with an Olympus E-330 integrated camera was used for photomicrographs. The top 40 metaphases from each species with optimal staining and well-defined morphology and contours were selected and photographed. Karyotypes were arranged using the software GNU Image Manipulation Program (GIMP) and COREL PHOTO PAINT13 and grouped by morphology and decreasing size according to the San Juan de Costa Rica Conference (Jones, 1965) as shown in Table 1. Chromosomal nomenclature was based on the domestic cat karyotype, which serves as the model for studying all other cat species. MICROMEASURE 3.3 software (Revees and Tears, 2000) was used to perform biometric analysis.

RESULTS AND DISCUSSION

The karyotype of domestic cats (FCA) is often considered the closest model to the original chromosome arrangement in the cat family, Felidae. This karyotype consists of 38 chromosomes, a finding consistent with previous research (Wurster-Hill and Centerwall, 1982; Wurster-Hill and Bernirshke, 1968) (Fig. 1). For our comparative analysis of the three studied species, we used the domestic cat's karyotype as a reference. Among the three species under study, only L. wiedii (LWI) exhibited a different chromosome number, 2n=36, and FN=72 (Fig. 2). In this case, all chromosomes, including autosomes and sex chromosomes, are bibrachial (having two arms). P. onca (PON) (Figure 3) and P. concolor (PCO) (Figure 4), on the other hand, both have 2n=38 chromosomes but differ in their FN. P. onca has FN=72, with 34 bibrachial and 4 telocentric chromosomes, while P. concolor has FN=74, with 36 bibrachial chromosomes and 1 pair of telocentric chromosomes. The sex chromosomes, X and Y, are described as median submetacentric and small submetacentric, respectively. The discrepancy in chromosome number between P. onca and P. concolor compared to L. wiedii is attributed to the absence of group F telocentric chromosomes. Thus, the variation in the fundamental number of P. onca and P. concolor can be explained by the presence of a single group F telocentric chromosome in P. concolor. Biometric data for all four cat species are summarized in Table 1.

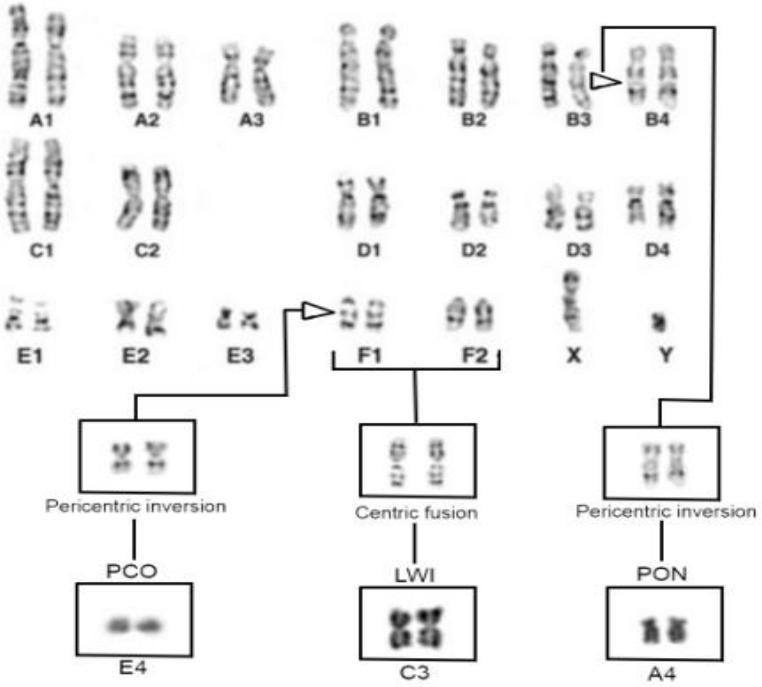

Figure 1. Karyotype of *F. catus* (FCA) showing the presumed types of chromosome rearrangements led to the karyotype of the species studied: Chromosome E4 of *P. concolor* (PCO) underwent a pericentric inversion from chromosome F1 of the common ancestor (FCA). Chromosome C3 of *L. wiedii* (LWI) was formed by a centric fusion from chromosomes F1 and F2 of the common ancestor. Finally, chromosome A4 of *P. onca* (PON) was formed by a pericentric inversion from chromosome B4 of the common ancestor. The arrows indicate the breakpoints of the chromosomes.

