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Research Article

Genetic structure and conservation of Mountain Lions in the South-Brazilian Atlantic Rain Forest

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Abstract

The Brazilian Atlantic Rain Forest, one of the most endangered ecosystems worldwide, is also among the most important hotspots as regards biodiversity. Through intensive logging, the initial area has been reduced to around 12% of its original size. In this study we investigated the genetic variability and structure of the mountain lion, *Puma concolor*. Using 18 microsatellite loci we analyzed evidence of allele dropout, null alleles and stuttering, calculated the number of allele/locus, PIC, observed and expected heterozygosity, linkage disequilibrium, Hardy-Weinberg equilibrium, F_{IS}, effective population size and genetic structure (MICROCHECKER, CERVUS, GENEPOP, FSTAT, ARLEQUIN, ONESAMP, LDNe, PCAGEN, GENECLASS software),we also determine whether there was evidence of a bottleneck (HYBRIDLAB, BOTTLENECK software) that might influence the future viability of the population in south Brazil. 106 alleles were identified, with the number of alleles/locus ranging from 2 to 11. Mean observed heterozygosity, mean number of alleles and polymorphism information content were 0.609, 5.89, and 0.6255, respectively. This population presented evidence of a recent bottleneck and loss of genetic variation. Persistent regional poaching constitutes an increasing in the extinction risk.

Key words: Araucaria Forest, Atlantic Rain Forest, conservation, genetic diversity, microsatellite.

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Introduction

The Brazilian Atlantic Rain Forest, one of the four most important biodiversity hotspots worldwide (Myers *et al.*, 2000), is also one of the most endangered ecosystems, through having undergone intense human exploitation and deforestation (Ribeiro *et al.*, 2009). The Araucaria Forest, one of the Atlantic Rain Forest physiognomies in south Brazil, has been heavily logged since the early 1900's. The whole biome is now legally protected (Federal Law 285/99, February, 2006) Even so, illegal exploitation still persists, and only 11.26% of the original area of the Atlantic Rain Forest itself, and 12.6% of the Araucaria Forest (Ribeiro *et al.*, 2009), still remain. Of this small remnant, only 0.39% of the latter lies within some kind of legally protected area (Ribeiro *et al.*, 2009).

Although almost 90% of the original habitat has disappeared in less than a hundred years, little is known on the effects of this fragmentation on animal populations. There are no population estimates prior to deforestation, and even today there are still none for most Brazilian mammals. The mountain lion (*Puma concolor*) is a top predator, whose very presence influences the ecosystem, and prey populations, hence the importance of its conservation (Henke and Bryant, 1999; Miller *et al.*, 2001; Terborgh *et al.*, 2001).

The mountain lion, the second largest Neotropical felid, occupies the largest geographical area in the Americas, this extending from Canada to southern Argentina. The subspecies that occurs in southern Brazil is *Puma concolor capricornensis* (Culver *et al.*, 2000). It is listed as of least concern (IUCN, 2008), and as vulnerable in the Brazilian National Red List (MMA and Fundação Biodiversitas, 2008). Little is known about local populations. Although having suffered severe habitat loss, there is no information regarding population sizes, and only scarce recent data on regional species (Mazzolli *et al.*, 2002; Mazzolli, 2010; Castilho *et al.*, 2011; and Marins-Sá, 2005, MSc Dissertation, Curso de Pós-Graduação em Ecologia UFRGS, Porto Alegre).

In addition to the severe habitat loss, the species is still illegally hunted because of livestock depredation (Mazzolli *et al.*, 2002; Marins-Sá, 2005, MSc Dissertation, Curso de Pós-Graduação em Ecologia UFRGS, Porto Alegre), which, in south Brazil, has been reported since the 1990's (Mazzolli *et al.*, 2002). Depletion very much de-

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creased when ranchers implemented management actions, such as corralling small animals (sheep and goats) at night (Mazzolli *et al.*, 2002).

Previous studies described the genetic variability, and structural and ecological characteristics of mountain lions in North America (Sinclair *et al.*, 2001; Ernest *et al.*, 2003; Anderson *et al.*, 2004; McRae *et al.*, 2005,) and South America (Culver *et al.*, 2000; Moreno *et al.*, 2006; Miotto *et al.*, 2007; Ruiz-Garcia *et al.*, 2009). However, specific information regarding genetic variability status related to recent specific processes, especially in such an important ecosystem as the Brazilian, is still lacking.

