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Molecular Markers and Genetic Diversity in Neotropical Felids

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1. Introduction

The Class Mammalia has a taxonomic diversity including 5416 existing species or recently extinct and this number increases each year with the description of new species. This extraordinary group shows not only a wide diversity of species but also of forms, ecologies, physiologies, life histories and behaviour (Wilson & Reeder, 1993).

Felines are important representatives of the Class Mammalia and the 38 existing species of felids occur naturally in almost all areas of the world except in some insular regions as Australia, New Guinea and New Zealand, Japan, Madagascar, Oceania, Andes and some Caribbean islands (Nowak, 1999; Johnson et al., 2006). The Neotropical region (covers the southern part of North America, Central America and South America) is occupied by 10 recognized cat species that are divided into three clades evolutionarily distinct, which have been distinguished using a variety of molecular genetic techniques. The first clade, ocelote lineage [*Leopardus pardalis* (Linnaeus, 1758)] that comprises all the species of the genus *Leopardus.* The second clade, puma lineage [*Puma concolor* (Linnaeus, 1771)] that comprises the two species of the genus *Puma*. The third clade, *Panthera* lineage is represented in this region by jaguar [*Panthera onca* (Linnaeus, 1758)] (Johnson et al., 2006). There are eight of these species in Brazil: *Leopardus pardalis* (ocelot), *Leopardus wiedii* (margay), *Leopardus tigrinus* (oncilla), *Leopardus geoffroyi* (Geoffrey's cat), *Leopardus colocolo* (colocolo), *Puma yagouaroundi* (jaguarundi), *Puma concolor* (cougar) and *Panthera onca* (jaguar) (Oliveira, 1994). The *Oreailurus jacobita* (andean mountain cat) and *Lynchailurus colocolo* (pampas cat) are the other species of ocelot lineage that occur in other South American regions.

Mattern & McLennan (2000), studying felines phylogeny and speciation, came to a phylogenetic tree that confirms the formation of the main groups of the family Felidae (Figure 1).

The IUCN (International Union for Conservation of Nature) published in 2001 the scheme transcribed below (Figure 2) concerning the categories used to evaluate the conservation status of the animals or plants species and that is used to elaborate the "Red list of the Brazilian fauna threatened with extinction" (MMA, 2008).

Among the endangered species, there is a classification in three levels: *critically endangered (CR):* taxon runs extremely high risk of extinction in the wild in a immediate future;

endangered (EN): taxon is not critically in danger but runs high risk of extinction in the wild in a near future; and, *vulnerable (VU):* taxon does not fit in the categories critically endangered or endangered, but runs high risk of extinction in the wild in the medium term.

