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Short Communication

Genetic variability of *Herpailurus yagouaroundi*, *Puma concolor* and *Panthera onca* (Mammalia, Felidae) studied using *Felis catus* microsatellites

Vanessa Roma Moreno[1], Alexéia Barufatti Grisolia[1], Francine Campagnari[1], Marcela Milazzotto[1], Cristina Harumi Adania[2], José Fernando Garcia[3] and Edislane Barreiros de Souza[4]

[1]*UNESP, Universidade Estadual Paulista, Instituto de Biociências, Departamento de Genética, Botucatu, SP, Brazil.*
[2]*Associação Mata Ciliar, Jundiaí, São Paulo, Brazil.*
[3]*UNESP, Universidade Estadual Paulista, Faculdade de Medicina Veterinária, Departamento de Apoio, Produção e Saúde Animal, Araçatuba, SP, Brazil.*
[4]*UNESP, Universidade Estadual Paulista, Faculdade de Ciências e Letras, Departamento de Ciências Biológicas, Assis, SP, Brazil.*

Abstract

We used four microsatellite loci (*Fca08*, *Fca45*, *Fca77* and *Fca96*) from the domestic cat, *Felis catus*, to investigate genetic variability in specimens of *Herpailurus yagouaroundi* (jaguarundi, otter cat, eyra), *Puma concolor* (cougar, mountain lion, puma) and *Panthera onca* (jaguar) held in various Brazilian zoos. Samples of DNA from the cats were PCR amplified and then sequenced before being analyzed using the CERVUS program. Our results show a mean polymorphic information content (PIC) of 0.83 for *H. yagouaroundi*, 0.66 for *P. concolor* and 0.69 for *P. onca* and a mean of 10.3 alleles for the *Fca08* locus, 5.3 for *Fca 45*, 9 for *Fca 77* and 14 for *Fca 96*. These results indicate a relatively high level of genetic diversity for the specimens studied.

Key words: genetic variability, mammals, felines, microsatellites.

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Brazil has the highest number of aquatic and terrestrial species and is the leading country in respect to biological diversity. Brazil, Indonesia and Mexico are the three countries with the richest mammalian fauna, Brazil currently having 460 species of mammals and this number is expected to increase as new species are described (Mittermeier, 1994).

Felines are an important component of the worldwide fauna, the family Felidae (Mammalia, Carnivora) comprising 37 species which occur naturally in almost every area of the world except for some insular regions such as Australia, New Guinea and New Zealand, Japan, Madagascar, Oceania, the poles and some Caribbean islands such as the West Indies (Nowak, 1991).

The Neotropics are home to ten feline species (*Herpailurus yagouaroundi* (jaguarundi, otter cat, eyra), *Leopardus pardalis* (the leopard cat), *Leopardus wiedii* (the margay), *Leopardus tigrinus* (the oncilla), *Oncifelis geoffroi* (Geoffroy's Cat), *Oncifelis colocolo* (the pampas cat), *Oncifelis guign* (the kodkod), *Oreailurus jacobita* (the Andes cat), *Puma concolor* (cougar, mountain lion, puma) and *Panthera onca* (jaguar)), of which only the Andes cat and the kodkod do not occur naturally in Brazil (Oliveira, 1994). All of the Neotropical Felidae are to some extent threatened with extinction, mainly due to indiscriminate hunting and large scale modification of their habitat (Crawshaw Jr., 1997).

Send corrrespondence to Vanessa Roma Moreno. Rua General Carneiro, n. 328, Vila Boa Vista, 19806-100 Assis, SP, Brazil. E-mail: bio_vanessa@hotmail.com.

Microsatellites consist of small DNA fragments of about 10-100 base pairs (bp) that contain repetitive elements displaying tandem repeats of 1-6 bp, variation in the number of repeats resulting in these loci having a high polymorphism information (PIC). The heterozygosity of microsatellite loci was first described in humans, but microsatellites have been found to be abundant, randomly distributed and highly polymorphic in all eukaryotic organisms so far investigated (Weber and May, 1989). Microsatellites are co-dominant markers (*i.e.* heterozygotes can be distinguished from the homozygotes) which can be amplified by the polymerase chain reaction (PCR) and generally show a level of heterozygosity in excess of 0.7 (Ferreira and Grattapaglia, 1998). One of the advantages of

using microsatellite loci is that primers for these loci can be used in species other than that for which they were originally designed. For example Moore *et al.* (1991) tested 48 ovine primers and found that 27 (42% of which presented polymorphism) successfully amplified the same markers in bovine DNA, while Bowcock *et al.* (1994) found that human primer pairs successfully their corresponding microsatellites in chimpanzee, gorilla and orangutan.