Knowledge of possible recent bottlenecks is extremely important for present-day management strategies. Identification of their very existence, the subsequent loss in genetic diversity, and the prevailing genetic structure, is important for evaluating the extinction risk of a population (Montgomery *et al.*, 2000; Reed and Frankham, 2003; Frankham *et al.*, 2005; O'Grady *et al.*, 2008).

Thus, the aim of this study was to determine whether there was a bottleneck that could possibly affect future population viability, besides assessing genetic structure, inbreeding and causes of mortality in regional mountain lions. The results would contribute to the conservation and management of both this endangered species and the ecosystem itself.

Material and Methods

Sample collection and laboratory procedures

We collected 63 samples of the mountain lion (*Puma concolor* Linnaeus, 1771) population in southern Brazil, 37 (58.7%) from the field and 26 from museum skins and skulls (41.3%). The samples came from the south Brazilian states of Rio Grande do Sul, Santa Catarina, and Paraná, from 1983 through 2007. Location, year and cause of death/capture were recorded. All the sample locations were recorded (coordinates registered in museum samples, and death/capture location in field samples using GPS) and mapped using TrackMaker (Figure 1). Genomic DNA was extracted using the CTAB protocol (Doyle and Doyle, 1987) for tissue samples, and phenol-chloroform (Sambrook *et al.*, 1989) for blood samples.

18 loci, four originally designated in *Felis catus* (Menotti-Raymond *et al.*, 1999) and 14 in *Puma concolor* (Kurushima *et al.*, 2006; Rodzen *et al.*, 2007), were amplified for subsequent analysis of genetic variability of the wild mountain lion population in south Brazil. Each microsatellite locus was individually amplified in PCR reactions, according to Castilho *et al.*, (2011). Allele sizes were defined by separating the amplification products on 6% polyacrylamide gels together with a 25 bp marker ladder. Intron DBY-7 (Luo *et al.*, 2007) was used for sex determination under the same PCR conditions as those for microsatellites. Samples were genotyped at least twice for validated allele scores.

Figure 1 - Study area in South Brazil, and sampling localities in detail. Dots represent sampling points.

Data analysis

Genetic polymorphism was estimated as the number of alleles per locus (A), observed heterozygosity (Ho), expected heterozygosity (H_E), polymorphic information content (PIC), and allelic frequencies, using the CERVUS 3.0.3 program (Marshall *et al.*, 1998). GENEPOP 3.3 (Raymond and Rousset, 1995) was used for testing linkage disequilibrium (LD) and ARLEQUIN 3.1 for checking genotypic distribution conformance to Hardy-Weinberg equilibrium (HWE).

Significance levels ($\alpha = 0.05$) were corrected with the Bonferroni approach for multiple simultaneous comparisons (Rice, 1989), in order to infer LD and departures from HWE. The probability of the presence of null alleles, allele dropout, and scoring errors due to stutter was tested using MicroChecker 2.2.3 (Van Oosterhout *et al.*, 2004). Allelic richness (A_R) and inbreeding coefficient (F_{IS}) were calculated with the FSTAT 2.9.3.2 program (Goudet, 2001).

In order to detect any genetic evidence of a population decline, the BOTTLENECK program (Cornuet and Luikart, 1996) was used with the infinite allele (I.A.M.), stepwise mutation (S.M.M.), and two-phase (T.P.M.) models, with 70% t and 95% S.M.M., 20% variance, and 1,000 iterations, as recommended by Piry *et al.* (1999). The Wilcoxon sign-rank test was applied to determine significance ($p \leq 0.05$). In order to assess whether the small sample size ($n = 37$) was masking the results, HYBRIDLAB 1.0 (Nielsen *et al.*, 2006) was used to simulate a population with 500 individuals, with $n = 37$ as a base population, and BOTTLENECK run with the same parameters described above.

The existence of population structure was inferred by principal component analysis (PCA) with PCAGEN software. GENECLASS 2 (Piry *et al.*, 2004) was carried out to infer the assignment or exclusion of individuals, assuming that all those sampled belonged to the same population. The ONESAMP1.1 (Tallmon *et al.*, 2008) and LDNe 1.31 (Waples, 2006) programs were used with genotypic data for estimating the effective population size (N_e).

