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Population structure and gene flow of Geoffroy's cat (*Leopardus geoffroyi*) in the Uruguayan Savanna ecoregion

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Felids are among the species most threatened by habitat fragmentation resulting from land-use change. In the Uruguayan Savanna ecoregion, about 30% of natural habitats have been lost, large felids have been eradicated from most of the region, and the impact of anthropogenic threats over the smaller species that remain is unknown. To develop management strategies, it is important to enhance knowledge about species population structure and landscape connectivity, particularly when land-use change will continue and intensify in the next years. In this study, we evaluate the population structure and gene flow of Geoffroy's cat in the Uruguayan Savanna ecoregion. We generated a matrix of 11 microsatellite loci for 70 individuals. Based on Bayesian approaches we found that within the Uruguayan Savanna, Geoffroy's cat shows high levels of genetic variability and no population structure. However, we observed genetic differences between individuals from the Uruguayan Savanna and those from the contiguous ecoregion, the Argentinian Humid Pampa. Four first-generation migrants from Humid Pampa were identified in the Uruguayan Savanna, suggesting a stronger gene flow in the west-east direction. We detected a past bottleneck followed by a subsequent recovery in Geoffroy's cat populations in both ecoregions. These results lay the groundwork to understand the population dynamics and conservation status of Geoffroy's cat in the Uruguayan Savanna ecoregion, and provide baseline data to establish population monitoring.

Key words: landscape fragmentation, microsatellite loci, molecular ecology, Neotropical felids, population genetics

Los félidos se encuentran entre las especies más amenazadas por la fragmentación del hábitat debido a cambios en el uso del suelo. En la ecorregión de la sabana uruguaya se ha perdido alrededor del 30% de los hábitats naturales, al tiempo que se han erradicado los grandes félidos de la mayor parte de la región, y se desconoce el impacto de las amenazas antropogénicas sobre las especies más pequeñas que aún quedan. Para desarrollar estrategias de gestión, es importante mejorar el conocimiento de la manera en que las especies se estructuran y conectan en el paisaje, en particular teniendo en cuenta que los cambios en el uso del suelo continuarán e incluso se intensificarán en los

próximos años. En este estudio evaluamos la estructura poblacional y el flujo génico del gato montés en la ecorregión de la sabana uruguaya. Para ello generamos una matriz de 11 loci microsatelitales en 70 ejemplares. Utilizando una aproximación Bayesiana, encontramos que en la sabana uruguaya el gato montés exhibe altos niveles de variabilidad genética y no presenta estructuración poblacional. Sin embargo, los individuos de esta ecorregión muestran diferencias genéticas con los de la ecorregión contigua, la pampa húmeda argentina. Identificamos cuatro migrantes de primera generación de la pampa húmeda en la sabana uruguaya, hecho que sugiere un flujo génico predominante en sentido oeste-este. En ambas ecorregiones detectamos un cuello de botella en las poblaciones de gato montés que ocurrió en el pasado, seguido de una recuperación más reciente. Estos resultados sientan las bases para comprender la dinámica poblacional y el estado de conservación del gato montés en la ecorregión de la sabana uruguaya, y proporcionan información de base para establecer un monitoreo de la población.

Palabras clave: ecología molecular, félidos neotropicales, fragmentación del paisaje, genética de poblaciones, microsatellites

Landscape fragmentation and habitat loss due to land-use change is a major threat to biodiversity (Lee and Jetz 2010). Land conversion is expected to continue and increase in subsequent years (Sala et al. 2000; Lee and Jetz 2008). It therefore is important to understand how landscape features affect population structure and gene flow of species, not only to generate ecological baseline data but also to design population management strategies (Manel et al. 2003).

The Uruguayan Savanna ecoregion is comprised of Uruguay and the southern portion of Rio Grande do Sul (Brazil), and it borders with the Argentinian Humid Pampa ecoregion (Olson et al. 2001). This region has been dramatically modified due to soybean agriculture and the expansion of exotic forests. As a result, 26% of natural habitats were lost by 2007 (Brazeiro et al. 2008). Terrestrial carnivores, particularly large species, are among the species most affected by habitat loss, and their population decline affects the structure and regulation of ecosystems (Ripple et al. 2014). Furthermore, due to the global decrease in large predator populations, mesopredators are acquiring greater influence in the regulation of food web structure, although their ecological role remains poorly studied and understood (Prugh et al. 2009; Roemer et al. 2009; Sarasola et al. 2016). In most of the Uruguayan Savanna, the largest predators — jaguar (*Panthera onca*) and puma (*Puma concolor*) — have become extinct. As for the smaller felid species, the severity and impact of the anthropogenic threats remain unknown (González et al. 2016).

Geoffroy's cat (*Leopardus geoffroyi*, d'Orbigny & Gervais 1844) is a small felid, endemic to southern South America, and the only wild cat that inhabits the entire Uruguayan Savanna ecoregion (Ximenez 1975; Pereira et al. 2015; Bou et al. 2019; Fig. 1). It lives both in wooded and open habitats, including riparian forests, shrubland, and pampas grasslands (Cuyckens et al. 2016). It also is found in peri-urban areas and agricultural landscapes (Castillo et al. 2008; Pereira and Novaro 2014; Guidobono et al. 2016). The IUCN Red List of Threatened Species classifies Geoffroy's cat as "Least Concern" (Pereira et al. 2015). On a local scale, it is classified as "Least Concern" in Argentina and "Vulnerable" in Rio Grande do Sul (Aprile et al. 2012; Almeida et al. 2013). Although Uruguay still does not have a red list of threatened mammals, the species is considered as "Priority for Conservation" based on national criteria (González et al. 2013).