Our study was designed to use *Felis catus* microsatellite markers (Menotti-Raymond and O'Brien, 1995) in a genetic variability study of three neotropical Brazilian feline species *H. yagouaroundi*, *P. concolor* and *P. onca*.

Blood samples were collected from specimens of *H. yagouaroundi* (n = 36), *P. concolor* (n = 18) and *P. onca* (n = 39) kept at a wide variety of Brazilian zoos (Table 1). The samples were humanly collected without suffering to the animal by the workers from the Riverine Forest Association (Associação Mata Ciliar, Jundiaí-SP, Brazil) and analyzed at the Genetics Laboratory at the Assis campus of São Paulo State University (Laboratório de Genética, FCL, UNESP, Assis, SP). Our methodology was standardized using a blood sample from the domestic cat (*Felis catus*) supplied by the Marylin Menotti-Raymond from the Laboratory of Genomic Diversity, Frederick, Maryland, USA.

Total DNA was isolated from white blood cells using proteinase K digestion and treatment with phenol/chloroform (Sambrook *et al.*, 1989). The primers used (Table 2) were the four *F. catus* dinucleotide primers developed by Menotti-Raymond and O'Brien (1995) who had tested them in four feline species. The microsatellite loci were PCR amplified in a final volume of 10 μL containing 10 ng of feline genomic DNA, 1.5 μL of PCR buffer (containing 200 mM Tris-HCl and 500 mM KCl), 25 mM $MgCl_2$, 250 μM of each dNTP, 2 units of *AmpliTaq Gold* (5U μL^{-1}), 2.5 pmol of each forward and reverse oligonucleotide primer (10 pmol μL^{-1}) and ultra-pure water. The forward sequences were fluorescently labeled using tetramethylrhodamine (TAMRA) for *Fca08*, hexachloro-6-carboxyfluorescine (HEX) for *Fca 45* and 6-carboxyfluorescine (FAM) for *Fca 77* and *Fca 96*. Amplification was performed using a PTC-100 thermocycler (MJ-Research) in accordance with the conditions described by Menotti-Raymond *et al.* (1999), *i.e.* 1 cycle of 94 °C for 10 min; 10 cycles of 94 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; 20 cycles of 89 °C for 15 s, 55 °C for 15 s, 72 °C for 30 s; and 1 cycle of 72 °C for 30 min. At the end of the reaction the samples were cooled to 4 °C.

The amplified microsatellite loci were separated using capillary electrophoresis in an automatic genetic analyzer (ABI PRISM® 310 Analyzer, Applied Biosystems). For each electrophoretic run 12 μL of amplified sample containing the PCR products was mixed with 12 μL of deionized formamide and 0.5 μL of ROX 500 molecular weight marker (Applied Biosystems), a control consisting of unamplified sample also being run. Before sequencing the samples were denatured in the thermocycler at 95 °C for 5 min and rapidly cooled in ice. The electrophoresis products were developed using Gel POP 4™ (Performance Optimized Polymer 4, Applied Biosyestems) and each sample was run for approximately 25 min. The electrophoresis data were stored on the ABI 310 system using the ABI Collec-

Table 1 - Zoo location (all in Brazil) and specimen registration number of the felines investigated in the current work.