The probability of loss in a generation of alleles with frequencies from 0.01 to 0.10 was calculated, using $Pr(A) = (1 - p)^{2N}$, where p is allele frequency (Frankham *et al.*, 2005), and considering N as a mean between that calculated by LDNe and ONESAMP. The loss of genetic variation in one generation was also calculated, using the equation $H_e = 1 - 1/(2N_e)^t$, where H_e is the level of heterozygosity, N_e the effective population size, and t the number of generations (Lowe *et al.*, 2004).

Results

DNA extraction was successful for 37 individuals, viz., 28 field samples (25 obtained from poachers, 1 road kill and 2 captured animals) and 9 from museums. DNA extraction was a failure in the case of field samples containing excess fat. Sixteen males and 21 females were successfully sexed using intron DBY-7 from the Y chromosome.

106 alleles were identified for the 18 microsatellite loci in the 37 samples (Table 1). The number of alleles/locus ranged from 2 (PcoB115) to 11 (PcoB203w), with a mean of 5.89. Mean observed heterozygosity (Ho) was 0.609 (ranging from 0.027 to 0.806), and mean expected heterozygosity (H_E) 0.6818 (ranging from 0.027 to 0.850) (Table 1). Polymorphism information content (PIC) for 16 of the 18 loci was higher than 0.5, with only Fca453 and Pco115 lower (0.463 and 0.026 respectively). The mean PIC for all the loci was 0.6255 (Table 1).

Tests showed no loci to be in linkage disequilibrium, although deviation from HWE ($p > 0.05$ after Bonferroni correction) occurred in three loci, Fca391, Fca424 and PcoB210w (Table 1). F_{IS}, calculated to test whether inbreeding was responsible for deviations from HWE, ranged from -0.30 to 0.42 (mean $F_{IS} = 0.10$), in the case of global F_{IS} and seven samples, significantly (Table 1). The F_{IS} value for sample Fca453 indicated heterozygote excess, and for the remainder, heterozygote deficit. CERVUS failed to find mother/ father – offspring pairs. For more details on parentage relations in this population see Castilho *et al.* (2011). MicroChecker results gave no evidence of allele dropout or scoring erros due to stuttering, although loci Fca391 and Fca424 presented a general excess of homozygotes for most allele-size classes, thereby implying the possible presence of null alleles.

Allelic frequencies varied from 0.013 to 0.986, with 17% at 0.01, and 34% and 46.2% lower than 0.05 and 0.10, respectively. One, at 0.98, was almost fixed in this population (locus Pco115). Seven alleles (6.6% of the total), apparent in samples dating from 1983 to 1995, were absent in the more recent.

Analysis with PCAGEN software ($p > 0.05$, data not shown) failed to detect a population genetic structure. The results from GENECLASS corroborated this, by indicating that all the individuals came from the same population source ($p > 0.05$).

Although evidence of a recent bottleneck in this population was found, when applying the Wilcoxon sign-rank test using the infinite allele model (I.A.M.) and two-phase model (T.P.M.), with 70% stepwise mutation model (S.M.M.), this was not so with either 95% S.M.M. ($p > 0.05$) or the stepwise mutation model (S.M.M.) for n = 37 (Table 2). However, when using n = 500 simulated genotypes, a recent bottleneck for I.A.M. and T.P.M. with 70% and 95% S.M.M. was noted (Table 2).

With ONESAMP1.1 (Tallmon *et al.*, 2008), it was estimated that the effective population size (N_e) was 23.5 (confidence limits 95% = 20.74-31.5), and with LDNe, 16.5. N_e as a mean between 23.5 and 16.5 was considered for further calculations.

The high percentage of alleles with allelic frequency of 0.01 (17%), or lower than 0.05 (32.1%) and 0.10 (46.2%), presume the risk of loss in future generations. The probability of future loss of alleles with frequencies from 0.01 to 0.10, considering N = 20.0 (mean between calculations by LDNe and ONESAMP) were 0.67 for alleles with a frequency of 0.01 (17%), and 0.44, 0.30, 0.20, 0.13, and 0.02 for alleles with allelic frequencies of 0.02 (3.8%), 0.03 (7.5%), 0.04 (5.7%), 0.05 (1.9%), and 0.10 (2.82%), respectively (Figure 2). It is possible that 6.6% of all the alleles have already been lost, as they appeared only in samples dating from 1983 through 1995, and not in more recent ones. Using the equation $H_e = 1\text{-}1/(2N_e)^t$, it was calculated that, in one generation, an effective population size of 20 individuals loses 0.025% of the variation present in the initial population.