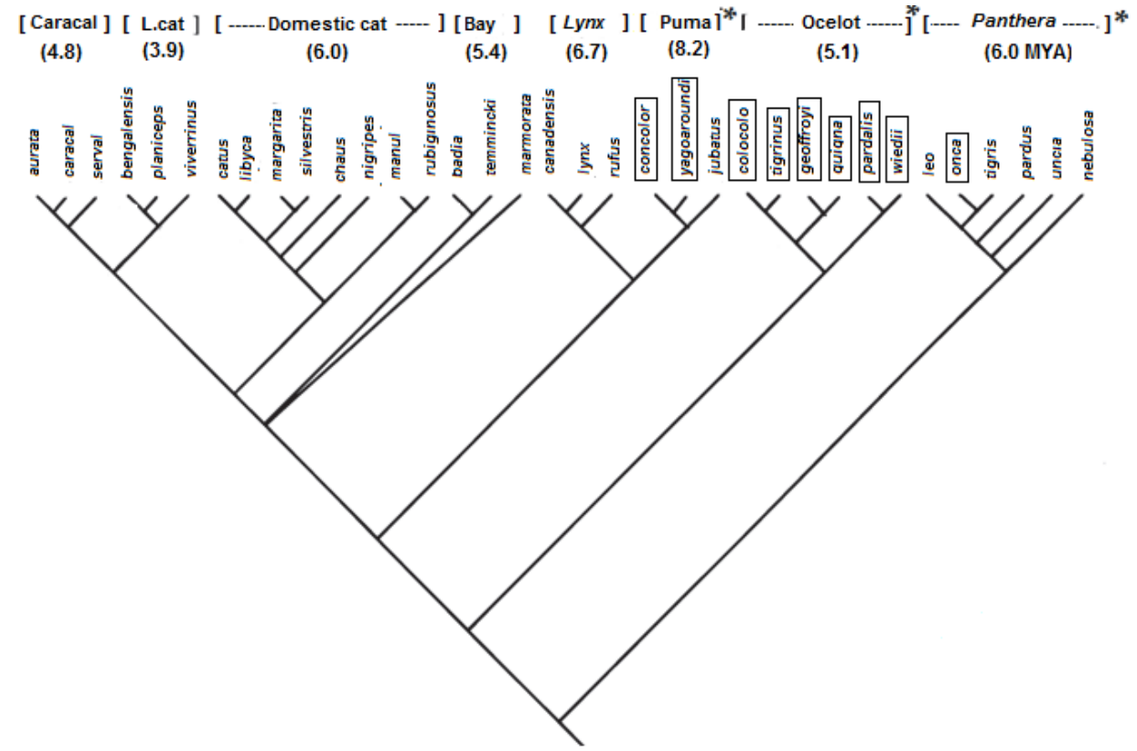

Fig. 1. Plylogenetic tree for Felidae based upon 1504 characters, ages of each lineage (MYA, million years ago) based upon amount of sequence divergence are written across the top of the tree. Only species names are indicated on the tree and Neotropical cats were framed. Adapted from Mattern & McLennan (2000). * Indicate clades of felids.

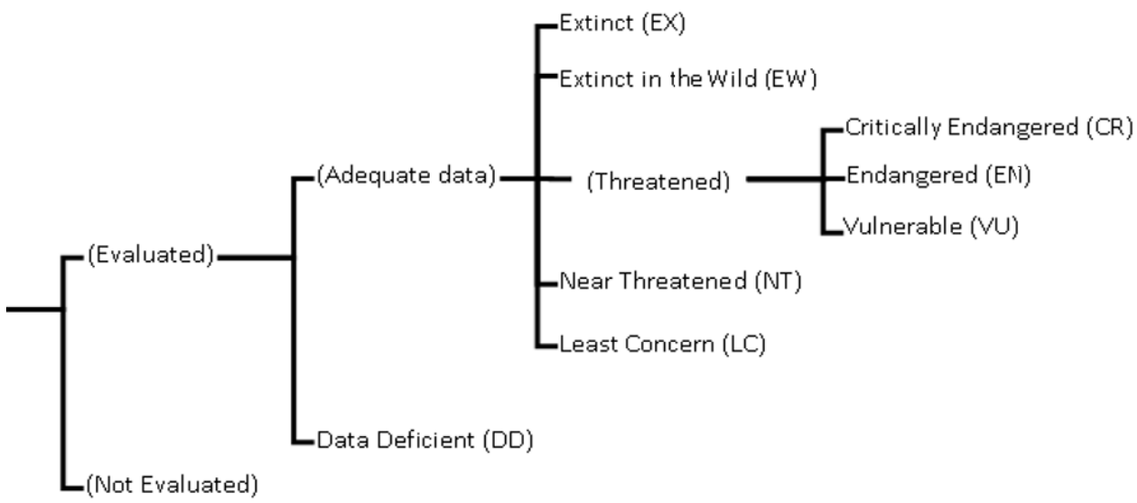

Fig. 2. Scheme for the classification of the species according to IUCN (2001).

All the neotropical felids are threatened with extinction on some level (IUCN, 2001), and this risk has increased mainly by anthropogenic changes that cause the reduction and fragmentation of the natural habitat of these animals, hunt and pollution, which significantly contribute to the decline and isolation of wild populations (Crawshaw Jr., 1997; Frankham et al., 2002; Schipper et al., 2008).

According to Frankham et al. (2008): "biodiversity is the variety of ecosystems, species, populations within species, as well as the genetic biodiversity existing within and between populations". One of the very promising research areas on the conservation of species threatened with extinction is the identification of "Hotspots" or areas with exceptional concentrations of endemic species, but with greatly reduced habitat. Among the 25 "Hotsposts" already indentified worldwide, the cerrado and the Atlantic rainforest of Brazil are included (Myers et al., 2000). The feline species that inhabit these areas where the deforestation is intense are particularly vulnerable. In the Brazilian rainforests, the ocelot (*Leopardus pardalis*), oncilla (*Leopardus tigrinus*), and margay (*Leopardus wiedii*), are among the felids that struggle to survive against the habitat destruction (Moreira et al., 2001).

The loss of biodiversity often culminates in the local extinction of species, reduction in the distribution and density of species, increasing the extinction risks. Therefore, the Conservation Biology has become an important science due to its multidisciplinary character, since it encompasses several other disciplines such as ecology and biology of populations, which allows accumulating data and information for the conservation and management of natural resources (Primack & Rodrigues, 2001; De Salle & Amato, 2004). Later and more recently the Conservation Genetics arose as an important area within the perspective of elaborating adequate management and conservation plans (Johnson et al., 2001; Perez-Sweeney et al., 2003).