There are few genetic studies involving this small cat, and most of them focus on understanding the radiation of the family Felidae (Eizirik et al. 2003; Johnson et al. 2006) and the validity of its constituent species and subspecies. Four or five subspecies of *L. geoffroyi* have been recognized, mainly based on their fur color: *L. g. geoffroyi*, *L. g. paraguae*, *L. g. euxanthus*, *L. g. salinarum*, and *L. g. leucobaptus* (Cabrera 1958; Ximenez 1975; Wozencraft 2005). However, genetic analyses have not shown a clear phylogeographic pattern congruent with subspecies as described (Johnson et al. 1999; Gómez-Fernández et al. 2020). More recently, a morphological analysis likewise failed to support the existence of subspecies (Nascimento 2014). The authors suggested that there is a large panmictic population with no significant barriers to gene flow. In addition, a high degree of hybridization was detected in a restricted area of sympatry between *L. geoffroyi* and *L. guttulus* (Trigo et al. 2008; Trigo et al. 2013).

The purpose of the present study is to assess the population structure and gene flow of Geoffroy's cat in the Uruguayan Savanna ecoregion using nuclear hypervariable molecular markers (microsatellites). We focused our analysis at a regional level to understand Geoffroy's cat population dynamics and provide conservation and management guidelines.

Materials and Methods

Sample collection.—Samples were collected from Uruguay, Brazil (Rio Grande do Sul), and Argentina (Buenos Aires and Entre Rios Provinces), all areas that correspond to the Uruguayan Savanna and Humid Pampa ecoregions. We obtained high-quality samples from tissues of road-killed animals and blood from animals of known origin kept in zoos; low-quality samples were taken from museum skins, feces, and hair. Brazilian samples came from animals with no evidence of being hybrids of *L. guttulus* and *L. geoffroyi* (Trigo et al. 2013), or from areas distant from those where the hybridization described by Trigo et al. (2014) between the two species takes place. A total of 172 samples were collected. However, because DNA from noninvasive samples or museum specimens showed a low success of PCRs amplifications, we worked with a matrix of 70 individuals: 49 from Uruguay, nine from southern Brazil, and 12 from eastern Argentina (Supplementary Data SD1).

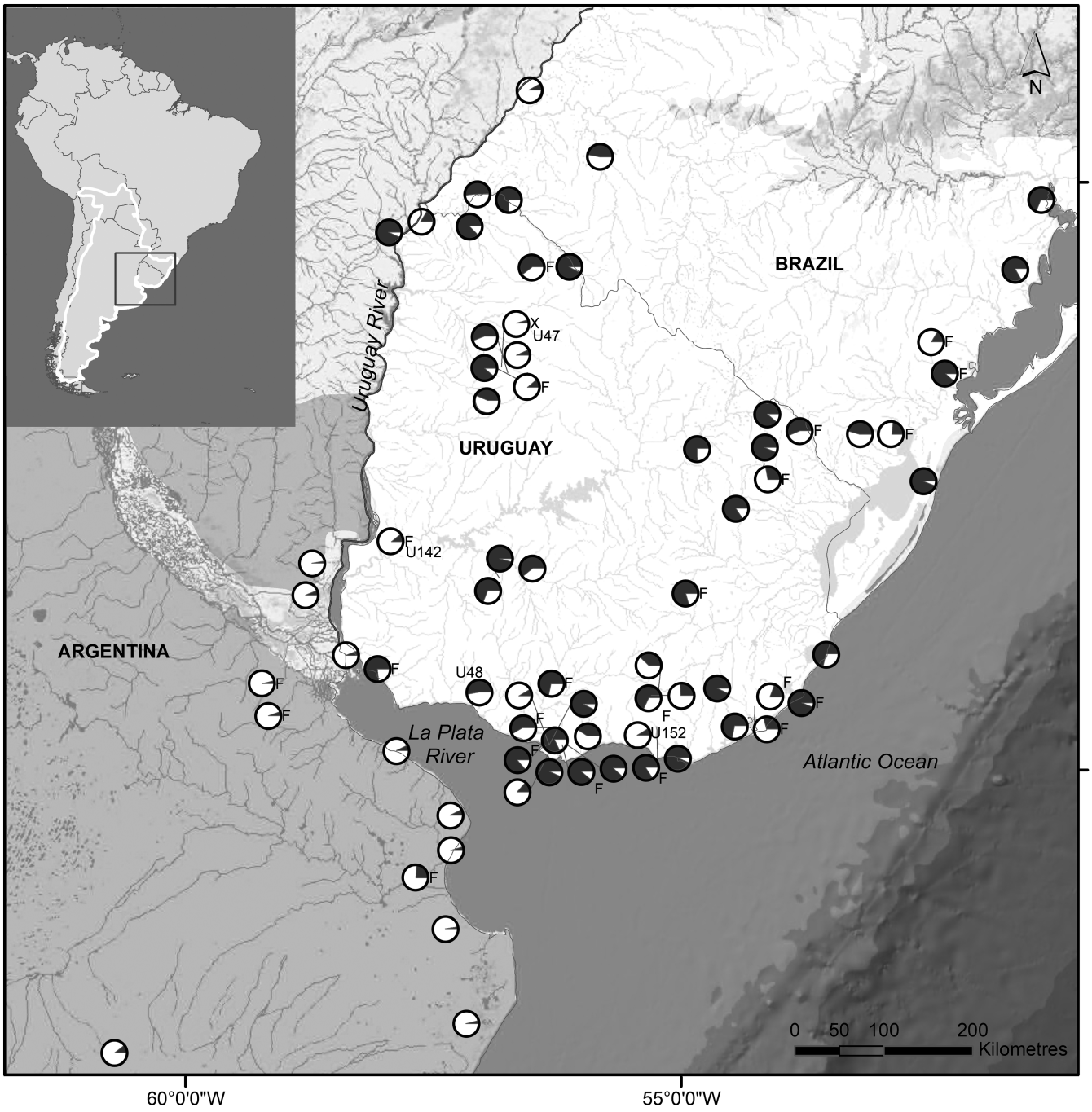

Fig. 1.—Map of South America (top-left) showing *Leopardus geoffroyi* distribution range (white boundary) from the IUCN Red List (Pereira et al. 2015), and the sampling area (square). The Uruguayan Savanna (white) and the Humid Pampa (light gray) ecoregions are identified in the sample area (Olson et al. 2001). Pie charts represent each individual's probability of membership to the two genetic groups identified with a Bayesian analysis. Samples are discriminated by gender: F for females, X for one individual with no sex information, and remaining individuals are males. Four first-generation migrants identified with GeneClass2 are indicated with their Sample ID.