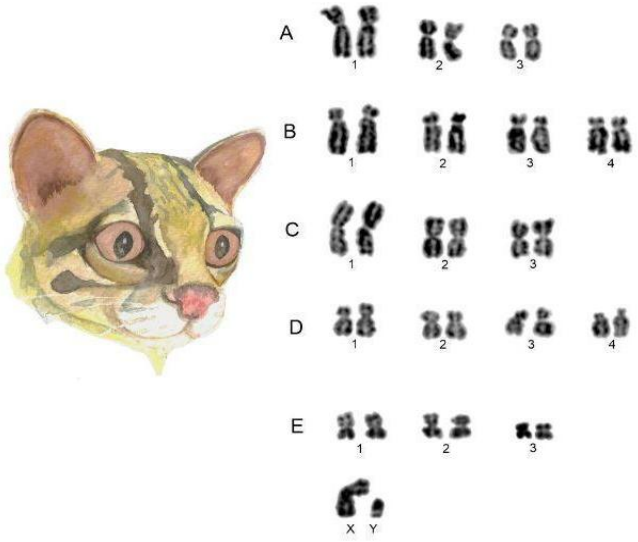

Figure 2. Karyotype of a male *Leopardus wiedii*, 2n=36 chromosomes. X chromosome is a medium submetacentric, and Y chromosome is a small submetacentric.

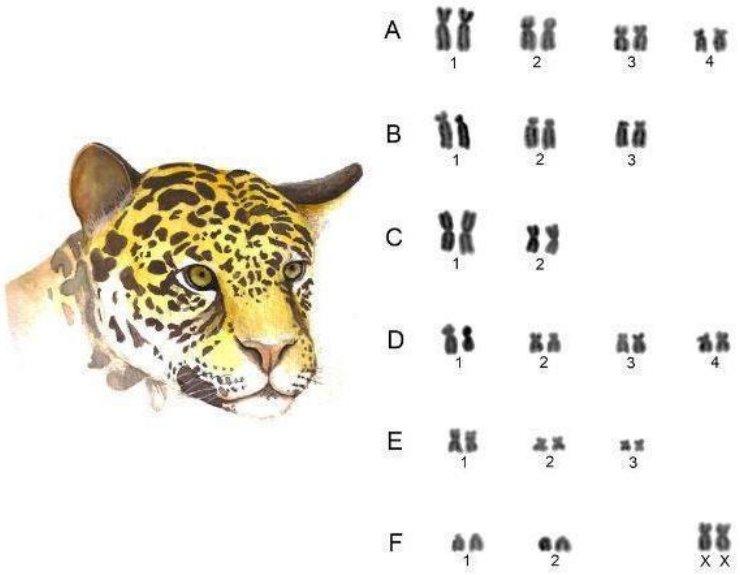

Figure 3. Karyotype of a female *Panthera onca*, 2n=38 chromosomes showing X is a medium submetacentric.

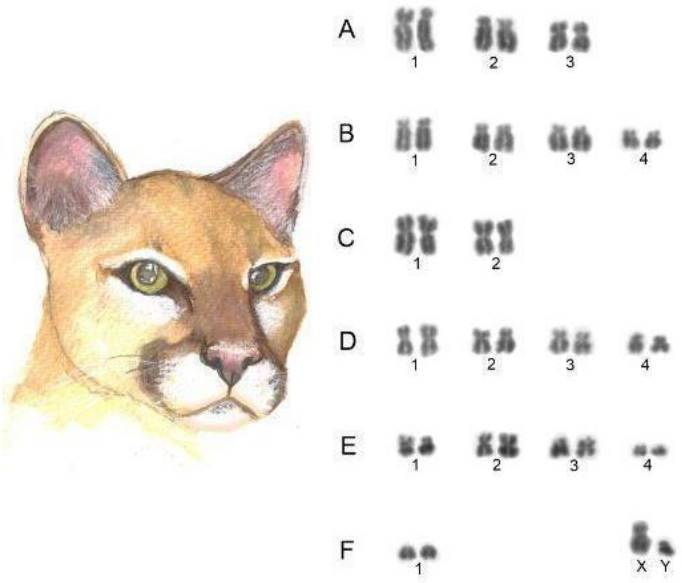

Figure 4. Karyotype of a male *Puma concolor*, 2n=38 chromosomes. X chromosome is a medium submetacentric and Y chromosome is a small submetacentric.