Zoo location (city/town-state) and registration number of specimen(s)		
Herpailurus yagouaroundi	*Puma concolor*	*Panthera onca*
Ilha Solteira-SP; 003	Varginha-MG; 013, 014	Campinas-SP; 001, 004, 002, Pintada 1 (no number)
São Paulo-SP; 141, 142	Goiânia-Go; O15	Pedreira-SP; Black (no number)
Pedreira-SP; 022, 021	Ubá-MG; 022	Uberlândia-MG; 032
Campinas-SP; 010	Pomerode-SC; 61	Ipatinga-MG; 016
Garça-SP; 20	Uberaba-MG; 040	Alfenas-MG; 05, 025
São José do Rio Preto-SP; 17	Campinas-SP; 01, 002	Muriaé-MG; 18, 17
Sorocaba-SP; 12, 014, 11, 15, 13, 18	Cascavel-PR; 062, 064, 063	Pouso Alegre-MG; 026
Catanduva-SP; 016	Curitiba-PR; 087, 086, 085, 088, 089, 090	Pomerode-SC; 059, 061, 60
Bauru-SP; 19	Ipatinga-MG; 011	Varginha-MG; 022
Jundiaí-SP; 027, 028, 051, 50, 029		Camboriú-SC; 67, 64, 68, 66, 65, 63, 69
Uberlândia-MG; 048		Goiânia-GO; 072, 037, 071, 007, 009
Varginha-MG; 035, 036		Cascavel-PR; 078, 073, 079, 080, 075, 076, 077, 074
Mogi Mirim-SP; 006		Curitiba-PR; 107, 106, 109
Goiânia-GO; 042, 043, 044		
Ipatinga-MG; 034, 033		
Brasília-DF; 041, 040, 039, 038		
Maringá-PR; 061		
Uberaba-MG; 047		

Key to Brazilian states: DF = Distrito Federal; GO = Goiás; MG = Minas Gerais; PR = Paraná; SC = Santa Catarina; SP = São Paulo.

Table 2 - Domestic cat (*Felis catus*) microsatellite loci used in amplifying the feline samples. Data from Menotti-Raymond and O'Brien (1995) and Menotti-Raymond *et al.* (1999).

Microsatellite loci	GenBank	Chromosome location	Number of repetitions	Primer sequence
Fca 08	AF130476	A1	$(CA)_{24}$	5'ACTGTAAATTTCTGAGCTGGCC3' 3'TGACAGACTGTTCTGGGTATGG5'
Fca 45	AF130489	A1	$(CA)_{15}$	5'TGAAGAAAAGAATCAGGCTGTG3' 3'GTATGAGCATCTCTGTGTTCGTG5'
Fca 77	AF130506	C2	$(CA)_{20}$	5'GGCACCTATAACTACCAGTGTGA3' 3'ATCTCTGGGGAAATAAATTTTGG5'
Fca 96	AF130519	A2	$(CA)_{17}$	5'CACGCCAAACTCTATGCTGA3' 3'CAATGTGCCGTCCAAGAAAC5'

tion software licensed by Applied Biosystems and allele sizes were determined using version 2 of the ABI GENESCAN® 500 and GENOTYPER® programs. The results were analyzed using the CERVUS program model 2.0 (Marshall *et al.*, 1998). For each microsatellite locus and species genetic diversity was measured based on the number of alleles and the polymorphism information content (PIC) (Botstein *et al.*, 1980) and the efficiency of each primer was calculated from the relationship between the number of samples analyzed and the number of positive amplifications of each loci for each species.

The success of inter-species microsatellite amplification depends on conservation of the primer sequence between species (Menotti-Raymond and O'Brien, 1995). Studies of *F. catus* microsatellite primers have shown that they produce amplification products of similar sizes in other feline species, loci containing the dinucleotide repetitions $(dC \bullet dA)_n$ $(dG \bullet dT)_n$ being highly polymorphic in domestic cats, lions, cheetahs, leopards and Geoffroy's cats (Menotti-Raymond and O'Brien, 1995; Shankaranarayanan *et al.*, 1997). The use of *F. catus* microsatellite primers in related felines has also been described by Eizirik *et al.* (2001) who used 29 *F. catus* microsatellite markers to investigate genetic diversity in 42 *P. onca* specimens.

Our results show that the *F. catus Fca 08*, *Fca 45*, *Fca 77* and *Fca 96* primers efficiently amplified the corresponding markers in *H. yagouaroundi*, *P. concolor* and *P. onca* at an efficiency of between 61 and 100%. This indicates that even though there was genetic diversity in the specimens conservation of primer sequences was significant in these species.

Microsatellite markers are known to generates a large number of alleles (multiallelism). Table 3 showing the number of *H. yagouaroundi*, *P. concolor* and *P. onca* alleles and the PIC for each of the microsatellite loci studied. Our results show that the number of alleles found for each of the loci in the three species studied was equal to or greater than that seen in *F. catus* and other representative felines (Menotti-Raymond and O'Brien, 1995), suggesting the presence of new and exclusive alleles the *H. yagouaroundi*, *P. concolor* and *P. onca* specimens studied by us and that many differently-sized alleles (in base-pairs) are shared and conserved among *H. yagouaroundi*, *P. concolor* and *P. onca*.