The Conservation Genetics involves the use of genetic technologies to minimize the risks of extinction of endangered species. Among the topics studied in this area we can cite: (1) minimization of inbreeding and loss of genetic diversity; (2) identification of species or populations in risk due to reduced genetic diversity; (3) resolution of the structure of fragmented populations; (4) resolution of taxonomic uncertainties; (5) definition of management unities within the limits of the species; (6) detection of hybridization; (7) non-invasive sampling for genetic analyses; (8) definition of locations and choice of the best populations for reintroduction; (9) forensic analysis; (10) understanding the biology of the species (Frankham et al., 2002).

One of the main focuses of conservation biology is the maintenance of the genetic diversity, so that IUCN recognizes the need to maintain this variation as one of the three global conservation priorities. Two aspects should be considered: first, due to the continuous process of environmental changes the populations evolve and adapt to these changes if there is the necessary genetic variability to their adaptation to such alterations. Second, in general, there is an association between loss of genetic diversity, inbreeding and general decrease in reproductive and survival indexes (Frankham et al., 2002).

In wildlife populations subjected to reduction of the habitat area, as well as the number of reproductively active individuals, there may be loss of genetic variability. It is manifested both by the effect of genetic drift as a result of consanguinity (Wanjtal et al., 1995). Some authors estimate that an effective population size to avoid inbreeding depression is 50 and

500 individuals if it is wanted to avoid the loss of genetic variability due to genetic drift. However, caution is needed when determining the population sizes for conservation purposes, since once the genetic variability is lost, it will be only recovered very slowly (by mutation or migration), so that, even taking measures to increase the population size of a species, it may continue an endangered species (Solé-Cava, 2001).

Given that the genetic diversity provides the adaptive/evolutionary potential of a species, its maintenance is one of the main focuses of conservation biology. Thus, it is fundamental the knowledge of the genetic composition of a species and how it is organized (structured) in their populations, so that the management can be done, when necessary, and for the conservation of the species. It is also important to understand if the genetic structure found is a natural characteristic of the species studied or if it is the result of the presence of physical barriers caused by man, as in the case of habitat fragmentation (Galetti Jr. et al., 2008).

With the advent, in recent times, of several technologies of Genetics and Molecular Biology, especially the recombinant DNA technology, polymerase chain reaction (PCR) and sequencing, numerous possibilities of genetic markers arose allowing the detection of genetic polymorphism directly in the DNA. Such methodologies have allowed numerous genetic studies, whether they are targeted or not to conservation, such as: evolution studies, inter- and intra-specific genetic diversity, genetic origin (phylogeny), etc (Faleiro, 2007).

2. Molecular markers

Genetic variability allows us to compare individuals, populations or different species. A relevant aspect from the conservation genetics is the fact that different molecular biomarkers can have different rates of substitution/evolution, so that by the judicious choice of these markers, we can study from problems of identification of individuals to identification of cryptic species or formulating phylogenetic hypothesis in supraspecific groups (Solé-Cava, 2001).

The main technologies available for obtaining molecular genetic markers can be isoenzymatic markers or based on DNA fragments. DNA markers can be classified into two groups, according to the methodology used: DNA hybridization or amplification. The markers related to the hybridization methodology are the RFLP markers (Restriction Fragment Length Polymorphism) and the Minisatellites or VNTR loci (Variable Number of Tandem Repeats). The markers revealed by DNA amplification are those of RAPD (Random Amplified Polymorphism DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Short Sequence Repeat) or Microsatellite, CAPS (Cleaved Amplified Polymorphic Sequence) or PCR-RFLP, SNP (Single Nucleotide Polymorphism) and others. Some characteristics of the main methodologies for the markers analyses are described in Table 1.

The choice of the methodology to be used in the approach of each problem depends on several criteria. Among the criteria, firstly, the adequacy of the variability degree of the molecular marker chosen at the divergence level that one wishes to study. Markers that evolve rapidly are useful for the study of individuals, families and populations, while markers that evolve more slowly are better used in the study of species or supraspecific taxa.

Another important criterion in the choice is the type of material available for the studies. Researches based on izoenzymatic markers, for example, need higher quantities of biological material, which must necessarily be fresh or frozen (the enzymes denature or are digested by proteases when kept at room temperature). This problem does not exist for most of the methods with DNA thanks to the advent of PCR, since it allows the use of very small amounts of tissue including those preserved in alcohol or dehydrated. On the other side, in population studies in which a very high number of individuals should be analyzed, techniques such as the DNA sequencing are very expensive.