Molecular methodology.—DNA was isolated from tissue samples following the procedure of González et al. (2015). Fecal DNA was extracted using QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA quality and quantification were analyzed by spectrophotometry (Nano-Drop Technologies, Inc., Wilmington, Delaware). Feces and museum samples were processed separately from fresh tissue to avoid cross-contamination. All samples were corroborated for their taxonomic identity with a multi-species TaqMan assay (Bou 2017) following the procedure of Cosse et al. (2017).

Thirteen microsatellite loci were amplified that originally were described for the domestic cat (Menotti-Raymond et al. 1999; Menotti-Raymond et al. 2012). Twelve of these had already had been tested for *L. geoffroyi* (Johnson et al. 1999; Trigo et al. 2008; Trigo et al. 2013); one was evaluated for the

first time for this species in this study. Four PCR multiplex were designed using fluorescently labeled primers (Supplementary Data SD2).

A PCR reaction of 10 µl final volume was carried out with 50–100 ng of total genomic DNA template, 0.2 µM of each primer, and 2.5× of PCR Platinum Multiplex Master Mix (Invitrogen Life Technologies, Carlsbad, California). The profile consisted of an initial denaturation at 95°C for 2 min, followed by 35–40 cycles of denaturation at 95°C for 30 s, an annealing step of 58°C or 60°C for 90 s (according to Multiplex, Supplementary Data SD2), an extension at 72°C for 50 s, and a final extension at 60°C for 30 min. Positive and negative controls were included in every PCR to check for contamination and standardize genotypes in different experiments. To minimize genotype errors, PCR replicates were undertaken for 50% of the samples (Taberlet et al. 1999). PCR products were analyzed by electrophoresis in 2% agarose gels and sent to the Institut Pasteur de Montevideo, Uruguay, for fragment analysis.

To establish the sex ratio of *L. geoffroyi* populations, we amplified a *Zfx–Zfy* gene fragment using ZFKF primers (Ortega et al. 2004) because several felid species have a 3-bp deletion on the *Zfy* gene compared with the *Zfx* gene (Pilgrim et al. 2005). To verify if *L. geoffroyi* had the *Zfy* deletion, we examined 17 samples of known sex. PCR conditions were the same as those used for microsatellite multiplex amplifications. We used a fluorescent dye (FAM) on the ZFKF 203L primer, and once the conditions were optimized, the fragment was amplified along with microsatellite multiplex PCRs.

Microsatellite analysis.—Genotyping and sex identification were carried out using GeneMarker 2.4.0 (Softgenetics LLC, State College, Pennsylvania). To ensure the reliability of the data, we only retained for analysis those samples that had at least 80% of the loci successfully amplified and consistently typed. Replicates per locus showed 2.6% of genotyping inconsistencies that, in most cases, were resolved by amplifying the microsatellite in question by itself rather than in a multiplex. Two loci, FCA249 and FCA080, were discarded from the final analyses due to low amplification efficiency (less than 50%) and poor genotyping concordance. Amplification efficiency and genotyping concordance of microsatellite markers are shown in Supplementary Data SD3.

Genotyping errors, null alleles, and allele dropouts, in the data set were identified by Micro-Checker 2.2.3 (Oosterhout et al. 2004). Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were evaluated for each locus and population (see "Results") using the Genepop 4.5.1 software (http://genepop.curtin.edu.au/) of Raymond and Rousset (1995) and Rousset (2008), and a sequential Bonferroni correction was used for multiple comparisons (Rice 1989). Polymorphism information content (PIC) value was calculated with Cervus 3.0.3 (Marshall et al. 1998).

F-statistics were estimated using FSTAT v.2.9.3 (Goudet 1995) and GenAlEx 6.5 (Peakall and Smouse 2006) software. Genetic variation levels were inferred from the average number of alleles per locus (A), observed heterozygosity (H_O), Nei (1978) unbiased expected heterozygosity (H_E), and the percentage/number of private alleles, all of which were calculated using GenAlEx 6.5 software (Peakall and Smouse 2006). Allelic richness (A_R) and private allelic richness (P_R) were computed with HP-Rare1.1 (Kalinowski 2005) using a rarefaction method, based on a minimum sample size of nine diploid individuals (18 gene copies), to compensate for unequal sample sizes.

Population analysis.—Structure (version 2.3.2) software was used to examine genetic subdivision patterns across the landscape (Pritchard et al. 2000). Twenty independent runs of K = 1–10 were undertaken with 200,000 Markov chain Monte Carlo (MCMC) repetitions, after a burn-in period of 100,000, using no prior information and assuming an admixture model. The proper number of clusters was estimated by ΔK calculations using Structure Harvester v. 0.6.94 (Evanno et al. 2005). Percentages of membership (q) from each individual for K clusters were plotted on a map using ArcMap (ESRI 2011). Differentiation among clusters was evaluated with genetic differentiation index F_{ST}, and its statistical significance was calculated with FSTAT (Goudet 1995). A genetic differentiation analysis also was carried out with the Geneland package, v. 4.0.6, in R software (version 3.3.1; e.g., Ihaka and Gentleman 1996; Guillot et al. 2005). Geneland uses genetic and geographic information to infer the number of clusters and their spatial distribution in the landscape (Guillot et al. 2005). Uncorrelated allelic frequencies were used, and no spatial uncertainty was assigned to the spatial coordinates of the samples. Five independent runs were carried out using 100,000 iterations. Every 100 iterations, values were saved for post-processing along the Markov chain Monte Carlo (MCMC). The number of clusters tested was K = 1 – 6. The choice of K was based on the highest mean posterior density across replicates. Samples were assigned to the cluster with the highest proportional probability of membership (Folt et al. 2019).

