Estimating puma *Puma concolor* population size in a human-disturbed landscape in Brazil, using DNA mark–recapture data

Renata A. Miotto, Marcelo Cervini, Maja Kajin, Rodrigo A. Begotti and Pedro M. Galetti Jr

Abstract The genetics and ecology of pumas are well documented in North America but there is a lack of studies in South America, especially in Brazil. By means of a noninvasive method, faecal DNA analysis, we estimated puma abundance in two protected areas embedded in a human-disturbed landscape in the north-east of São Paulo state, in south-east Brazil. In 8 months of mark–recapture faeces sampling, 15 individual pumas were identified using seven microsatellite loci. The estimated abundance of pumas with the Jolly–Seber open population model was 23.81 ± SE 6.22. This is the first estimate of the abundance of pumas in a human-dominated landscape in São Paulo state, the most populous, developed and industrialized state of Brazil. The absence of high-quality habitats in the north-east of the state, the absence of direct competitors and the high availability of prey in protected areas are probably contributing to the high number of pumas concentrated in a relatively small area (c. 260 km^2). Our results will contribute to the long-term monitoring of this puma population and, combined with other ecological, behavioural and genetic data, will help guide conservation action to maintain a viable population of the puma in this region.

Keywords Abundance, faecal DNA, Jolly–Seber model, MARK, microsatellite, noninvasive analysis

Introduction

In the 20th century the north-east area of São Paulo state, Brazil, changed drastically (Dean, 1996; Martinelli & Filoso, 2008). The development of urban centres, expansion of sugar cane and eucalyptus plantations, and extensive enlargement of the road network resulted in considerable habitat loss and fragmentation of natural vegetation cover. Despite this intensive transformation some large carnivore species, such as the puma *Puma concolor*, still inhabit the area (Miotto et al., 2007; Lyra-Jorge et al., 2008). How these carnivores are able to survive in the remnant habitat fragments, and their population sizes, require study. Estimation of the size of carnivore populations is important for conservation management, especially in human-dominated landscapes, and monitoring this parameter provides managers with a measure of the effectiveness of conservation actions (Caughley, 1994; Karanth et al., 2006; Kelly et al., 2008).

Mark–recapture methods have been used to estimate population size of large carnivores, based on camera-trap photographs (Karanth & Nichols, 1998; Trolle & Kéry, 2003; Silver et al., 2004; Trolle et al., 2007; Kelly et al., 2008; Paviolo et al., 2009; Silveira et al., 2009) or radio-telemetry tracking data (Franklin et al., 1999; Logan & Sweanor, 2001; Soisalo & Cavalcanti, 2006). As an alternative, noninvasive genetic sampling has become a powerful tool for studying and monitoring elusive and low-density species (Waits & Paetkau, 2005; Schwartz et al., 2007; Janečka et al., 2008). DNA from sources such as hair or faeces can be used as molecular tags in mark–recapture population censuses (Bellemain et al., 2005; Prugh et al., 2005; Boulanger et al., 2008; De Barba et al., 2010). In this context collecting noninvasive samples is equivalent to capturing the animal that deposited the genetic sample. The probability of encountering faeces can therefore be referred to the capture probability. Because each animal has a unique multilocus genotype, each individual can be identified. Closed or open-population models can then be applied, depending on the length of the sampling period, and births, deaths, emigration and immigration can be used to estimate population parameters such as abundance, survival and recruitment.

The puma is the most widely distributed large terrestrial mammal in the Americas (Iriarte et al., 1990). It has been studied extensively in North America (Ross & Jalkotzy, 1992; Roelke et al., 1993; Lindzey et al., 1994; Beier et al., 1995; Ernest et al., 2000; Logan & Sweanor, 2001; Anderson et al., 2004; McRae et al., 2005) but not in such detail in

Renata A. Miotto (Corresponding author) and Pedro M. Galetti Jr Laboratório de Biodiversidade Molecular e Conservação, Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rodovia Washington Luís, km 235, CEP 13565-905 Monjolinho, São Carlos, São Paulo, Brazil. E-mail remiotto@yahoo.com.br

Marcelo Cervini Laboratório de Genética Molecular, Departamento de Ciências Biológicas, Universidade Estadual do Sudoeste da Bahia, Campus Jequié, Jequié, Bahia, Brazil

Maja Kajin Departamento de Ecologia, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier 524, PHLC-220, CEP 20550-900 Maracanã

Rodrigo A. Begotti Laboratório de Hidrologia Florestal, Departamento de Ciências Florestais, Escola Superior de Agricultura "Luíz de Queiroz", Universidade de São Paulo, Piracicaba, São Paulo, Brazil

Received 17 November 2011. Revision requested 11 May 2012. Accepted 6 June 2012. First published online 24 February 2014.