The cause of death was recorded in the case of field samples (71.4% and 81.25% of all the females and males, respectively). The main cause was farmer retaliatory hunt-

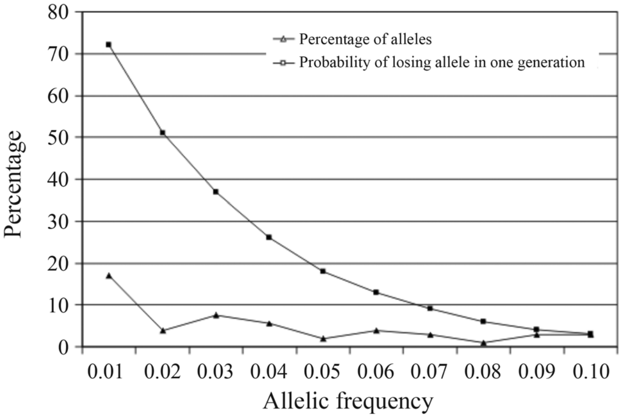

Figure 2 - Probability of allele loss in one generation and percentage of alleles with frequencies ranging from 0.01 to 0.10, calculated as $Pr(A) = (1\text{-}p)^{2N}$ where p is the allele frequency and N = 20.0.

Table 1 - Characterization of microsatellites for mountain lions in south Brazil for 18 microsatellite loci. Locus name, number of individuals genotyped (N), number of alleles (A), observed heterozygosity (Ho), expected heterozygosity (H_E), HWE *P*-values, polymorphic information content (PIC), allelic richness (A_R), and inbreeding coefficient (F_{IS}). Bold values: $p \leq 0.05$ after Bonferroni correction.

Locus	N	A	Ho	H_E	*p*-values	PIC	A_R	Fis
Fca391	34	7	0.529	0.739	**0.00018**	0.682	6.648	**0.287**
Fca424	32	5	0.406	0.698	**0.00175**	0.626	4.780	**0.422**
Fca441	37	5	0.676	0.766	0.32040	0.712	4.914	0.119
Fca453	31	5	0.742	0.570	0.18719	0.463	4.654	**-0.309**
PcoD8	26	5	0.769	0.747	0.35067	0.696	5.000	-0.030
PcoB105	36	4	0.778	0.725	0.11840	0.666	4.000	-0.074
PcoD329	35	6	0.571	0.742	0.08537	0.683	5.725	0.233[1]
PcoB203w	32	11	0.750	0.850	0.33006	0.819	10.399	0.120
PcoB210w	31	7	0.806	0.840	**0.00247**	0.803	6.996	0.040
PcoD103w	35	5	0.543	0.677	0.46885	0.608	4.740	0.201
PcoD301	33	7	0.667	0.719	0.00625	0.666	6.574	0.074
PcoA2	31	8	0.677	0.667	0.54950	0.630	7.808	-0.015
PcoD217w	32	6	0.594	0.758	0.09523	0.706	5.812	**0.220**
PcoD303	29	6	0.586	0.753	0.06781	0.698	5.982	**0.225**
PcoD310	33	6	0.515	0.752	0.01904	0.694	5.745	**0.318**
PcoB323	34	6	0.706	0.655	0.39336	0.578	5.477	-0.079
PcoD323	32	5	0.625	0.587	0.28156	0.503	4.779	-0.066
PcoB115	37	2	0.027	0.027	1.00000	0.026	1.703	0.000
Mean	-	5.89	0.609	0.6818	-	0.6255	5.652	**0.108**

Table 2 - *P*-values for heterozygosity (H) deficiency and/or excess from Wilcoxon sign-rank test, using the infinite allele model (I.A.M.), stepwise mutation model (S.M.M.), and two-phase model (T.P.M.) with 70% and 95% stepwise mutation models and 20% variance. Results for n = 37 (field samples) and n = 500 (simulated genotypes). Values in bold face are $p \leq 0.05$.