Variable	RFLP	PCR-RFLP	RAPD	Micro satellite	SNP	AFLP
Quantity of DNA	10ug	50ng	50ng	50ng	50ng	500ng
Quality of DNA	Excellent	Reasonable	Reasonable	Reasonable	Good	Good
Based on PCR	No	Yes	Yes	Yes	Yes	Yes
Radioactivity	Yes	No	No	Yes/No	No	Yes/No
Multiplex	No	No	No	Yes	Yes	No
Ease of Use	No	Yes	Yes	Yes	Yes	No
Automation	No	Yes	Yes	Yes	Yes- high	Yes
Reproducibility	Good	Good	Low	Good	Good	Medium
Number of alleles per locus	Biallelic	Biallelic	Biallelic	Multiallelic	Biallelic	Biallelic
Gene expression	Codominant	Codominant	Dominant	Codominant	Codominant	Dominant
Abundance throughout the genome	High	High	Low	Medium	High	Medium
Cost by data	High	Low	Low	Low	Very low	Low

Table 1. Comparative analysis of the characteristics and methodology of main molecular markers.

The choice of molecular marker to be used depends, therefore, on several factors. The fundamental is that the problem to be studied is well defined, that there is an adequacy in the polymorphism degree of the marker chosen to the type of evolutionary divergence to be studied, that the assumptions of the data analyses are clearly explicit (Solé-Cava, 2001).

2.1 Nuclear DNA and microsatellite marker

DNA analysis shows us genetic variability and allows the refinement of the genealogies of wild and captive populations (Mace et al., 1996). Microsatellites, due to their abundance in the genome and their high mutation rates, are molecular markers used to detect levels of genetic variability and to plan future breeding strategies. Although several types of molecular markers from the nuclear genome are available, short, tandem, repetitive regions within the nuclear genome known as microsatellites currently are the most popular molecular marker for ecological studies (Avise, 2004). Microsatellites consist of small DNA fragments of about 10-100 base pairs (bp) that contain repetitive elements displaying tandem repeats of 1-6 bp (Figure 3), variation in the number of repeats resulting in these loci having a high polymorphism information content (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 investigated so far (Weber & May, 1989). Microsatellites are codominant markers (*i.e.* heterozygotes can be distinguished from the homozygotes) which can be amplified by PCR and generally show a level of heterozygosity in excess of 0.7 (Ferreira & Grattapaglia, 1998).

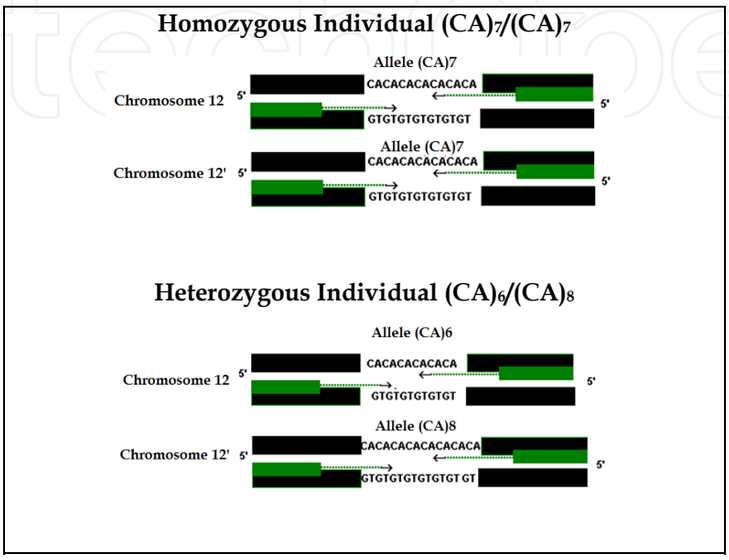

Fig. 3. Representation of the homozygous genotypes (identical alleles) and heterozygous (different alleles) for a genomic region that comprises a microsatellite of (CA)/ (GT) (adapted from Ferreira & Grattapaglia, 1998).

An important characteristic of microsatellites is that primers developed for a specific species can be used for other species of related taxons. Ten highly polymorphic microsatellite loci of dinucleotide repetitions (dC.dA)n/(dG.dT)n developed from the domestic cat genome showed amplified products of the same size in lions, cheetahs, pumas, leopard cat, and Geoffroy's cat (Menotti-Raymond & O'Brien, 1995). A broad range of heterozygosity was observed among the species for a single locus and among loci within a single feline species. This characteristic demonstrates that a microsatellite is an important informative marker (Menotti-Raymond & O'Brien, 1995).

Identifying factors that decrease the potential for inter-population gene flow is of singular importance in the conservation of felids populations. Drift and inbreeding can combine in small populations to reduce fitness in the short term (Keller & Waller, 2002) and theoretically reduce evolutionary potential in the long term (Lacy, 1997). Epps et al. (2005) used a combination of microsatellites and mitochondrial markers to infer greatly reduced gene flow between desert bighorn populations bisected by human-constructed barriers (e.g., major highways, urban development, etc.). While genetic analyses using molecular markers can inform ongoing conservation efforts for established populations felines, such analyses may also contribute to efforts targeted at re-populating vacant feline habitat through reintroductions.