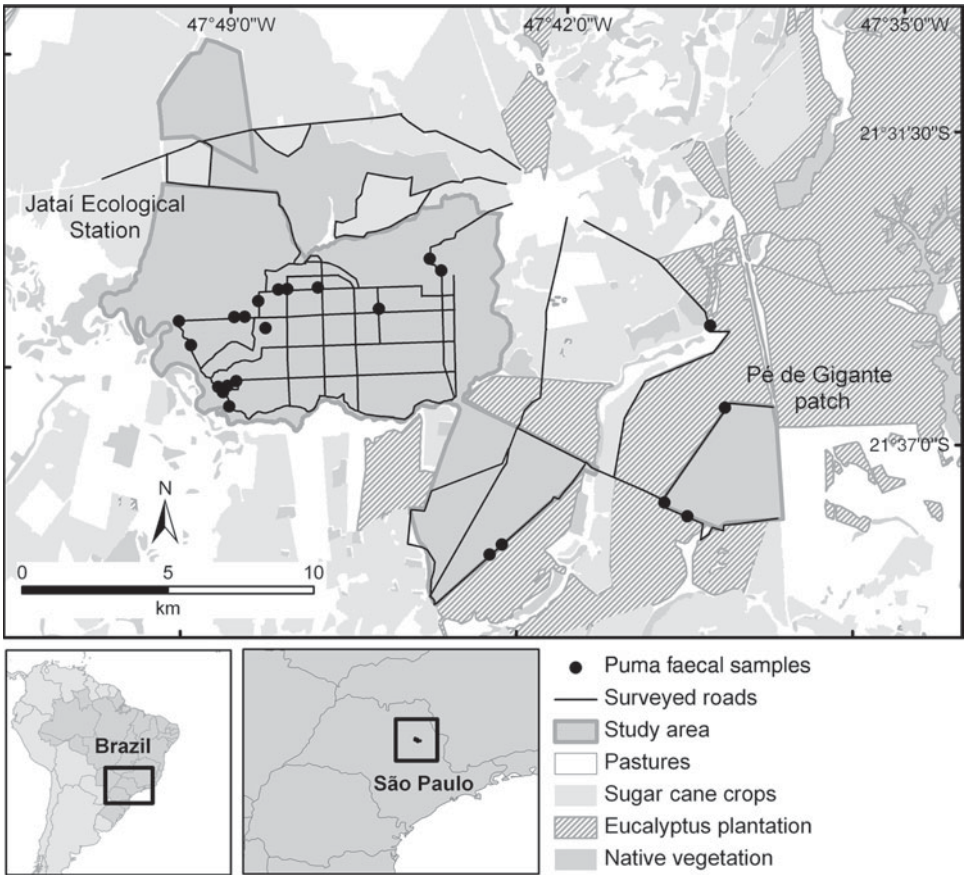

FIG. 1 The location of Jataí Ecological Station and the Pé de Gigante forest patch (the latter a part of Vassununga State Park) in São Paulo state, showing the roads where the surveys were made and the locations where faeces were collected. The insets show the location of the main map in Brazil (left) and in north-east São Paulo state (right).

South America. Puma population sizes and densities have been estimated in a few studies (Franklin et al., 1999, in Patagonia, Chile; Kelly et al., 2008, in Bolívia, Argentina and Belize; Paviolo et al., 2009, in the Green Corridor of Atlantic Forest in Misiones Province, Argentina) but there have been few studies in Brazil (Mazzolli, 2010; Negrões et al., 2010), especially in human-disturbed areas. Puma research in Brazil has concentrated on food habits or habitat use (Emmons, 1987; Ciocheti, 2007; Lyra-Jorge et al., 2008), movement (Schaller & Crawshaw, 1980) and human–puma conflicts (Mazzolli et al., 2002; Conforti & Azevedo, 2003; Azevedo, 2008).

Here, we present an estimate of the population size of pumas inhabiting a human-disturbed landscape in the north-east of São Paulo state, based on a DNA mark–recapture method. Using microsatellites we individualized marked and recaptured samples of puma faeces and estimated population abundance by applying an open population model framework.

Study area

The study area encompasses c. 260 km^2 and is located in the Luís Antônio and Santa Rita do Passa Quatro municipalities, in north-east São Paulo state, Brazil (Fig. 1). The area is characterized as transitional between Cerrado and semi-deciduous Atlantic forest. There are two protected areas 3 km apart, the Jataí Ecological Station and the Vassununga State Park, in addition to some habitat patches on private properties. The 9,000 ha Jataí Ecological Station is the largest protected area in the state with continuous Cerrado vegetation. The 2,070 ha Vassununga State Park contains six distinct patches of Cerrado and semi-deciduous forest (Korman, 2003). In our sampling in Vassununga State Park we concentrated on the largest patch (Pé de Gigante, 1,210 ha), as the smaller patches are surrounded by intensive human activity and unlikely to be used by pumas. Both protected areas are surrounded by sugar cane crops, cattle ranches, eucalyptus plantations, dirt roads and highways but still retain high faunal diversity (Talamoni et al., 2000).

Methods

Sample collection From 2004 to 2008 we conducted a noninvasive puma monitoring project in the study area (Miotto et al., 2012), collecting faeces preferentially in the dry seasons (May–August) of each year, with the exception of 2008, when we conducted monthly 2-day field excursions from March to October. For the analysis described here we used only the 2008 data because they were collected

https://doi.org/10.1017/S0030605312000841 Published online by Cambridge University Press

at regular intervals and on the same roads. There were eight sampling occasions, and each 2-day excursion was considered one sampling occasion. In the study area fragments and plantations are either surrounded or intersected by low-traffic dirt roads. Our sampling scheme encompassed these roads, covering c. 200 km in each field trip (Fig. 1). Pumas tend to use dirt roads to move from one place to another (Dickson et al., 2005) and, especially in the study area, they commonly defecate on these roads (authors, pers. obs.). Samples were collected exclusively from these roads. All fieldwork was conducted by car and by two researchers. Based on tracks and the diameter and morphology of faeces we collected 37 potential puma faecal samples, and we recorded the locations of all collections, using a global positioning system. Samples were stored in sterile, preservative-free plastic tubes without any conservation solution and kept at −22°C in the laboratory until the DNA extraction was performed.