Indirect estimation of gene flow among clusters was expressed as the number of migrants per generation (N_m) calculated by the private allele method (Barton and Slatkin 1986) with Genepop. First-generation migrants were assessed with GeneClass2 (Paetkau 2004; Piry et al. 2004) using a Bayesian approach (Rannala and Mountain 1997).

Differentiation by distance was tested between matrices of genetic and geographic distances for each pair of individuals applying Mantel's test (Mantel 1967), calculated with GenAlEx 6 (Peakall and Smouse 2006). The genetic distance used was Nei's standard distance (D_s—Nei 1972) calculated with Population 1.2.32 software (Langella 1999). The geographical distance applied was the Euclidean distance obtained with GenAlEx 6.

Departure from drift-mutation equilibrium was tested using BottleneckV1.2.02 software (Cornuet and Luikart 1996; Piry et al. 1999) that examines deviations from expected heterozygosity excess relative to allelic diversity. During population bottlenecks, rare alleles are lost due to drift at a faster rate than loss of heterozygosity (Nei et al. 1975). Bottleneck uses this difference to detect past bottlenecks. We also evaluated the mode-shifted distortion on allele frequency distribution

to detect bottlenecks in the recent past (Luikart et al. 1998). Microsatellite could evolve by two extreme models: the infinite alleles model (IAM), and the stepwise mutational model (SMM). Basically, all loci will evolve by mutational events, and that will occur between these two extreme models (Piry et al. 1999). Perfect or imperfect dinucleotide repeats tend toward IAM, while tetra motifs tend toward SMM (Cornuet and Luikart 1996; Cristescu et al. 2010). The two-phase model (TPM) combines the two extreme models, and it has been suggested as a better approximation to what is observed in nature (Piry et al. 1999). In our microsatellite set, we had six dinucleotide and five tetranucleotide repeat motifs. We therefore carried out a bottleneck analysis for a first scenario with the TPM and chose a 40% SMM and 60% IAM proportion. A second scenario was built excluding microsatellites with tetra motif and using only dinucleotide repeats, based on the hypothesis that these repeats generate a more powerful analysis (Cornuet and Luikart 1996; Cristescu et al. 2010). In that case, the proportion was set in favor of IAM (5% SMM and 95% IAM). In both scenarios, we ran 1,000 iterations and tested significance with Wilcoxon signed-rank test as recommended by Maudet et al. (2002). The Wilcoxon test provides relatively high resolution and can be used with as few as four polymorphic loci and any number of individuals (Mahmoudi et al. 2012).

Results

Microsatellite analysis.—Five loci (FCA304, FCA096, F124, FCA126, and FCA424) showed significant deviation from HWE, three of them showed null alleles (FCA304, FCA096, and F124), and one pair of loci (FCA742/FCA043) showed significant LD after Bonferroni correction. The sample set also deviated from HWE. Estimated diversity was as follows: average number of alleles per locus (A) = 11.2, PIC = 0.74, observed heterozygosity (H_O) = 0.70, Nei (1978) unbiased expected heterozygosity (H_E) = 0.77, and F_{IS} = 0.093.

Population analysis.—Structure analysis resulted in Δk maximized at k = 2 (Fig. 2). Samples from the Uruguayan Savanna were included in one genetic group, whereas samples from the Humid Pampa were in a separate cluster (Fig. 1). Geneland analysis showed congruent results, supporting the same two clusters (Supplementary Data SD4). When we analyzed HWE separately for each one of the two identified