Investigating natural history traits - Some aspects of natural history are difficult or impossible to investigate without using molecular markers. These investigations of wild feline natural history demonstrate the utility of microsatellites: useful not only in population analyses, but also in analyses focusing in scale down to the individual.

2.2 Mitochondrial DNA

A small molecule of circular DNA is present in the mitochondria – the mitochondrial DNA (mtDNA). This material is very abundant in cells in general, since there are many mitochondria per cells; it is inherited maternally in most species and is easily isolated. The restriction enzymes (RFLP), SSCP and sequencing are some of the methods that can be employed in the investigation of genetic diversity in the mtDNA, and, moreover, the availability of several primers for different loci of this DNA allows the amplification of these DNA fragments by PCR and subsequent sequencing of the products (Frankham et al., 2002).

Two interesting features of the mtDNA have led to the constant use of this molecule in phylogenetic and phylogeographic analyses. One is the absence of recombination in this molecule and the other is the fact that the mtDNA has a faster evolutionary rate in relation to the nuclear sequences (there are differences in relation to the molecular evolutionary rates even within the same mtDNA sequence). Based on the purpose of the research, one or another region of the molecule of the mitochondria DNA can be used. In general, the control region of the mtDNA (D-loop region, contains the location of replication origin) is the most employed in population studies, while the NADH genes, ribosomal and cytochrome genes tend to be used in matters related to the species as a whole and its distribution and in the intergeneric systematic (Perez-Sweeney et al., 2003).

Worldwide, researchers involved with the Conservation Genetics area have used mitochondrial markers for different types of analyses in several groups of animals, including the several species of felids. In the case of the representatives of the family Felidae, the phylogenetic and phylogeographic analyses are included in many researches.

3. Applications of molecular markers in the species conservation

Molecular methods have opened the entire biological world to the genetic analysis, as well as the complete spectrum of ecological and evolutionary scales in the genetic differentiation. In a final analysis, biodiversity is genetic diversity, and the magnitudes and patterns of this diversity can now be examined in any organisms (Avise, 1995). With the fast advance of techniques of molecular genetics in the last years, it was noticed that many of them could considerably contribute to the management of endangered species and for the conservation of biodiversity.

There are, essentially, two ways to use genetic markers in the conservation area. The first is based on the assumption that the genetic variability is determinant in the suitability and feasibility of a population. Thus, genetic markers are used to indicate the variability levels and their distribution in populations of a certain species (genetic structure). The second is the measurement of processes, such as migration, which produce measurable effects in the patterns of genetic variability (Neigel, 1996).

Among the molecular markers employed in population studies, evolutionary and/or studies targeted for conservation, stand out: the traditional protein electrophoresis, which

initiated great part of the genetic studies in natural population; studies with RFLP and the sequencing of mitochondrial DNA or nuclear genes; DNA fingerprinting using probes for minisatellite regions; RAPD; and polymorphisms in microsatellite regions. Currently, great emphasis is given to studies of microsatellites and mtDNA as markers of genetic diversity (mainly in animal populations), and the latter is extremely useful for investigations of phylogenetic relationships between different taxa and identification of geographic subdivision between population unities (Eizirik, 1996). Microsatellites are markers widely used in genetic studies. The potential use of these markers in small populations, especially in endangered species, is enormous, since these sequences are highly informative and the material required for its analysis can be collected by non-invasive methods (Bruford et al., 1996).

4. Molecular markers in felines

The use of these markers in endangered species is widespread, since these sequences are highly informative. For these studies blood samples, faeces and even hair are used, enabling accurate results from non-invasive methods. Using microsatellites it is possible to evaluate the structure within and between populations, besides the possibility to determine parentage. This fact is crucial for suggesting adequate conservation and management strategies (O'Brien, 1996).

As the genetic progress in recognizing the formulation of conservation strategies is evident that the current information on the genetic structure of natural populations are still insufficient, therefore, becomes necessary to perform reproductive management of the species that are increasingly represented by small populations in forest reserves. It also becomes clear that the initiative to revert the decline of biodiversity should be involved with several areas of knowledge, including molecular genetics. It should be kept in mind that the molecular approach in the study of endangered species is to provide an additional tool that can help to avoid extinction. Based on multidisciplinary approaches, conservation plans have been drawn up with a better knowledge about the real threats to the survival of the species (O'Brien, 1996).

With the increasing destruction of wildlife, captive populations have become an important strategy for the conservation of endangered species. The general discussion on this aspect is how the captive populations and their maintenance programs can be integrated with wild populations and how these programs can maximize the preservation of biodiversity (Foose, 1983).