DNA extraction Faecal DNA was extracted using the QIAmp DNA Stool Mini Kit (Qiagen) or PSP Spin Stool DNA Kit (Invitek), following manufacturer's recommendations. For the blood and tissue DNA extractions used as reference sequences in the genetic analysis we followed the phenol/chloroform/isoamylic alcohol protocol described in Sambrook et al. (1989). We collected these samples directly from pumas killed or injured in collisions with vehicles in the region and from the government agency Centro Nacional de Pesquisa e Conservação de Mamíferos Carnívoros, which has a blood/tissue bank.

Species identification To confirm the species of the collected faecal samples we amplified and sequenced a 146 bp portion from the cytochrome *b* gene of the mitochondrial DNA (mtDNA), using primers described by Farrell et al. (2000). Details of the amplification, sequencing and analysis procedures are described in Miotto et al. (2007).

Individual identification To individualize each faecal sample we amplified a set of seven species-specific microsatellite loci, with primers developed by Kurushima et al. (2006): Pco C108 (a tetranucleotide-repeat microsatellite locus), and Pco B010, Pco B210, Pco A339, Pco A208, Pco A216 and Pco B003 (dinucleotide-repeat microsatellite loci). Primers were marked with universal fluorescent M13 tails following Schuelke (2000). Each PCR reaction (15 μL) contained 7.5 μL of *GoTaq Master Mix* (Promega), containing 1×buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 unit Taq polymerase, 8 pmol of reverse primer, 2 pmol of forward primer, 8 pmol of M13 sequence marked with the 6-FAM fluorophore, and 150 μg ml^{-1} of BSA. The remaining volume of the 15 μL reaction comprised DNA. Amplifications were performed in a PTC-100 Thermocycler (MJ Research) according to the following protocol (for all primer pairs): an initial denaturation cycle at 94°C for 5 minutes, 40 cycles at 92°C for 1 minute, 48°C for 1 minute, and 72°C for 1 minute, and a final 30-minute extension at 72°C. Negative controls were included in all reactions to monitor possible contamination. The resulting genotypes were analysed in a MegaBACE ET-550R Size Standard automatic sequencer (GE Healthcare) with *Genetic Profiler* (GE Healthcare). We conducted individual identification of genotypes using *Gimlet* (Valière, 2002). To prevent misidentification as a result of allelic dropout, in which one of the two alleles in a heterozygous individual is not detected (Taberlet et al., 1996), we genotyped homozygote samples in 3–5 independent PCR reactions and genotyped heterozygotes twice. For individual identification only samples that were successfully genotyped for at least five loci were included in the analysis. To quantify the power to discriminate individuals with the microsatellite loci used we determined the identity probability ($P_{(ID)}$); i.e. the probability of two individuals in a population randomly sharing identical genotypes for all the analysed loci (Paetkau et al., 1998; Waits et al., 2001). The $P_{(ID)}$ values were calculated based on the allele frequencies of unique genotypes for each locus, in *Gimlet* (Valière, 2002), and then multiplied by the total number of loci to obtain a total $P_{(ID)}$ (Paetkau et al., 1998). Because we expected to sample related animals in the study area (Miotto et al., 2012) we calculated both the $P_{(ID)unbiased}$ and $P_{(ID)sib}$ equations, taking into account the population size and the presence of related individuals, respectively (Waits et al., 2001). We determined the total and per-locus genotyping error rates (allelic dropout) by dividing the number of detected errors by the number of cases in which an error might have been detected (i.e. the total number of genotyping reactions). Finally, we investigated null allele and stutters occurrence with *Micro-Checker 2.2.3* (Van Oosterhout et al., 2004)

Sex determination To identify the sex of the individuals sampled we amplified a portion of the amelogenin gene present in both sex chromosomes, using primers described by Pilgrim et al. (2005). In this gene fragment males have a 20 bp deletion in the Y-chromosome copy and consequently produce PCR products of different sizes, whereas females amplify fragments of the same size. To prevent false positive for females we repeated each reaction three times.

Abundance of pumas After individualizing puma faecal samples, a capture–recapture history of each animal was established for the 8-month sampling period. To estimate abundance ($\hat{N}$), we opted for an open population model as we assumed that animals could leave or immigrate into the study area during the sampling period. Pumas are vagile and have large home ranges and we had previous information that suggested some may frequently disperse throughout the matrix (Miotto et al., 2012). We estimated $\hat{N}$ with the Jolly–Seber probability model (Jolly, 1965; Seber, 1965), using the POPAN formulation (Schwarz & Arnason, 1996),

https://doi.org/10.1017/S0030605312000841 Published online by Cambridge University Press

TABLE 1 Model selection results for the *POPAN* parameterized models for puma *Puma concolor* capture data in north-east São Paulo state (Fig. 1).

Model*	AICc	ΔAICc	AICc weight	Model likelihood	No. of parameters	Deviance
$\{\Phi(.)p(.)b(t)N(.)\}$	112.399	0.000	0.955	1.000	10	8.097
$\{\Phi(t)p(t)b(t)N(.)\}$	118.517	6.117	0.045	0.047	23	5.138
$\{\Phi(t)p(.)b(t)N(.)\}$	183.328	70.929	0.000	0.000	16	6.235
$\{\Phi(.)p(t)b(t)N(.)\}$	209.307	96.908	0.000	0.000	17	5.928
$\{\Phi(.)p(.)b(.)N(.)\}$	4,225.807	4,113.408	0.000	0.000	4	4,148.323
$\{\Phi(t)p(.)b(.)N(.)\}$	4,232.918	4,120.519	0.000	0.000	10	4,128.617
$\{\Phi(t)p(t)b(.)N(.)\}$	4,327.568	4,215.169	0.000	0.000	17	4,124.189
$\{\Phi(.)p(t)b(.)N(.)\}$	11,131.408	11,019.009	0.000	0.000	11	11,020.030

*ϕ, survival probability; *p*, capture probability; *b*, probability of an individual leaving or entering the study area during the sampling period; (.), constancy; (*t*), temporal variation

TABLE 2 The range size, number of genotyped samples, number of alleles, probability of identity (unbiased and with sibs), and per locus and total allelic dropout rate for the seven microsatellite loci analysed.