	n = 37				n = 500			
	I.A.M	T.P.M		S.M.M.	I.A.M	T.P.M.		S.M.M.
		70%	95%			70%	95%	
H deficiency	0.99979	0.95512	0.51694	0.18461	1.0	0.99998	0.99203	0.75246
H excess	**0.00026**	**0.04937**	0.50000	0.82673	**0.0**	**0.00003**	**0.00912**	0.26131
H excess and deficiency	**0.00052**	0.09874	1.00000	0.36922	**0.00001**	**0.00005**	**0.01823**	0.52261

ing, due to livestock depredation (74% of all deaths, and 92.8% and 53.8% of female and male deaths respectively). Human action was instrumental for 92.86% of all deaths/captures (100% and 84.6% of females and males, respectively). Road kills were responsible for 7.2% and 7.7% of female and male deaths, respectively. Only males were captured (23.2%), or killed by disease (15.4%).

Discussion

The absence of genetic structure in the study area corroborates data obtained by Castilho *et al.* (2011) for this population, in that the surroundings are still permeable for mountain lions, possibly arising from the long distances that this species is capable of traveling (Sweanor *et al.*, 2000; Logan and Sweanor, 2001), even though through discontinuous habitats (Logan and Sweanor, 2001; Castilho *et al.*, 2011). Ruiz-Garcia *et al.* (2009) found genetic similarity among individuals from the Bolivian Andes, and samples from Colombia, Peru, Ecuador, Venezuela, and the west Brazilian Amazon.

Severe habitat loss is one of the major causes of genetic loss and extinction risk in animals in general, and carnivore populations in particular (Nowell and Jackson, 1996). Bottlenecks caused by habitat loss have been recorded for several species (Hoelzel, 1999; Kuo and Janzen, 2004; Culver *et al.*, 2008), these always indicating the need for attention to the population that has undergone a reduction in size, because of the increased extinction risk of both the population or species (Montgomery *et al.*, 2000; Reed and Frankham, 2003; Frankham *et al.*, 2005; O'Grady *et al.*, 2008).

Evidently there has been a recent bottleneck in the south Brazilian mountain lion population. The excess of heterozygosity observed when a population has suffered a recent bottleneck can be detected during 0.25 to 2.5 x 2 N_e generations (41 to 412 years for *P. concolor*), after which allelic frequencies again regain equilibrium. The bottleneck that was detected in the present study possibly started when intense deforestation occurred in the Araucaria Forest in south Brazil. From the early 1900's, this has brought about the loss of almost 90% of the original vegetation cover. Concomitantly, in addition to the extensive loss of habitat, many loggers hunted mountain lions and their prey species for food or protection. Although illegal, the hunting of mountain lions and prey species still persists (Mazzolli *et al.*, 2002). A severe decrease in population through human intervention can induce genetic loss (Allendorf *et al.*, 2008). Thus, poaching can be held directly responsible for bottlenecks, and the consequential loss of genetic diversity, in several animal species (Bonnell and Selander 1974; Larson *et al.*, 2002; Culver *et al.*, 2008; Bishop *et al.*, 2009). Furthermore, bottlenecks induce the loss of low-frequency alleles, and, consequentially, of genetic diversity through inbreeding and genetic drift (Allendorf and Luikart, 2007), thereby increasing the susceptibility to inbreeding depression effects, such as reproductive and cardiac problems, and epidemic diseases (O'Brien and Evermann, 1988; Roelke *et al.*, 1993).

Molecular markers show that North American mountain lions comprise a large panmictic population, with reduced genetic variation compared to the South Americans (Culver *et al.*, 2000). Most likely, the present-day North Americans descended from a founder event involving a small number of individuals that had migrated out of South America approximately 10,000 years ago (Culver *et al.*, 2000). Therefore, higher genetic diversity in southern Brazil could be expected, when compared with North American studies. However, on comparing genetic diversity found for *P. concolor* with that for North American samples (Kurushima *et al.*, 2006; Rodzen *et al.*, 2007) (Table 3), the observed mean number of alleles/locus and the expected heterozygosity estimated in the present study were found to be lower, when using the same species-specific primers (n = 243 individuals from California and Nevada, and n = 23-25 individuals from California) (Kurushima *et al.*, 2006; Rodzen *et al.*, 2007).