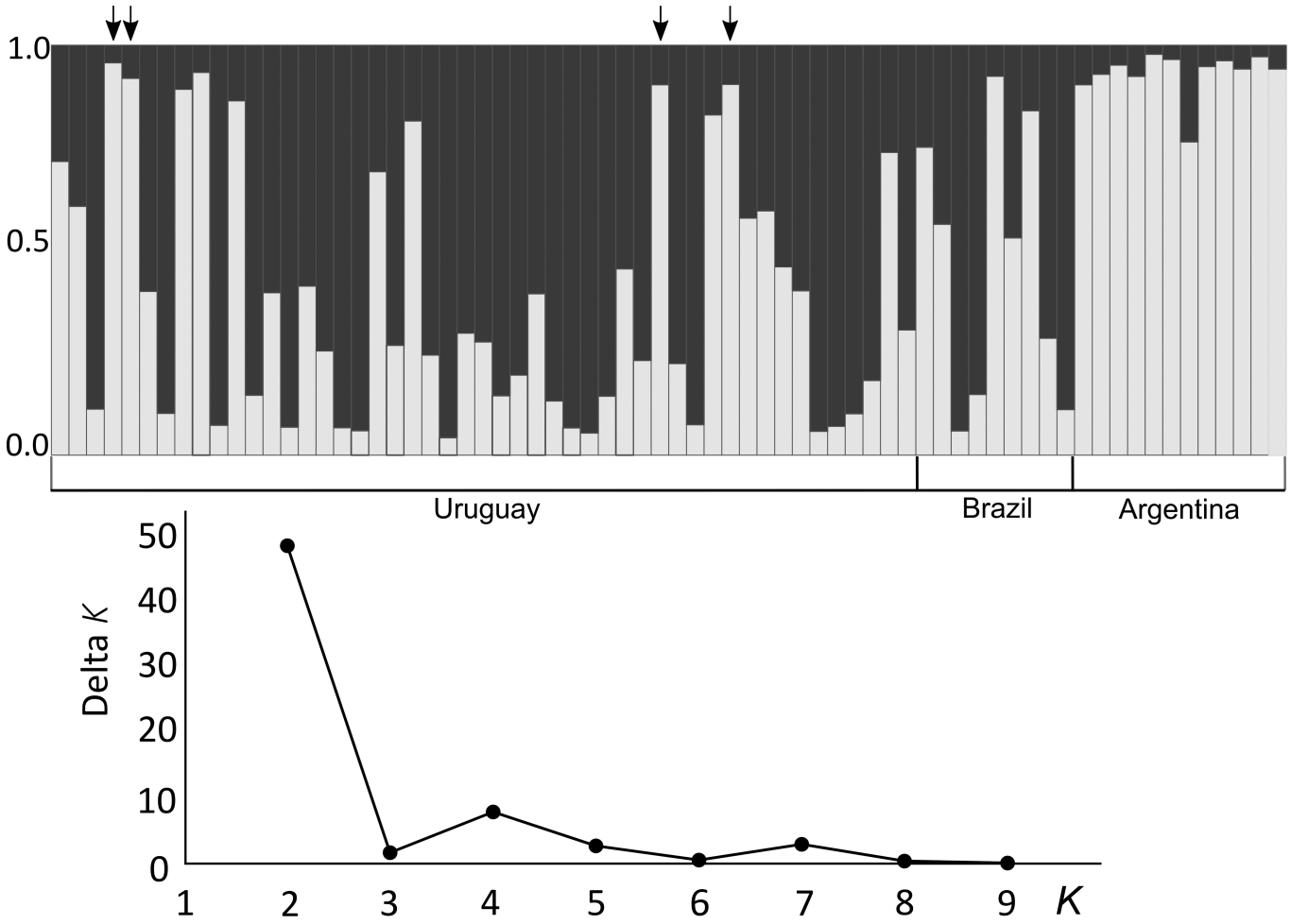

Fig. 2.—STRUCTURE bar plot (upper) showing two admixed genetic populations. Each column in the bar plot, from left to right, represents the individuals described in Supplementary Data SD1. Four first-generation migrants identified with GeneClass2 are indicated with an arrow. The magnitude of ΔK (rate of change in the log probability) as a function of K (clusters) is shown in the lower panel.

clusters, we found three loci that showed significant deviation from HWE with null alleles (FCA304, FCA096, and F124) in the Uruguayan Savanna cluster; this population also showed deviation from HWE. The Humid Pampa cluster exhibited HWE for all loci and at a population level (Table 1). Once allele frequencies were corrected for null alleles with Micro-Checker, the results supported the existence of two clusters and deviation from HWE in the Uruguayan Savanna population.

The F_{ST} genetic differentiation index between the Uruguayan Savanna and the Humid Pampa clusters was 0.042, which was statistically significant ($P < 0.01$). The number of migrants per generation was 1.83. Four first-generation migrants from Humid Pampa were detected in the Uruguayan Savanna cluster. Mantel's test showed a positive and significant relationship between genetic and geographic distance ($P = 0.012$, $r = 0.109$).

We detected a significant increase in the heterozygosity relative to expectation for the observed allele numbers in both Geoffroy's cat clusters on both TPMs (40% SMM and 60% IAM; 95% IAM and 5% SMM; Table 2). That indicated the existence of a past bottleneck. However, all simulations resulted in a normal L-shaped allele frequency distribution as would be expected in the absence of a recent bottleneck. Also, F_{IS} values for each cluster were low (Uruguayan Savanna = 0.081; Humid Pampa = 0.071).

Sex determination.—The 3-bp deletion in the *Zfy* gene was confirmed for Geoffroy's cat. All known females tested were homozygotes (genotype 195/195 bp), while all males were heterozygotes (genotype 192/195 bp). Of the 70 samples, 53 were of unknown sex; we were able to assign gender to 52 of them. The sex ratio obtained was 2:1 (47 males, 22 females, and 1 unassigned). The spatial distribution of the samples by sex did not show a geographic pattern (Fig. 1).

Discussion

Genotyping performance.—Given that ca. 80% of the samples came from museums and noninvasive sampling, we obtained poor DNA quality with low endogenous content and highly fragmented DNA samples, generally with the presence of inhibitors (Taberlet et al. 1999; van der Valk et al. 2017). To minimize genotyping errors, we only worked with samples with high PCR efficiency (amplification of nine out of 11 loci). As a result, just 40.7% of the samples collected were included in the study.

Genetic diversity and demographic history.—The genetic diversity of Geoffroy's cat from the Uruguayan Savanna and Humid Pampa populations is similar to that estimated by previous authors (Johnson et al. 1999; Trigo et al. 2008; Tirelli et al. 2018) and concordant to what is expected for healthy populations of species in the family Felidae (Garner et al. 2005). Genetic diversity in Geoffroy's cat was higher than that in endangered felid species that are habitat specialists, such as the kodkod (*Leopardus guigna*—Johnson et al. 1999; Napolitano et al. 2014, 2015), the Andean mountain cat (*Leopardus jacobita*—Cossíos et al. 2012), and the pampas cat (*Leopardus colocola*) from the Andean region (Cossíos et al. 2009). Surprisingly, genetic diversity in Geoffroy's cat is comparable to that of the oncilla (*Leopardus tigrinus*), a vulnerable habitat specialist, and lower than that of the pampas cat from central-south America (Trigo et al. 2008). However, both species have taxonomic delimitation issues and their

Table 1.—Genetic variability in Geoffroy's cat clusters obtained from 11 microsatellite loci: sample size (N), inbreeding coefficient (F_{IS}), observed heterozygosity (H_O), unbiased expected heterozygosity (H_E), P-value for Hardy–Weinberg equilibrium (HWE) (*significant departure from HWE, $P < 0.05$), the average number of alleles per locus (A), average allelic richness (A_R), and private allelic richness (P_R).