Locus	Range size (base pairs)	No. of samples	No. of alleles	$P_{(ID)unbiased}$	$P_{(ID)sib}$	Allelic dropout (%)
Pco C108	124–160	22	4	1.612×10^{-1}	4.792×10^{-1}	7.51
Pco B010	203–229	23	8	5.023×10^{-2}	4.119×10^{-1}	2.10
Pco B210	165–177	23	7	5.831×10^{-2}	4.014×10^{-1}	9.43
Pco A216	237–251	22	6	4.745×10^{-2}	3.816×10^{-1}	3.70
Pco A208	187–201	23	6	6.920×10^{-2}	4.042×10^{-1}	13.40
Pco B003	279–303	23	6	1.099×10^{-1}	4.430×10^{-1}	2.10
Pco A339	264–280	24	7	3.436×10^{-2}	3.654×10^{-1}	7.70
Mean/total		24	6.28	5.854×10^{-9}	0.001	6.56

implemented in *MARK* (White & Burnham, 1999). This open population model assumes the existence of a 'super-population' (N) in which unmarked animals have the same probability of capture as marked animals in the population (assumption of equal catchability), survival rates are homogeneous for marked and unmarked animals, the sampled area is constant, and there is no loss of mark (genetic tag in our case) during the sampling period. The parameters of this model are p_i, the probability of capture of marked and unmarked individuals on occasion i, ϕ_i, the probability of survival of marked and unmarked individuals from occasion i to $i+1$, and b_i, the probability of a new animal entering the population between occasions i and $i+1$. To test the validity of the above assumptions a goodness of fit test was performed using *RELEASE v. 3.0* (Burnham et al., 1987) and by accessing the deviance of 1,000 bootstrapped simulations based on the global time-dependent Cormack–Jolly–Seber model (Dunstan et al., 2011). We selected between eight models, all nested inside the global model, allowing for temporal variation in all three parameters $[p(t)\phi(t)b(t)N(.)]$. The tested models are described in Table 1. To guarantee numerical convergence a multinomial link function was used for the b_i parameters (constraining a set of real parameters to sum to 1.00), and in the case of the abundance parameter an identity link function was used, permitting the parameter to have values outside the [0, 1] interval. Models were ranked using the Akaike information criterion (AICc) adjusted for small population sizes. The model with the lowest AICc value was considered the model that best balanced bias and precision (White & Burnham, 1999) and, therefore, the most appropriate model for accurate estimation of abundance.

Results

Of the 37 faecal samples collected mtDNA was amplified in 27 samples (84.37%). By comparing the sequenced mtDNA fragments we identified 24 puma faeces. Three samples belonging to ocelots *Leopardus pardalis*, a sympatric species present in the area, were discarded from further analysis. Genotyping success for the set of seven microsatellite loci was > 80%. We quantified a general rate of 6.56% for allelic dropout and estimated a total $P_{(ID)unbiased}$ of 6×10^{-9} and a total $P_{(ID)sib}$ of 0.001. Our data presented no evidence of null alleles or stutter peaks. The range size, number of alleles per locus, and P_{ID} values per locus are described in Table 2. From the 24 samples we identified 15 different pumas. Table 3 presents their mark–recapture data (encounter history).

No evidence of overdispersion was found for the global model and therefore our models were considered to

https://doi.org/10.1017/S0030605312000841 Published online by Cambridge University Press

TABLE 3 Encounter history (1, detected; 0, not detected) of 15 individualized female (F) and male (M) pumas in the study area, from eight periods of faecal collection.

		Occasions							
Individual	Sex	1	2	3	4	5	6	7	8
1	F	0	0	0	0	1	0	1	0
2	F	0	1	0	0	0	0	0	0
3	F	1	0	1	0	0	0	0	0
4	M	0	0	1	1	1	0	0	0
5	F	0	0	0	1	0	0	0	0
6	F	0	1	0	0	0	0	0	0
7	F	1	0	0	0	1	0	0	0
8	F	1	0	0	0	0	0	0	1
9	M	0	0	1	0	0	1	0	0
10	F	0	1	0	1	0	0	0	0
11	M	0	1	0	0	0	0	0	0
12	F	0	1	0	0	0	0	0	0
13	F	0	0	0	1	0	0	0	0
14	F	0	0	0	0	0	1	0	0
15	F	0	0	0	0	0	1	0	1

adequately fit the data and no important assumptions were being violated. Model selection in *MARK* resulted in $\{\Phi(.)p(.)b(t)N(.)\}$ (constant capture probability, constant survival rate, and temporal variation in the probability of new animals entering the population between sampling occasions) being the most appropriate model (AICc = 112.399; Table 1). The high ΔAICc values for the remaining models allowed us to distinguish the first model as the only one that sufficiently supported the data and, therefore, this was the only model appropriate for obtaining further estimates. Estimated puma abundance using this model was 23.81 ± SE 6.22 (95% CI 17.53–45.66). The capture probabilities for this model were constant and equal to 0.19 ± SE 0.06 (95% CI 0.098–0.339).