Table 1. Biometric analysis of *P. onca*, *P. concolor* and *L. wiedii* analyzed in this study according to the San Juan Conference on Karyotype of Felidae (Jones, 1965). Groups: A (large submetacentric); B (large acrocentric); C (large metacentric); D (small submetacentric and subtelocentric); E (small metacentric); F (telocentric or acrocentric); X (medium submetacentric) and Y (small submetacentric)

Group	N° chromosomes	Panthera onca		Puma concolor		Leopardus wiedii		Felis catus	
		CI	CM	CI	CM	CI	CM	CI	CM
A	1	0.34	sm	0.3	sm	0.36	sm	0.35	sm
	2	0.34	sm	0.23	sm	0.35	sm	0.34	sm
	3	0.34	sm	0.31	sm	0.36	sm	0.36	sm
	4	0.31	sm						
B	1	0.21	st	0.27	st	0.23	st	0.22	st
	2	0.23	st	0.29	st	0.25	st	0.25	st
	3	0.22	st	0.27	st	0.24	st	0.25	st
	4			0.25	st	0.24	st	0.25	st
C	1	0.47	m	0.43	m	0.47	m	0.46	m
	2	0.44	m	0.44	m	0.46	m	0.44	m
	3					0.44	m		
D	1	0.33	sm	0.32	sm	0.33	sm	0.27	sm
	2	0.35	sm	0.3	sm	0.36	sm	0.27	sm
	3	0.33	sm	0.34	sm	0.35	sm	0.29	sm
	4	0.29	sm	0.33	sm	0.23	st	0.26	sm
E	1	0.44	m	0.44	m	0.45	m	0.43	m
	2	0.41	m	0.41	m	0.41	m	0.42	m
	3	0.42	m	0.44	m	0.41	m	0.40	m
	4			0.43	m				
F	1	0	t	0	t			0	t
	2	0	t					0	t
SEXUAL	X	0.36	sm	0.3	sm	0.36	sm	0.39	sm
	Y			0.33	sm	0.42	sm		

CM: chromosome morphology, CI: centromeric index, m: metacentric, sm: submetacentric, st: subtelocentric, t: telocentric, a: acrocentric.

P. onca, the species closest to F. catus, differs from it primarily due to a morphological change in one of the chromosome groups, specifically the B4 chromosome pair in F. catus. In P. onca, this pair underwent a pericentric inversion and is now part of the A group (Fig. 1). While three chromosome pairs in the E group of P. concolor resemble those in F. catus, it's possible that the E4 pair is absent in P. concolor. Additionally, P. concolor has only one pair of telocentric chromosomes in the F group. This presence of a small fourth metacentric pair in group E and the absence of the telocentric pair in group F suggest a pericentric inversion in P. concolor, deviating from the original karyotype (Fig. 1). In contrast, L. wiedii shares morphologically similar chromosomes in groups A, B, D, and E with F. catus. The fundamental difference lies in the absence of chromosomes in group F and the presence of an extra pair of chromosomes in group D. This variation may be attributed to a fusion event between the two pairs of telocentric chromosomes in group F, giving rise to the C3 pair in L. wiedii (Fig. 1).

South American cats, especially those in the Leopardus genus, possess unique characteristics not shared by the other 48 genera in the Felidae family. However, some species, like P. concolor (which has an extra pair in group E and is missing two pairs in group F), and P. onca, still share similarities with other members of the family.

Through silver staining analysis, we found that the nucleolus organization regions (NORs) are located on the short arm of the E1 chromosome pair, specifically within the secondary constriction, in P. onca, P. concolor, and F. catus (Fig. 5). This pattern is consistent with what is commonly observed in most cat species studied, and it serves as a characteristic chromosomal marker for the NOR carrier family.

Figure 5. NOR-banding of a male individual of *F. catus* (A), *P. onca* (B), and *P. concolor* (C). The arrows indicate the E1 pair carrying the nucleolar organization region.

We made multiple attempts using the C-banding technique, but unfortunately, we couldn't achieve satisfactory results. This difficulty likely arose because the amount of heterochromatin in the cat's karyotype is extremely low or practically non-existent. To better understand the evolutionary relationships among the studied species, we turned to alternative techniques such as fluorescence in situ hybridization (FISH), restriction enzymes, and molecular methods, which proved to be more effective in our investigation.

CONCLUSION

Conventional Giemsa staining clearly shows the low variability and changes occur in the cat species studied. Chromosome bands can be used to clarify the evolutionary origins of additional chromosome pairs, such as the C3 pair in the genus *Leopardus* and the E4 pair in the genus *Puma*, as well as the absence of acrocentric pairs in these two genera. Together with the procyonids, the felids have the best conserved karyotype of the carnivore order and resulting in a low amount of heterochromatin in both families.

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