Furthermore, diversity in individuals from south Brazil was lower than that found for previously analyzed South American mountain-lion populations (Table 4). The present results cannot be directly compared to other studies in South America, since different sets of microsatellites were used by all. Even so, by using species-specific primers, higher heterozygosity could be expected. Diversity in the individuals from south Brazil was lower than that indicated for other previously analyzed South-Americans. The mean number of alleles/locus was lower in the former than the latter, except when compared with São Paulo and Bolivian samples. Mean heterozygosity was also lower, except when compared with Bolivian samples, although this may have been due to the small number of samples used (9 and 8 individuals respectively) (Miotto *et al.*, 2007; Ruiz-Garcia *et*

Table 3 - Mean observed and expected heterozygosity and alleles/locus for 14 species-specific loci used in this study, and in two others (Kurushima *et al.*, 2006[1]; Rodzen *et al.*, 2007[2]), using samples from North American mountain lion individuals.

	Ho		H_E		Alleles	
	This study	Kurushima *et al.* 2006 Rodzen *et al.* 2007	This study	Kurushima *et al.* 2006 Rodzen *et al.* 2007	This study	Kurushima *et al.* 2006 Rodzen *et al.* 2007
PcoD8[2]	0.769	0.79	0.747	0.83	5	8
PcoB105[2]	0.778	1.00	0.725	0.74	4	7
PcoD329[2]	0.571	0.71	0.742	0.77	6	8
PcoB203w[1]	0.750	0.46	0.850	0.57	11	7
PcoB210w[1]	0.806	0.62	0.840	0.74	7	7
PcoD103w[1]	0.543	0.58	0.677	0.71	5	6
PcoD301[2]	0.667	0.58	0.719	0.78	7	7
PcoA2[2]	0.677	0.76	0.667	0.68	8	6
PcoD217w[1]	0.594	0.45	0.758	0.59	6	5
PcoD303[2]	0.586	0.67	0.753	0.68	6	4
PcoD310[2]	0.515	0.5	0.752	0.62	6	5
PcoB323[2]	0.706	0.71	0.655	0.69	6	5
PcoD323[2]	0.625	0.33	0.587	0.57	5	5
PcoB115[2]	0.027	0.75	0.027	0.67	2	5
Mean	0.615	0.636	0.678	0.689	6	6.07

Table 4 - Genetic diversity estimates in mountain lions in South and North American populations. N = number of individuals, L = number of loci analyzed.

Continent/Country/State/Region	N	L	Mean number alleles/locus	Mean heterozygosity	Reference
North America					
Utah	50	9	4.44	0.653	Sinclair *et al.*, 2001
California	431	12	4.4	0.44	Ernest *et al.*, 2003
Colorado, Wyoming, South Dakota	312	9	4	0.535	Anderson *et al.*, 2004
Utah, Colorado, Arizona, New Mexico	540	16	3.25-5.06	0.52-0.63	McRae *et al.*, 2005
South América (SA)					
East[1] SA	22	10	8.6	0.71	Culver *et al.*, 2000
North SA	25	10	9.1	0.75	Culver *et al.*, 2000
Central SA	17	10	6.7	0.75	Culver *et al.*, 2000
South SA	22	10	6.0	0.64	Culver *et al.*, 2000
Bolivia – Andes	8	7	3.857	0.592	Ruiz-Garcia *et al.*, 2009
Colombia, Peru, Ecuador, Venezuela, and western Brazilian Amazon	45	7	11	0.629	Ruiz-Garcia *et al.*, 2009
Brazil – MG, GO, SC, PR, SP	18[2]	4	9.25	-	Moreno *et al.*, 2006
Brazil – SP	9	4	4	-	Miotto *et al.*, 2007
Brazil – RS, SC, PR	37	18[3]	5.98	0.609	This study

Brazilian state abbreviations: MG = Minas Gerais, GO = Goiás, SC = Santa Catarina, PR = Paraná, SP = São Paulo, RS = Rio Grande do Sul.

[1] This region corresponds to the distribution of the subspecies *Puma concolor capricornensis.*

[2] Samples from captive individuals.

[3] In this study, loci from *Felis catus* and *Puma concolor* were used, whereas in all the others only loci from *Felis catus* were.

al., 2009). This appears to indicate a loss of genetic diversity in south Brazilian mountain lion populations.