Discussion

Noninvasive genetic analysis

The estimated values of probability of identity were low for the seven loci analysed ($P_{(ID)unbiased}$ of 6×10^{-9} and $P_{(ID)sib}$ of 0.001). Both values are considered satisfactory for individual identification in noninvasive genotyping (Waits et al., 2001) and indicate that the analysed loci successfully distinguished each individual, even in the presence of closely related animals (Miotto et al., 2012). Because of low DNA quality and quantity, microsatellite analyses of noninvasive samples, such as faeces, are commonly affected by genotyping errors, such as allelic dropout or false allele amplification, and consequently the identification of individuals and the estimates of population size could be biased (Taberlet et al., 1996, 1999; Waits et al., 2001; Broquet & Petit, 2004; Prugh et al., 2005). To avoid errors we established conditions to obtain consistent genotypes: we genotyped heterozygotes twice and confirmed the genotypes of homozygotes with 3–5 independent PCR reactions, we included a locus in our analysis only if > 80% of samples were successfully genotyped, and in the final analysis we only included those samples that were genotyped for at least 80% of all the analysed loci.

Unlike other felid species pumas have no pelage patterns, such as the rosettes that are features of jaguars and ocelots (Trolle & Kéry, 2003; Silver et al., 2004; Trolle et al., 2007; Silveira et al., 2009) or the stripes of tigers (Karanth & Nichols, 1998; Karanth et al., 2006) that could allow reliable individual identification in camera-trap studies. Kelly et al., (2008) proposed a protocol to identify individual pumas based on body patterns analysed by distinct researchers. This protocol was later used by Paviolo et al. (2009) but some subjectivity in individual identification remained once each researcher identified a distinct number of animals (Kelly et al., 2008). Mazzolli (2010) and Negrões et al., (2010) also based their estimates of puma population size on photographs but both studies were subject to some degree of subjectivity. Foster & Harmsen (2011) listed a number of biases that can compromise the individual identification of species that lack individually identifiable natural markers. Thus, despite some inherent challenges in the technique, mainly in tropical humid and hot regions (Norris & Michalski, 2010), as long as a rigid amplification protocol against genotype misidentification is applied (i.e. reducing allelic dropout or false allele occurrence), noninvasive genetic analysis may guarantee more accuracy in individual identification, especially for species without evident pelage patterns, and thus avoid potential under- or overestimation of population size.

Population size estimators

The best model in our selection analysis supports temporal variation in recruitment (i.e. variation in the pattern by which new animals enter the population, either by births or immigration) and implies constancy of survival and capture probability. The support for this model was substantial, as the ΔAICc of the second best model was > 2 (Burnham & Anderson, 1998). The model is consistent with the species' biology as pumas do not have a specific reproductive season, with mating occurring throughout the year (Logan & Sweanor, 2001). Male pumas tend to disperse from natal areas whereas females may exhibit phylopatry or disperse over short distances (Logan & Sweanor, 2001). Therefore, the detected variation could be a direct response to reproduction, with the entry of males into the area for mating and the subsequent entry of newborn males and females, or a response of subadults leaving the area after the maternal care period, which occurs at 16–22 months of age (Logan & Sweanor, 2001). Unfortunately, by using

https://doi.org/10.1017/S0030605312000841 Published online by Cambridge University Press

molecular markers we were not able to establish age structure and, consequently, could not distinguish cubs, juveniles or adults. In a previous study (Miotto et al., 2012), however, we detected few males and many resident females, suggesting that males may be more responsible for the flow of individuals into and out of the area.

Our abundance estimate represents the entire population (males and females), as grouping the data would have weakened the analysis through over-parameterization of models. The models used for estimating abundance showed no evidence of lack of fit to the data, which reinforced the accuracy of the abundance estimates. Our sample size was small but, in addition to being constant, P (0.19 ± SE 0.06) was higher than the mean reported in other felid studies (0.13 ± SE 0.09; Foster & Harmsen, 2011).

To compare our results with other studies in South America (Franklin et al., 1999; Kelly et al., 2008; Paviolo et al., 2009), including Brazil (Mazzolli, 2010; Negrões et al., 2010), we would need to estimate density. However, as the population was demographically open we are unable to make such a comparison. For geographically open populations the effective sampled area ($\hat{A}$) is poorly defined relative to the sampled population (White et al., 1982) and studies using telemetry data have shown that density is overestimated by $\hat{N}/\hat{A}$ (Soisalo & Cavalcanti, 2006; Dillon & Kelly, 2008).

Puma population size

Estimates of population size and dynamics of the puma are crucial for long-term conservation of the species, especially in human-disturbed landscapes. Our estimates of population size (23.81 ± SE 6.22) is high considering the small size of the study area. There appears to have been a recent increase in the number of pumas in north-east São Paulo state and other regions of Brazil. For example, from 2004 to 2012 in a 1,700 km^2 area that encompasses our study area, 26 pumas were killed on roads and 11 were rescued in urban areas as a result of livestock depredation (R.A. Miotto, pers. obs. & unpubl. data). Little is known about the reasons for this possible expansion, or if it will continue, but we believe it is influenced by the characteristics of the area.

In the previous 50 years the area planted with sugar cane in Brazil increased from c. 1.4 to 7 million ha (Martinelli & Filoso, 2008). More than 50% of the country's sugar cane land cover is in São Paulo state, with plantations increasing at an annual rate of c. 85,000 ha (Martinelli & Filoso, 2008). In addition to eucalyptus plantations, pastures and the development of urban centres, this intensive human activity has severely transformed the original vegetation cover of the north-east of the state. The area is now characterized by many small and a few large patches of vegetation surrounded by human-disturbed areas. Despite this transformation pumas are still present in the area, probably as a consequence of their generalist habits.