During the last years, a wide range of studies have been carried out to estimate the genetic variability in feline populations, including the several species represented in this group. O'Brien et al. (1987), using the isoenzymes electrophoresis, analyzed four populations of African lions and Asian lions. They found a moderate variability in the specimens from Africa, while no variations were found in the Asian lions. This fact led to the conclusion that possibly these populations suffered a decrease in the population size followed by inbreeding in the recent past.

To characterize the genetic variability in cheetahs (*Acinonyx jubatus*) studies using alloenzymes, soluble proteins, histocompatibility complex genes, mitochondrial DNA, minisatellites and microsatellites were carried out (O'Brien et al., 1983; Menotti – Raymond

& O'Brien, 1993). Regarding alloenzymes, the variation observed in cheetahs was low, while in mitochondrial DNA it was moderate and high for microsatellites and minisatellites. Perhaps this higher variability in these markers could be explained by the high mutation rate in these sequences. As apparent consequences of this genetic uniformity in cheetahs in relation to isoenzymatic markers, are: great difficulty in captive breeding, high degree of infant mortality in captivity and nature, and high frequency of abnormal spermatozoids in ejaculation.

O'Brien et al. (1990) carried out studies with populations of Florida panther (*Puma concolor coryi*), a highly endangered animal. They reported the existence of two genetically 12 distinct groups using techniques of isoenzymes electrophoresis, 11 mitochondrial DNA and nuclear markers: a group descendant of ancestors of *P. c. coryi*, phylogenetically close to other subspecies of North America, and another group, resembling the cougars of Central America or South America and that must have been introduced in Florida in the recent past. The analysis concluded that, despite the genetic differences presented by the two subpopulations of Florida, they have in their historical separation, a common ancestor sufficiently recent to eliminate the development of mechanisms of reproductive isolation, i.e., to allow the crossbreeding between individuals of two populations. Moreover, according to O' Brien et al. (1990), the formation of hybrids could even enhance the survival chances of Florida panther, which has strongly suffered the consequences of inbreeding.

Menotti-Raymond & O'Brien (1995) isolated, characterized and amplified 10 highly polymorphic loci of microsatellites of dinucleotide repeats (dC.dA)n/(dG.dT)n of the domestic cat (*Felis catus*). They demonstrated that 10 pairs of oligonucleotide primers developed from the domestic cat genome amplified products of similar size in lions, cheetahs, Asian leopard cat and Geoffroy's cat. This fact suggested that the flanking sequences of these microsatellites are conserved in felines. Individual loci showed large heterozygosity in the species of felines studied, thus showing themselves as informative markers. It was observed a mean rate of heterozygosity of 0.77 in *Felis catus*, 0.39 in cheetah, 0.61 in puma and 0.66 in lion, from the 10 oligonucleotide primers analyzed. The polymorphism of the microsatellites loci of felines offers much potential as molecular marker for: i) identification of species or subspecies, ii) accurate measure of the genetic divergence of the population and iii) evaluation of the origin in native populations.

In order to examine the extension of inbreeding in populations of Asian lions and Indian tigers, to identify pure Asian lion from the hybrid and pure Indian tiger from the hybrid and identify lions and tigers with extensive genetic variation for selective inbreeding for their own management, Shankaranarayanan et al. (1997) developed a study using RAPD markers and microsatellites. The RAPD patterns were evaluated in lions and tigers using 30 random primers, of which four produced polymorphic pattern and were used for population studies. It was analyzed a total of 38 individuals and a mean heterozygosity of 25.82% was observed, varying from 16.71% to 34.39% in the individual analysis of the primers. The primers that presented polymorphism in lions did not show any polymorphism in tigers. The analysis of microsatellites was done by 5 loci of CA repetition that are polymorphic in felines. The Asian lions, however, did not show variation for all the 5 loci of microsatellites studied; only 2 of them showed differences between Asian lions and hybrid lions. Analyzing the microsatellites in 30 Indian tigers, Shankaranarayanan et al. (1997) observed mean

heterozygosity of 22.65% in three of the five loci tested. The exam of microsatellites in 15 skin samples of museum tigers, aged from 50 to 125 years, showed a mean heterozygosity of 21.01% and, therefore, the difference between present and past populations were not statistically significant. Only the loci that were polymorphic in the actual population showed polymorphism in the individuals of 50 – 125 years ago. These analyses also served to identify the presence of hybrid tigers in the population studied. Moreover, results like this can be used for breeding programs to increase the genetic variability in the population.