According to evident inbreeding and the estimated global value, this population may be in the process of losing genetic variability. Both estimates of effective population size were lower than $N_e = 50$, the number necessary for diminishing the loss of genetic diversity by inbreeding (Soulé, 1980), and $N_e = 500$, the number necessary for preventing long-term loss of variability by genetic drift (Franklin, 1980; Frankel and Soulé, 1981). This observed loss of genetic diversity is probably a consequence of the recent bottleneck this population apparently underwent. On increasing, with inbreeding and low N_e, this loss can lead to reduced adaptive potential and increased inbreeding depression, with vulnerability to environmental, demographic and stochastic variation, and a consequential increase in the probability of extinction (Reed and Frankham, 2003; Spielman *et al.*, 2004; Frankham *et al.*, 2005). Inbreeding may also affect both individual and population performances (Keller and Waller, 2002).

Conserving Brazilian mountain lions

As carnivores exert considerable influence on ecosystems and the maintenance of their ecological processes (Henke and Bryant, 1999; Miller *et al.*, 2001; Terborgh *et al.*, 2001; Ray *et al.*, 2005), environments where mountain lions have disappeared through human presence and intervention manifest decreased biodiversity (Ripple and Beschta, 2006). Carnivores in general are secretive and nocturnal, comprise small populations, and are frequently endangered. These characteristics, although making it difficult to study them, increase the need for further information, thereby making conservation genetics an essential tool for the purpose. Little is known on mountain lion genetic variability in south Brazil, this constituting a crucial item for both understanding the evolutionary potential of the population and for determining the best strategy for their conservation and management.

A recent bottleneck and loss of genetic diversity were identified in this population. As it is well-known that a decrease in population size and the consequential loss of genetic diversity increase the risk of extinction (Hoelzel, 1999; Dalén *et al.*, 2006; Hájková *et al.*, 2007; Culver *et al.*, 2008), special attention should be dedicated to conservation action, in order to reduce the risk in this case.

Apart from human persecution induced by financial loss, poaching and human exploitation are the major causes of death in carnivores (Nowell and Jackson, 1996). Although hunting is illegal in Brazil, it still occurs in many areas, including in the southern part of the country. In the studied samples, human action was responsible for 92.86% of all the deaths/captures (100% and 84.6% of females and males, respectively), 74% the result of poaching. Although this high percentage may be owing to the sampling method employed, obviously it still indicates the importance of the impact in the area. Weaver *et al.* (1996) found that 75% of all mountain lion deaths in North America were caused by human persecution, and Morrison and Boyle (2009) that

50% were by direct human action. Poaching also caused a general decline in the mammal population of the Atlantic Rain Forest (Cullen *et al.*, 2000; Paviolo *et al.*, 2008, 2009). The population in south Brazil is, without doubt, still prone to poaching and persecution by way of farmer retaliatory hunting (Mazzolli *et al.*, 2002; Marins-Sá, 2005, MSc Dissertation, Curso de Pós-Graduação em Ecologia UFRGS, Porto Alegre), and although the observed bottleneck was probably caused by intense deforestation and habitat loss, it is currently believed that illegal poaching poses the largest local threat. Since this population has undergone a recent and intense reduction in size (identified by the evident bottleneck), with the consequentially low effective population size and decrease in genetic diversity, poaching will probably further increase the risk of extinction.

Conservation efforts may focus on the population level, instead of the species (Garner *et al.*, 2005), since extinction rates for populations are estimated to be three to eight times higher than for species (Hughes *et al.*, 1997). For *P. concolor*, a species that has a geographically diversified environment and various subspecies (Culver *et al.*, 2000), as well as manifold genetic diversity across its range, and a variable intensity of threats, the best conservation strategy could be to develop regional conservation plans according to the identified threats for each region. Therefore, it is believed that mountain lion conservation efforts in south Brazil should be directed towards mitigating human-versus-predator conflicts due to livestock depredation, since this appears to be a grave threat and the principal cause of mountain lion deaths in the area nowadays. Mazzolli *et al.* (2002), when studying the causes of mountain lion depredation in south Brazil, observed that ranches without management plans lost as much as 78% of the goats and 84% of the sheep, whereas losses were substantially reduced if the herds were corralled at night. The authors observed that mountain lions often killed several free-ranging sheep or goats in a single attack, but would take only a single animal from a corral, thereby indicating that ranchers that have introduced management plans for their livestock are prone to few or no losses to mountain lions, thus implying that less conflict is possible with rancher cooperation. Furthermore, education programs should be intensified, with a focus on local populations and farmers, and genetic monitoring programs, implemented for surveying the fluctuation of genetic variability, since there are indications of an imminent loss in coming generations.

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