In general, carnivore densities are positively correlated with prey biomass (Franklin et al., 1999; Logan & Sweanor, 2001; Carbone & Gittleman, 2002; Karanth et al., 2006). The two protected areas in our study area are amongst the largest natural refuges for many species of the ungulate prey of pumas (Novack et al., 2005; Ciocheti, 2007) in the north-east of São Paulo state. These protected areas are therefore high-quality habitat patches in the matrix and may allow pumas to concentrate in the area and tolerate overlapping territories (Miotto et al., 2012). Intraspecific conflict may contribute to puma mortality but when prey availability is high female pumas tend to have smaller home ranges (Logan & Sweanor, 2001). Even embedded in a relatively permeable matrix, with eucalyptus plantations and sugar cane crops, pumas may be confined to these protected areas when there are no surrounding patches of similar quality. Fragments surrounding the protected areas are more susceptible to hunting pressure, which leads to decreased prey biomass and abundance (Cullen et al., 2000, 2001). In addition, the absence of competitors such as the jaguar *Panthera onca* may facilitate a greater number of pumas in the study area (coexistence between different felid species may regulate population sizes; Donadio & Buskirk, 2006). The study area may act as a source area of pumas (Miotto et al., 2012); i.e. an area where pumas are resident and raise their cubs, which then disperse as juveniles. Because molecular markers do not allow us to define age structure, we could not recognize cubs in our data set, but their probable presence in the area may have increased the population estimate.

The closest protected area >1,000 ha is c. 70 km from Jataí Ecological Station and Vassununga State Park. To maintain the puma population in this region the priority should be to improve connectivity by establishing new protected areas and increasing the size of existing protected areas such as Jataí Ecological Station and Vassununga State Park. The improvement of habitat quality on private properties, with recovery and conservation of remnant forest fragments in areas occupied by the sugar cane and wood pulp industries, would also improve landscape management for the puma.

Acknowledgements

We thank Fundação de Amparo à Pesquisa do Estado de São Paulo and Conselho Nacional de Desenvolvimento Científico e Tecnológico for their financial support, the Capes agency for a PhD scholarship to RAM and a post-doctoral fellowship to MK (PNPD), the Neotropical Grassland Conservancy for supplying laboratory reagents, Marina Gomes de Figueiredo and Giordano Ciocheti

for their help with fieldwork, and two anonymous reviewers for their comments and suggestions.

References

Anderson, Jr, C.R., Lindzey, F.G. & McDonald, D.B. (2004) Genetic structure of cougar populations across the Wyoming Basin: metapopulation or megapopulation. *Journal of Mammalogy*, 85, 1207–1214.

Azevedo, F.C.C. (2008) Food habits and livestock depredation of sympatric jaguars and pumas in the Iguaçu National Park area, south Brazil. *Biotropica*, 40, 494–500.

Beier, P., Choate, D. & Barret, R.H. (1995) Movement patterns of mountain lions during different behaviors. *Journal of Mammalogy*, 76, 1056–1070.

Bellemain, E., Swenson, J.E., Tallmon, D.A., Brunberg, S. & Taberlet, P. (2005) Estimating population size of elusive animals with DNA from hunter-collected faeces: four methods for brown bears. *Conservation Biology*, 19, 150–161.

Boulanger, J., Kendall, K.C., Stetz, J.B., Roon, D.A., Waits, L.P. & Paetkau, D. (2008) Multiple data sources improve DNA-based mark–recapture population estimates of grizzly bears. *Ecological Applications*, 18, 577–589.

Broquet, T. & Petit, E. (2004) Quantifying genotyping errors in noninvasive population genetics. *Molecular Ecology*, 13, 3601–3608.

Burnham, K.P. & Anderson, D.R. (1998) *Model Selection and Multimodel Inference. A Practical Information-Theoretic Approach*. 2nd edition. Springer, New York, USA.

Burnham, K.P., Anderson, D.R., White, G.C., Brownie, C. & Pollock, K.H. (1987) *Design and Analysis Methods for Fish Survival Experiments Based on Release–Recapture*. American Fisheries Society, Bethesda, USA.

Carbone, C. & Gittleman, J.L. (2002) A common rule for the scaling of carnivore density. *Science*, 295, 2273–2276.

Caughley, G. (1994) Directions in conservation biology. *Journal of Animal Ecology*, 63, 215–244.

Ciocheti, G. (2007) *Large and medium mammal habitat use and activities pattern, and trophic niche of maned wolf (*Chrysocyon brachyurus*), puma (*Puma concolor*) and ocelot (*Leopardus pardalis*) in an agroforestry landscape of São Paulo state*. MSc thesis. Universidade de São Paulo, São Paulo, Brazil.

Conforti, V.A. & Azevedo, F.C.C. (2003) Local perceptions of jaguars (*Panthera onca*) and pumas (*Puma concolor*) in the Iguaçu National Park area, south Brazil. *Biological Conservation*, 111, 215–221.

Cullen, Jr, L., Bodmer, E.R. & Valladares-Pádua, C. (2000) Effects of hunting in habitat fragments of the Atlantic forests, Brazil. *Biological Conservation*, 95, 49–56.

Cullen, Jr, L., Bodmer, E.R. & Valladares-Pádua, C. (2001) Ecological consequences of hunting in Atlantic forest patches, São Paulo, Brazil. *Oryx*, 35, 137–144.

Dean, W. (1996) *With Broadax and Firebrand: The Destruction of the Brazilian Atlantic Forest*. University of California Press, Berkeley, USA.

De Barba, M., Waits, L.P., Genovesi, P., Randi, E., Chirichella, R. & Cetto, E. (2010) Comparing opportunistic and systematic sampling methods for non-invasive genetic monitoring of a small translocated brown bear population. *Journal of Applied Ecology*, 47, 172–181.