Population	Locus	N	F_{IS}	H_O	H_E	HWE	A	A_R	P_R
Uruguayan Savanna	FCA304	53	0.247	0.604	0.8	0.0001*	11		
	FCA742	57	0.085	0.772	0.843	0.2954	11		
	FCA096	45	0.324	0.622	0.917	0.0002*	21		
	F124	58	0.177	0.586	0.711	0.1975	6		
	F42	58	–0.041	0.914	0.878	0.6724	12		
	FCA391	56	0.068	0.732	0.785	0.4313	10		
	FCA126	58	0.005	0.741	0.745	0.0385*	8		
	FCA424	58	0.124	0.328	0.374	0.0568	6		
	FCA146	56	0.085	0.679	0.741	0.2939	8		
	FCA043	58	–0.118	0.914	0.818	0.0578	9		
	FCA187	58	–0.044	0.776	0.743	0.8998	7		
	Population	58	0.083	0.70	0.76	High significance	9.91	6.31	2.24
Humid Pampa	FCA304	12	0.048	0.75	0.786	0.6566	8		
	FCA742	12	0	0.917	0.917	0.9061	12		
	FCA096	9	0.164	0.778	0.922	0.3048	10		
	F124	12	0.088	0.75	0.819	0.263	7		
	F42	12	0.064	0.833	0.888	0.0899	11		
	FCA391	12	0.46	0.333	0.605	0.08	4		
	FCA126	12	0.112	0.75	0.841	0.1162	7		
	FCA424	12	–0.222	0.417	0.344	1.0000	2		
	FCA146	12	0.238	0.583	0.757	0.4275	4		
	FCA043	12	–0.131	0.917	0.815	0.0795	7		
	FCA187	12	–0.134	0.833	0.739	0.468	5		
	Population	12	0.071	0.71	0.77	Non-significance	7	6.41	2.34
Global population		70	0.093	0.70	0.77	High significance	11	6.36	

Table 2.—BOTTLENECK results for two Geoffroy's cat clusters detected with Structure: Uruguayan Savanna (US) and Humid Pampa (HP). Different scenarios were simulated with two-phase model (TPM): all loci with 40% proportion of stepwise mutational model (SMM) in TPM (P. SMM in TPM) and only loci with dinucleotide repeat motifs with 5% proportion of SMM in TPM. Mean *N* and *k* represent the mean numbers of gene copies and alleles, respectively; expected heterozygosity (H_E); *P*-values of Wilcoxon test, significant results ($P < 0.05$) with an asterisk (*), and the allele frequency distribution obtained.

	P. SMM in TPM (%)	Pop.	Mean *N*	Mean *k*	H_E	Wilcoxon test TPM (*P*-value)	Allele frequency distribution
All loci	40	US	111.82	9.91	0.76	0.0268*	L-shaped
		HP	23.45	7.00	0.77	0.0415*	L-shaped
Only loci with dinucleotide repeat motifs	5	US	110.50	10.00	0.78	0.0020*	L-shaped
		HP	23.25	6.50	0.79	0.0195*	L-shaped

diversity values probably include more than one species (Trigo et al. 2008, 2013; Santos et al. 2018; Nascimento et al. 2020). On the other hand, the expected heterozygosity seems to be a little lower in Geoffroy's cat than in the ocelot (*Leopardus pardalis*—Ruiz-Garcia et al. 2012), a nonthreatened species with a broad distribution range. Genetic diversity among felid species of the genus *Leopardus* is summarized in Fig. 3. These comparisons should be taken with caution because each study used different sets of microsatellite markers.

We detected a bottleneck event based on the Wilcoxon test, but also observed an L-shaped allele frequency distribution that showed the lack of a recent genetic bottleneck. Some demographic events in the population, such as exponential growth, immigration, or sampling bias, could result in false negatives (Luikart et al. 1998; Piry et al. 1999; Cristescu et al. 2010). The L-shaped allelic frequency distribution observed should be expected in a population that suffered either a low severity bottleneck or a bottleneck more than 12 generations ago (approximately 30 years) with a subsequent recovery (Luikart et al. 1998). The bottleneck event could be related to the reduction in the native forests during World War II, when problems in oil and coal supply led to the use of wood and charcoal as energy sources (Bertoni and Román 2006). Also, Geoffroy's cat experienced a large hunting pressure for its fur that increased since the 1960s, reaching the second most frequently commercialized fur in the international market (Sunquist and Sunquist 2017). In 1992, the species was promoted to Appendix I of CITES, thus prompting the end of its fur's legal commercialization (UNEP-WCMC 2013; Pereira et al. 2015). This period of extensive poaching could have resulted in a significant reduction in the size of the population, with its subsequent recovery.

Population structure and gene flow.—Evidence of at least two Geoffroy's cat genetic clusters consistent with the Uruguayan Savanna and the Humid Pampa ecoregions does not support the hypothesis of Johnson et al. (1999) and Nascimento (2014) of a large panmictic population with no significant barriers to gene flow. Johnson et al. (1999) carried out a phylogenetic analysis with samples 33 *L. geoffroyi* from throughout the species' range. In contrast, our study comprises a larger number of samples in a smaller area and involves a detailed population analysis. Genetic differentiation between Humid Pampa and Uruguayan Savanna populations ($F_{ST} = 0.042$; $P < 0.01$) is lower compared to the pairwise F_{ST} values estimated with microsatellite markers among other Neotropical felids populations, where anthropogenic impact and habitat fragmentation are the main factors that drive the observed structure; e.g., *L. guigna*, *P. onca*, *L. pardalis*, and *P. concolor* (Haag et al. 2010; Napolitano et al. 2014; Wultsch et al. 2016). The pairwise F_{ST} value observed in this study is similar to the values obtained between sampling localities of *P. onca* in the Pantanal, a reasonably preserved region where individuals wander across the landscape (Valdez et al. 2015). The high levels of genetic variability in Geoffroy's cat, low differentiation between clusters, and the lack of genetic structure within ecoregions suggest that, despite changes introduced into the species' habitat by human activities, the populations genetics of the species has not been severely affected. Other factors therefore could be causing the genetic differentiation observed.