Menotti-Raymond et al. (1999) developed a genetic linkage map in felines using 253 microsatellite loci. It was identified and genotyped 235 loci of dinucleotide repetition (dC · dA)n · (dG · dT)n and 18 tetranucleotide, in two families, with 108 members resulting from crossbreeding of lineages of domestic cat (*Felis catus*) and leopard cat (*Prionailurus bengalensis*). Two hundred and twenty-nine loci were linked, identifying 34 linkage groups, and from the 19 pairs of chromosomes, 16 were mapped. According to the authors, the genome measures approximately 2900 cM, and they estimate that the genetic length of the map in 3300 cM.

A study carried out by Eizirik et al. (2001) aimed to investigate the genetic diversity, population structure and demographic history of jaguars throughout their geographic distribution. It was analyzed 715 pb of the control region of mitochondrial DNA and 29 microsatellite loci in approximately 40 individuals sampled from Mexico to southern Brazil. The results showed low to moderate diversity levels for the mtDNA and medium to high for microsatellites and also showed a recent demographic expansion. No well-defined geographic structure was observed but some geographic barriers, such as the Amazon River and the Darien gap between northern South America and Central America, seem to have limited the historical genetic flow in these species, producing measurable genetic diversity. The representatives of *Panthera onca* studied could be divided into four incomplete isolated phylogeographic groups.

The molecular markers have also helped in taxonomic studies in several animal species, including felines. The incorporation of accurate definitions for taxonomic unities in the legislation of wildlife has required a re-evaluation of the taxonomy of threatened or endangered species. Miththapala et al. (1996) carried out a study with 60 leopards (*Panthera pardus*) representing 12 recognized subspecies. Three molecular methods were used: alloenzymes, mtDNA restriction sites and minisatellites feline-specific that showed a considerable genetic variability in the individuals sampled. The mainland populations and subspecies of Africa and Asia presented the highest amount of molecular genetic variation, while in insular populations relatively low amounts of diversity were present. The phylogenetic analysis of molecular data showed the phylogenetic distinction of six groups of leopards geographically isolated: African, central Asian, Hindu, Sri Lankan, Javan and East Asia. Based on the combined molecular analyses and favored by morphological data, the authors suggest a taxonomic revision for eight subspecies of leopards.

Gugolz et al. (2008) used samples of animals preserved in museums of the Alps lynx and other existing populations of representatives of *Lynx lynx* populations in Europe and Asia, in order to evaluate the phylogenetic position of the Alps lynx. The phylogenetic analysis using a region of 345pb of the cytochrome b gene placed the Alps lynx within the lineage of lynxes from Eurasia, while the analysis of a fragment of 300pb of the control regions showed

seven different haplotypes but no exclusive haplotype of the Alps lynx. The haplotypes of the extinct population were identical to those previously described in the Scandinavian lynx signifying a recent genetic ancestor with the current European populations.

Patterns of molecular genetic diversity of the largest remaining free-ranging cheetah population were described in a survey of 313 individuals throughout Namibia using 19 polymorphic microsatellite loci. There was limited differentiation among regions, evidence that this is a generally panmictic population. Measures of genetic variation were similar among all regions and were comparable with Eastern African cheetah populations. The long-term maintenance of current patterns of genetic variation in Namibia depends on retaining habitat characteristics that promote natural dispersal and gene flow of cheetahs (Marker et al., 2008).

Driscoll et al. (2009), using DNA samples extracted from specimens held in museums, carried out a study with mtDNA analysis aiming to investigate and interpret the phylogeographic natural history of the Caspian tiger (*Panthera tigris virgata*) in the context of contemporary subspecies of tigers, the Amur tigers (*P. t. altaica*). The data obtained with the mtDNA fragments showed that the Caspian tigers have a large haplotype differing in only one nucleotide of the monomorphic haplotype found in Amur tigers, suggesting, by the phylogeographic analysis, that both species of tigers colonized Central Asia less than 10, 000 years ago. The authors also suggest that, based on their findings, the original habitat of the Caspian tiger in Central Asia is open to reintroductions of genetic stocks of the Amur Tiger, since there is a evolutionary proximity between the two subspecies.

Colocolo *(Leopardus colocolo*) and the Andean mountain cat (*Leopardus jacobita*) are being highly studied in comparison to the South American felids (Redford & Eisenberg, 1992; Nowell & Jackson, 1996). One of these studies included 33 samples of *L.jacobita* and 75 of *L.colocolo* collected in northern Chile and one of the objectives was the determination of general patterns of genetic variation in the two species. Mitochondrial genes were used and, in general, a relatively low genetic variation (2 haplotypes in the mtDNA) was shown for the Andean mountain cat when compared to colocolo (17 haplotypes), which suggests a distinct evolutionary history (Napolitano et al., 2008).