Dickson, B.G., Jenness, J.S. & Beier, P. (2005) Influence of vegetation, topography, and roads on cougar movement in Southern California. *Journal of Wildlife Management*, 69, 264–276.

Dillon, A. & Kelly, M.J. (2008) Ocelot home range, overlap and density: comparing radio telemetry with camera trapping. *Journal of Zoology*, 275, 391–398.

Donadio, E. & Buskirk, S.W. (2006) Diet, morphology, and interspecific killing in Carnivora. *American Naturalist*, 167, 524–536.

Dunstan, A., Bradshaw, C.J.A. & Marshall, J. (2011) Nautilus at risk—estimating population size and demography of *Nautilus pompilius*. *PLoS One*, 6, e16716.

Emmons, L.H. (1987) Comparative feeding ecology of felids in a Neotropical rainforest. *Behavioral Ecology and Sociobiology*, 20, 271–283.

Ernest, H.B., Penedo, M.C.T., May, B.P., Syvanen, M.S. & Boyce, W.M. (2000) Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Molecular Ecology*, 9, 433–441.

Farrell, L.E., Roman, J. & Sunquist, M.E. (2000) Dietary separation of sympatric carnivores identified by molecular analysis of scats. *Molecular Ecology*, 9, 1583–1590.

Foster, R.J. & Harmsen, B.J. (2011) A critique of density estimation from camera-trap data. *The Journal of Wildlife Management*, 76, 224–236.

Franklin, W.L., Johnson, W.E., Sarno, R.J. & Iriarte, J.A. (1999) Ecology of the Patagonia puma *Felis concolor patagonica* in southern Chile. *Biological Conservation*, 90, 33–40.

Iriarte, J.A., Franklin, W.L., Johnson, W.E. & Redford, K.H. (1990) Biogeographic variation of food habits and body size of the American puma. *Oecologia*, 85, 185–190.

Janečka, J.E., Jackson, R., Yuquang, Z., Diqiang, L., Munkhtsog, B., Buckley, V. & Murphy, W.J. (2008) Population monitoring of snow leopards using noninvasive collection of scat samples: a pilot study. *Animal Conservation*, 11, 401–411.

Jolly, G.M. (1965) Explicit estimates from capture–recapture data with both death and immigration-stochastic model. *Biometrika*, 52, 225–247.

Karanth, K.U. & Nichols, J.D. (1998) Estimation of tiger densities in India using photographic captures and recaptures. *Ecology*, 79, 2852–2862.

Karanth, K.U., Nichols, J.D., Kumar, N.S. & Hines, J.E. (2006) Assessing tiger population dynamics using photographic capture–recapture sampling. *Ecology*, 87, 2925–2937.

Kelly, M.J., Noss, A.J., Di Bitetti, M.S., Maffei, L., Arispe, R.L., Paviolo, A. et al. (2008) Estimating puma densities from camera trapping across three study sites: Bolivia, Argentina, Belize. *Journal of Mammalogy*, 89, 408–418.

Korman, V. (2003) *Proposal for the interconnection of the Vassununga State Park fragments, state of São Paulo, Brazil*. MSC thesis. Escola Superior de Agricultura "Luiz de Queiroz", Universidade de São Paulo, Piracicaba, Brazil.

Kurushima, J.D., Collins, J.W. & Ernest, H.B. (2006) Development of 21 microsatellite loci for puma (*Puma concolor*) ecology and forensics. *Molecular Ecology Notes*, 6, 1260–1262.

Lindzey, F.G., Van Sickle, W.D., Ackerman, B.B., Barnhurst, D., Hemker, T.P. & Laing, S.P. (1994) Cougar population dynamics in southern Utah. *Journal of Wildlife Management*, 58, 619–624.

Logan, K.A. & Sweanor, L. (2001). *Desert Puma: Evolutionary Ecology and Conservation of an Enduring Carnivore*. Island Press, Washington, DC, USA.

Lyra-Jorge, M.C., Ciocheti, G. & Pivello, V.R. (2008) Carnivore mammals in a fragmented landscape in northeast of São Paulo state. *Biodiversity and Conservation*, 17, 1573–1580.

Martinelli, L.A. & Filoso, S. (2008) Expansion of sugarcane ethanol production in Brazil: environmental and social challenges. *Ecological Applications*, 18, 885–898.

Mazzolli, M. (2010) Mosaics of exotic forest plantations and native forests as habitat of pumas. *Environmental Management*, 46, 237–253.

Mazzolli, M., Graipel, M.E. & Dunstone, N. (2002) Mountain lion depredation in southern Brazil. *Biological Conservation*, 105, 43–51.

McRae, B.H., Beier, P., Dewald, L.E., Huynh, L.Y. & Keim, P. (2005) Habitat barriers limit gene flow and illuminate historical events in a wide-ranging carnivore, the American puma. *Molecular Ecology*, 14, 1965–1977.

Miotto, R.A., Cervini, M., Begotti, R.A. & Galetti, Jr, P.M. (2012) Monitoring a puma (*Puma concolor*) population in a fragmented landscape in southeast Brazil. *Biotropica*, 44, 98–104.

Miotto, R.A., Rodrigues, F.P., Ciocheti, G. & Galetti, Jr, P.M. (2007) Determination of the minimum population size of pumas (*Puma concolor*) through faecal DNA analysis in two protected cerrado areas in the Brazilian southeast. *Biotropica*, 39, 647–654.

Negrões, N., Sarmento, P., Cruz, J., Eira, C., Revilla, E., Fonseca, C. et al. (2010) Use of camera-trapping to estimate puma density and influencing factors in central Brazil. *Journal of Wildlife Management*, 74, 1195–1203.