Table 3 shows that the *Fca 96* locus exhibited the largest number of alleles (17 in *H. yagouaroundi* and 13 in *P. concolor*) and was therefore the most polymorphic loci, although in *F. catus* this locus has been reported to be less polymorphic (Menotti-Raymond and O'Brien, 1995). The *Fca 96* locus showed 12 alleles in *P. onca*, the same number as the *Fca 08* locus. Since genetic variance within a population is described by the number and frequency of alleles in each locus (Futuyma, 1986) our microsatellite marker results indicate that despite *H. yagouaroundi*, *P. concolor* and *P. onca* being threatened species they still presents a reasonable level of genetic diversity. In spite of the fact that there were differences in terms of the quantity of alleles per loci and the felines studied between our work and that of other published researchers, our results are equal or, very often, superior to those previously reported for the same oligonucleotides in other felines.

The polymorphism frequency of a microsatellite sequence is frequently related to the number of repeats and microsatellites consisting of over 10 repeats are considered to be highly informative, *polymorphism information content* (PIC) values being used to determine the potential usefulness of markers for each locus (DeWoody *et al.*, 1995). Because of their co-dominant expression and multiallelism microsatellite markers exhibit the highest PIC values of all mark-

Table 3 - Allele and polymorphic information content (PIC) data for the four microsatellite loci in the three feline species investigated.

	Feline species					
	Herpailurus yagouaroundi		*Puma concolor*		*Panthera onca*	
Microsatellite loci	Number of alleles	PIC	Number of alleles	PIC	Number of alleles	PIC
Fca 08	9	0.73	10	0.75	12	0.72
Fca 45	8	0.82	5	0.41	3	0.48
Fca 77	9	0.83	9	0.63	9	0.71
Fca 96	17	0.92	13	0.86	12	0.45

ers (Ferreira and Grattapaglia, 1998), and it has also been pointed out that PIC values higher than 0.5 indicate high polymorphism and that markers with this, or higher, level of polymorphism are highly informative for genetic studies and extremely useful in distinguishing the polymorphism rate of a marker at a specific locus (DeWoody *et al.*, 1995).

We found that for *H. yagouaroundi* the highest PIC value was 0.92 for the *Fca 96* locus and that the lowest PIC value for this species was 0.73 for the *Fca 08* locus (Table 3) and that in this species the overall PIC for the four microsatellite loci was quite high, indicating that the all the microsatellites studied are good and representative markers able to genetically differentiate or characterize individuals of a *H. yagouaroundi* population. In *P. concolor* the *Fca 96* locus showed the highest polymorphism rate with a PIC value of 0.86 while the *Fca 08* locus had a PIC value of 0.75 (Table 3), both these loci being efficient and informative in this species. The *P. concolor Fca 45* locus had the lowest PIC value (0.41) of all the loci studied in the three species (Table 3). For *P. onca* the highest PIC values were 0.72 for the *Fca 08* locus and 0.71 for the *Fca 77* locus, while the PIC values of 0.48 for the *Fca 45* locus and 0.45 for the *Fca 96* locus indicated a low level of polymorphism for these loci (Table 3). Thus *Fca 08* and *Fca 77* could be used successfully in *P. onca* genetic diversity assessment studies.

Table 3 shows that the *Fca 08* microsatellite locus gave the most consistent PIC values in all three species studied and was in all cases equal to or higher than 0.72. For *H. yagouaroundi* the highest PIC values were those for the *Fca 45*, *Fca 77* and *Fca 96* loci, ranging from 0.82 to 0.92. However, for *P. concolor* and *P. onca* the *Fca 45* locus gave PIC values of less than 0.5 and the same was true for the *P. onca Fca 96* locus, these three loci being considered as having an average level of polymorphism in these species (Table 3).

In their study on deer, DeWoody *et al.* (1995) found PIC values between 0.63 and 0.86 for five out of seven microsatellites and regarded these as indicating a high level of polymorphism and the markers as being highly informative for genetic studies. Our results indicate that in the three feline species studied by us the microsatellite loci used presented a medium to high level of polymorphism and appear to be suited for genetic diversity studies in these species.

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