Another example of application of mtDNA is the identification of individuals, either as forensic evidence or in the classification as a certain species. Grahn et al (2011) analyzed a fragment of 402pb of the mtDNA control region for identifying mitotypes of domestic cats (*Felis catus*) of United States and other geographical regions. The total sampling was of 1394 animals (174 previously studied) and the analyses showed that this region has suitable discriminatory power for use in wildlife forensics.

Puma concolor is one of the felines that suffer most from habitat reduction and forest fragmentation and its population is often reduced to few individuals in various regions. A study in the northeastern São Paulo State – Brazil, in two protected areas, used samples of faeces and analysis of a portion of the mitochondrial gene (cytochrome b) to determine the presence of pumas and estimate their minimal population (Miotto et al, 2007). The results indicated that the mtDNA was able to differentiate the faeces of pumas from other felines present in the region, such as ocelot (*Leopardus pardalis*), amplifying in 60% of the samples collected.

Several other species of felines have been recently studied based on mitochondrial genes as markers to assess different aspects in these animals, besides those already mentioned (e.g.,

population structure, evolutionary history, hybridization rates and introgression): *Panthera leo ssp* (Barnett et al., 2009), *Acinonyx jubatus* (Charrau et al., 2011), *Prionailurus bengalensis* and *Felis chaus* (Mukherjee et al., 2010), *Felis silvestris* (Hertwig et al., 2009; Eckert et al., 2010), *Neofelis diardi* (Wilting et al., 2011), among others.

Trigo et al., (2008) employed mitochondrial DNA (mtDNA) sequences and nine microsatellite loci to identify and characterize a hybrid zone between two Neotropical felids, *Leopardus geoffroyi* and *L. tigrinus*, both of which are well-established species having diverged from each other c. 1 million years ago. They observed that these two felids are mostly allopatric throughout their ranges in South America and present strong evidence for the occurrence of hybridization between these species. Mondol et al. (2009) studying leopards observed 25 of the 29 tested cross-specific microsatellite markers showed positive amplification in 37 wild-caught leopards, these results demonstrated that the selected panel of eight microsatellite loci can conclusively identify leopards from various kinds of biological samples.

There are also less detailed studies, but also of equal importance since they serve as basis for more refined researches in the future. One of them is the study with Amur tigers (*Panthera tigris altaica*) carried out by Russello et al. (2004), who found by the mitochondrial DNA analysis that this population has low rates of genetic diversity, even lower than of the populations in captivity. This study served as a warning to the importance of the integrate management of *in situ* and *ex situ* populations for the conservation of this species. Another study recently performed with two populations of ocelots (*Leopardus pardalis*) in the USA also found low genetic variability and a small and insufficient effective population size for the long-term viability of these populations (Janecka et al., 2008). In Brazil this type of study is still scarce. Most feline genetics researches that are performed in the country are focused on phylogenetic and evolutionary issues. Literature only contains few studies that focus the genetic variability of local populations, of which two of them are with captive animal (Grisolia, et al., 2007; Moreno et al., 2006) and one with two free-living populations of jaguars (Eizirik et al., 2008). This is a worrying situation since the knowledge of the genetic diversity level is one of the main factors to assess the viability of populations, besides bringing crucial information for future decision-making in conservation and management plans.

5. Conclusion

The potential for future contributions of molecular genetics to wild felines conservation and management will only increase as molecular methods become more accessible, cost effective, and practical. Researches about genetic diversity and geographic structure of neotropical felids are basis for further investigations at regional and local levels, including studies of population structure, relatedness between individuals, dispersal patterns, adaptation to different ecosystems, and other ecological and evolutionary aspects that can be addressed using molecular markers. Likewise, we hope that research in this area will contribute to enable the development of efficient strategies for the conservation of these species, their genetic diversity and maintaining ecological and natural processes that influence the continuity of its evolution.

Molecular analysis of genetic structure and integration of ecology, natural history data and feline reproduction could provide a greater comprehension of the factors to be considered in efficacious management plans for these endangered species groups.

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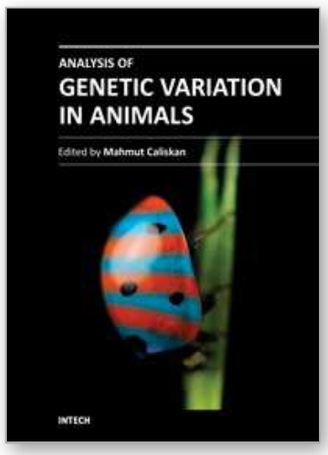

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Analysis of Genetic Variation in Animals includes chapters revealing the magnitude of genetic variation existing in animal populations. The genetic diversity between and within populations displayed by molecular markers receive extensive interest due to the usefulness of this information in breeding and conservation programs. In this concept molecular markers give valuable information. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in animals and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation in animals by presenting the thoughts of scientists who are engaged in the generation of new idea and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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