Norris, D. & Michalski, F. (2010) Implications of faecal removal by dung beetles for scat surveys in a fragmented landscape of the Brazilian Amazon. *Oryx*, 44, 455–458.

Novack, A.J., Main, M.B., Sunquist, M.E. & Labisky, R.F. (2005) Foraging ecology of jaguar (*Panthera onca*) and puma (*Puma concolor*) in hunted and non-hunted sites within the Maya Biosphere Reserve, Guatemala. *Journal of Zoology*, 267, 167–178.

Paetkau, D., Waits, L.P., Clarkson, P.L., Craighead, L., Vyse, E., Ward, R. & Strobeck, C. (1998) Variation in genetic diversity across the range of North American brown bears. *Conservation Biology*, 12, 418–429.

Paviolo, A., DiBlanco, Y.E., De Angelo, C.D. & DiBitetti, M.S. (2009) Protection affects the abundance and activity patterns of pumas in the Atlantic Forest. *Journal of Mammalogy*, 90, 926–934.

Pilgrim, K.L., McKelvey, K.S., Riddle, A.E. & Schwartz, M.K. (2005) Felid sex identification based on noninvasive genetic samples. *Molecular Ecology Notes*, 5, 60–61.

Prugh, L.R., Ritland, C.E., Arthur, M.A. & Krebs, C.J. (2005) Monitoring coyote population dynamics by genotyping faeces. *Molecular Ecology*, 14, 1585–1596.

Roelke, M.E., Martenson, J.S. & O'Brien, S.J. (1993) The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biology*, 3, 340–350.

Ross, P.L. & Jalkotzy, M.G. (1992) Characteristics of a hunted population of cougars in southwestern Alberta. *Journal of Wildlife Management*, 56, 417–426.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. 2nd edition. Cold Spring Harbor Press, New York, USA.

Schaller, G.B. & Crawshaw, P.G. (1980). Movement patterns of jaguar. *Biotropica*, 12, 161–168.

Schuelke, M. (2000). An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, 18, 233–234.

Schwartz, M.K., Luikart, G. & Waples, R.S. (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology and Evolution*, 22, 25–33.

Schwarz, C.J. & Arnason, A.N. (1996) A general methodology for the analysis of open-model capture–recapture experiments. *Biometrics*, 52, 860–873.

Seber, G.A.F. (1965) A note on the multiple recapture census. *Biometrika*, 52, 249–259.

Silveira, L., Jácomo, A.T.A., Astete, S., Sollmann, R., Torres, N.M., Furtado, M.M. & Marinho-Filho, J. (2009) Density of the Near Threatened jaguar *Panthera onca* in the caatinga of north-eastern Brazil. *Oryx*, 44, 104–109.

Silver, S.C., Ostro, L.E.T., Marsh, L.K., Maffei, L., Noss, A.J., Kelly, M.J. et al. (2004) The use of camera traps for estimating jaguar *Panthera onca* abundance and density using capture/recapture analysis. *Oryx*, 38, 148–154.

Soisalo, M.K. & Cavalcanti, M.C. (2006) Estimating the density of a jaguar population in the Brazilian Pantanal using camera-traps and capture–recapture sampling in combination with GPS radiotelemetry. *Biological Conservation*, 129, 487–496.

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., et al. (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, 24, 3189–3194.

Taberlet, P., Waits, L.P. & Luikart, G. (1999) Noninvasive genetic sampling: look before you leap. *Trends in Ecology and Evolution*, 14, 323–327.

Talamoni, S.A., Motta, Jr, J.C. & Dias, M.M. (2000) Fauna de mamíferos da Estação Ecológica de Jataí e da Estação Experimental de Luiz Antônio. In *Estudos Integrados em Ecossistemas, Estação Ecológica de Jataí* (eds J.E. Santos & J.S.R. Pires), pp. 317–329. Vol. 1. Editora Rima, São Carlos, São Paulo, Brazil.

Trolle, M. & Kéry, M. (2003) Estimation of ocelot density in the Pantanal using capture–recapture analysis of camera trapping data. *Journal of Mammalogy*, 84, 607–614.

Trolle, M., Noss, A.J., De Lima, E.S. & Dalponte, J.C. (2007) Camera-trap studies of maned wolf density in the Cerrado and the Pantanal of Brazil. *Biodiversity and Conservation*, 16, 1197–1204.

Valière, N. (2002) Gimlet: a computer program for analyzing genetic individual identification data. *Molecular Ecology Notes*, 2, 377–379.

Van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M. & Shipley, P. (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4, 535–538.

Waits, L.P., Luikart, G. & Taberlet, P. (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology*, 10, 249–256.

Waits, L.P. & Paetkau, D. (2005) Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. *Journal of Wildlife Management*, 69, 1419–1433.

White, G.C., Anderson, D.R., Burham, K.P. & Otis, D.L. (1982) *Capture–recapture and Removal Methods for Sampling Closed Populations*. Los Alamos National Laboratory Report LA-8787-NERP, Los Alamos, USA.

White, G.C. & Burnham, K.P. (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study*, 46, 120–138.

Biographical sketches

Renata A. Miotto is investigating the genetics and ecology of carnivorous mammals living in fragmented and disturbed areas in Brazil. Marcelo Cervini is a geneticist working on the conservation and evolution of a range of groups, from mammals to bees. Maja Kajin works on population ecology and genetics, ecological modelling, and demography, focusing mainly on Atlantic forest mammals. Rodrigo A. Begotti is studying the conservation of forest resources, and works with landscape ecology and the conservation of medium and large-bodied mammals in fragmented landscapes. Pedro M. Galetti Jr's research group studies the conservation genetics of a diverse range of organisms.