One such factor is isolation by distance (Rousset 1997). In fact, we found a positive correlation between geographic and genetic distance. However, differentiation by distance, isolation by barriers, and landscape resistance can give the same signal in Mantel's test (Murphy et al. 2008; Cushman and Landguth 2010); other causes therefore have to be explored. The Uruguay River, which divides the two ecoregions, is not a full barrier for this cat because gene flow does exist, but it may restrict its movement, for the river on average is 1–2 km wide and widens toward its mouth (Iriondo and Kröhling 2008). Biological corridors should be further investigated, but the connection probably is through the north, where the Uruguay River is narrower and some islands found there could work as stepstones between the river banks, particularly during periods of drought (Iriondo and Kröhling 2008). Historical events also could be the underlying causes of landscape structure. Gómez-Fernández et al. (2020) proposed that Geoffroy's cat dispersed from central Argentina toward the periphery until it reached its current geographic distribution range. Because we are working with microsatellite markers, we cannot make inferences about historical factors (Balkenhol et al. 2009; Landguth et al. 2010). It would be interesting to corroborate if mitochondrial markers also retain the structure signal between ecoregions.

The four individuals identified as first-generation migrants, and the admixture observed in the Uruguayan Savanna, which is greater than that in the Humid Pampa, suggests a stronger gene flow in the west-east direction, although flow in the opposite direction also may be possible. The central-periphery colonization pattern (Gómez-Fernández et al. 2020) could generate a gene flow dynamic preserved until

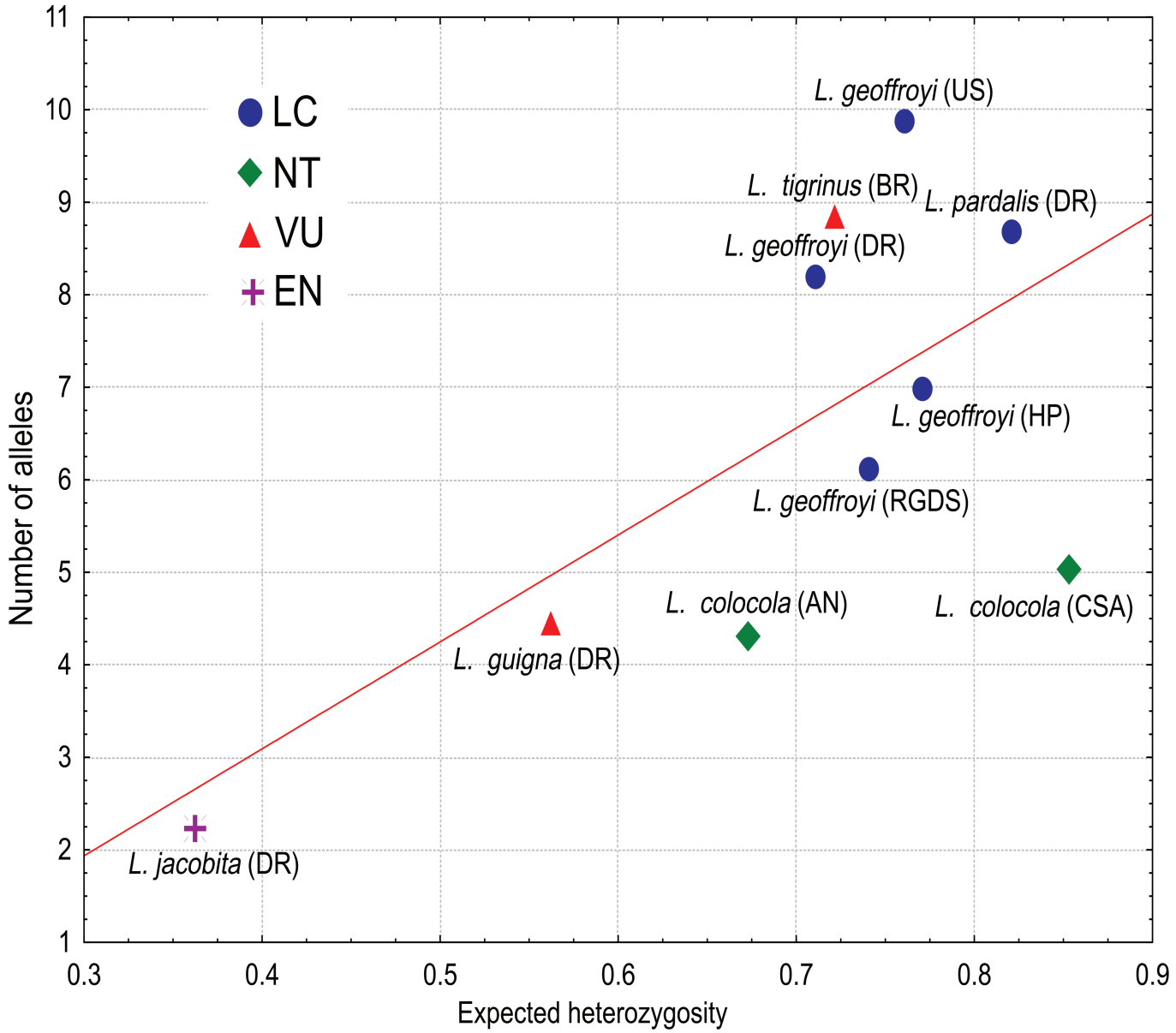

Fig. 3.—Comparison of microsatellite genetic diversity among felid species of the genus *Leopardus*. Mean expected heterozygosity and number of alleles were estimated from this study: *L. geoffroyi* from Uruguayan Savanna (US) and Humid Pampa (HP); Tirelli et al. (2018): *L. geoffroyi* from Rio Grande Do Sul (RGDS); Trigo et al. (2008): *L. geoffroyi* from the whole distribution range (DR), *L. colocola* from central-south America (CSA), and *L. tigrinus* from Brazil (BR); Cossíos et al. (2009): *L. colocola* from the Andean region (AN); Cossíos et al (2012): *L. jacobita* from the whole distribution range (DR); Napolitano et al. (2015): *L. guigna* from the whole distribution range (DR); Ruiz-Garcia et al. (2012): *L. pardalis* from the whole distribution range (DR). LC: Least Concern; NT: Near Threatened; VU: Vulnerable; EN: Endangered.

today. Nevertheless, it cannot be discarded that the apparent unidirectional flow is an effect of the different sample sizes between clusters, as larger sample size increases the chances of finding migrants. To have a better understanding of the structure process and flow dynamics, it is critical to increase the number of samples from the Humid Pampa and include samples from other ecoregions, particularly from the northern limit of the Uruguayan Savanna.

Sex determination.—Sex ratio in our sample was 2:1 (m:f). This could represent the actual sex ratio of the population in the territory, or it could be the result of a sampling bias. In general, male Geoffroy's cats have greater dispersion and larger home ranges with less overlap than females (Manfredi et al. 2006; Pereira et al. 2012b; Tirelli et al. 2018). Males therefore would be more susceptible to higher mortality caused by encounters with humans (Kamler and Gipson 2000; Haines et al. 2005; Pereira and Novaro 2014). Because our samples did not come from livetrapped animals but mainly from roadkill, poaching, or captured animals in zoos (Supplementary Data SD1), our set of samples could reflect this bias toward males.

Conservation and management implications.—The Geoffroy's cat population from the Uruguayan Savanna is well connected throughout the territory, shows healthy levels of genetic variability, and is not undergoing any structuring process due to anthropogenic causes. Our findings support the inclusion of this cat in a nonthreatened category of the red list of Uruguayan mammals that currently is being developed. However, it is important to note that there is a time lag between the emergence of an environmental barrier and its corresponding genetic signal. It takes approximately five generations to detect the appearance of a barrier for organisms with relatively high dispersion capacity (> 20 Km—Murphy et al. 2008; Landguth et al. 2010), such as Geoffroy's cat (Johnson and Franklin 1991; Cuellar et al. 2006; Pereira and Novaro 2014). Considering a generational time of 2–4 years (Nowell and Jackson 1996; Foreman 1997; Pereira et al. 2012a), our analysis shows a scenario from approximately 20 years ago. The most dramatic land conversion process in Uruguay began in 2000 and has been increasing since 2002 (Soutullo et al. 2013). Thus, the effect of habitat modification in the last decades should be assessed in the next generations. Also, we suggest complementing this information with ecological studies on a finer scale that could reveal any ecological and anthropogenic pressures that Geoffroy's cat is facing, and the compensatory behavioral measures it develops (Pereira and Novaro 2014).

Our analysis highlights the importance of maintaining ecological connectivity between Uruguay and Brazil. There are two main orographic systems in eastern Uruguay acting as biological corridors with Brazil, systems that also are one of the most suitable areas for Geoffroy's cat in Uruguay: Cuchilla Grande and Cuchilla de Haedo (Evia and Gudynas 2000; Cantón et al. 2010; Bou et al. 2019). The existence of other corridors must be evaluated, but in the meantime, we recommend that land-use planning must be carried out taking into consideration the preservation of natural areas in these two geographic systems. Gene flow from the Humid Pampa ecoregion contributes to maintaining high levels of genetic variability in the Uruguayan Savanna population. Therefore, it is important to identify the main corridors to gain a better understanding of gene flow patterns and promote their maintenance over time.

A central question regarding management implications arising from our work is whether Geoffroy's cat population in the Uruguayan Savanna has to be considered a Management Unit (MU—Moritz 1994). There are several genetic values used as a reference to recognize independent MUs. Wang (2004) proposed a threshold of one effective migrant per generation between groups. Waples and Gaggiotti (2006) suggested less than five migrants per generation, which correspond to a F_{st} > 0.05. Geoffroy's cat populations in the Uruguayan Savanna and Humid Pampa show genetic differentiation (F_{st} = 0.042) and gene flow values (N_m = 1.83) close to the threshold values proposed to define MUs. In addition, several authors advise that the determination of MUs should be done case-by-case, and that genetic data must be complemented with demographic, ecological, and historical information (Taylor and Dizon 1999; Crandall et al. 2000; Palsböll et al. 2006). The threats and the conservation context of the populations also should be evaluated (Taylor and Dizon 1999). We consider that our findings support the need for testing the validity of this MU in a more comprehensive framework (e.g. Crandall et al. 2000; Napolitano et al. 2014). Regardless of whether the population of the Uruguayan Savanna is or not a MU, Garner et al. (2005) advised regarding the relevance of targeting conservation efforts at the population level. Preservation of genetic variability in each population ensures the maintenance of all genetic variants and resources existing within a species and therefore achieves efficient protection of the genetic diversity of the species as a whole (Garner et al. 2005). In the Uruguayan Savanna, Geoffroy's cats have a genetic identity of their own, slightly different from that of conspecifics inhabiting the contiguous ecoregion, the Humid Pampa. This must be taken into account when considering conservation and management policies at a local and regional scale. In an increasingly human-modified environment, our work contributes to a better understanding of the spatial dynamics of the Geoffroy's cat at the regional level and establishes a starting point for population monitoring.

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Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Samples of Geoffroy's cat analyzed in this study. Sample ID is the sample code in IIBCE's collection. MNHN: National Museum of Natural History of Uruguay, one individual (U130) had not been entered into the official collection yet and remains with a private acronym. Geographic area specifies divisions within countries (departments in Uruguay, states in Brazil, and provinces in Argentina). Uy: Uruguay, Br: Brazil, Ar: Argentina; F: female, M: male.

Supplementary Data SD2.—Primers used in this study. Chr.: chromosome location of microsatellite loci in *Felis silvestris catus*. Size: expected PCR product size (in base pairs). AT: multiplex annealing temperature in °C. [1]Original description by Menotti-Raymond et al. (1999); [2]original description by Menotti-Raymond et al. (2012). Tested in *Leopardus geoffroyi* by [A]Johnson et al. (1999); [B]Trigo et al. (2008); [C]Trigo et al. (2013); [D]present study.

Supplementary Data SD3.—Amplification efficiency and genotyping concordance of the 11 microsatellite markers used in this study.

Supplementary Data SD4.—Geneland analysis output. (a) Bar plot showing the posterior density distribution of the clusters (K = 1–6). (b) Map of estimated cluster membership of individuals, K = 1 in green and K = 2 in gray. (c and d) The assignment of individuals to each one of the clusters. The highest probability of membership is in light yellow.

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